

Urinary pathology in pregnancy

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1 Preface

1.1 Declaration

I, Jane Louise Danielle Currie, confirm that the work presented in this PhD thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

1.2 Word count

99,954 words excluding title pages, table of contents, appendices and references.

1.3 Abstract

Urinary tract infection (UTI) in pregnancy is associated with pyelonephritis and preterm birth (PTB). However, modern evidence questions these associations. UTI in pregnancy research lacks clinical outcomes. I questioned the gold standard UTI diagnosis and examined the underlying assumptions.

Alternative tests for urinary pathology were developed to research chronic UTI. I applied them to pregnancy; either for the first time (Artemis LUTS inventory, urinary adenosine triphosphate (ATP), urinary lactoferrin, fluorescent microscopy for bacteria-associated epithelial cells and uroplakin-3-stained epithelial cells) or in novel settings (quality-of-life assessment, fresh unspun microscopy for cell counts, enhanced sediment culture with identification of microbial isolates to species level, urinary IL-6 and IL-8).

In the "Abdominal pain" study I recruited women with abdominal pain (n=50), gestation-matched against women with other acute problems (n=58) and attending routine appointments (n=51).

In the "PTB study" I studied women with high risk of preterm birth owing to previous preterm birth (n=37), gestation-matched against women with other preterm birth risk factors (n=30) and low risk women (n=13).

In the MSU-CSU study I compared mid-stream urine (MSU) samples against catheter specimen urine (CSU) samples in women undergoing catheterisation for elective caesarean (n=32) or cervical cerclage (n=2).

I used parallel methods to enable pooling of MSU results to study relationships between tests and compare against outcomes (n=273).

There were minimal between-group differences in Abdominal pain and PTB studies. MSU and CSU samples were markedly different, which may reflect different sampling of the urinary sediment rather than contamination. There was a high prevalence and range of urinary pathology across settings, for all alternative tests; these detected more pathology than standard culture. However, the significance for outcomes is not clear.

Findings provide further evidence to challenge assumptions underlying UTI in pregnancy diagnosis, to encourage a more thoughtful approach clinically, and to demand outcome-focussed future research.

1.4 Impact statement

Clinicians and patients care about UTI in pregnancy because of the risk of poor outcomes for mother and baby. Current practice is based on 1950s research which demonstrated increased risk of pyelonephritis and preterm birth from asymptomatic bacteriuria, using a culture threshold which became the gold standard. UTI in pregnancy research subsequently focused on comparing new diagnostic tests against the gold standard, without re-examining outcomes. This research demonstrates that studies about diagnosis of UTI in pregnancy need to consider outcomes, to improve care.

Correct diagnosis of UTI is imperative to minimise harm by (1) avoiding overtreating women who do not need antibiotics (for example some women with asymptomatic bacteriuria, although we do not know which) and (2) avoiding undertreating women who may benefit (for example with urinary-pathology-related preterm birth risk).

There may be economic benefit to improving diagnosis. Universal screening for asymptomatic bacteriuria is expensive, yet modern evidence questions its benefit. 2-10% of women are positive for asymptomatic bacteriuria, and are offered antibiotics, many in first trimester. This brings into consideration risks of antibiotic use and antimicrobial resistance. Any reduction in preterm birth, a significant cause of neonatal morbidity and mortality, which also brings economic cost to parents and health services, would be valuable.

This work has been presented at local and national conferences, including the UK Preterm Birth conference (winning best oral presentation) and the RCOG Annual Academic Meeting (winning best poster pitch). This gave an opportunity to reach audiences who were not familiar with UTI diagnosis controversies. This has led to many conversations, both academics interested in research collaboration, and clinicians wanting to understand the implications for their practice. Following locally-delivered teaching, I have seen the small-scale impact of the research on clinician colleagues changing their practice.

Whilst we cannot currently recommend alternative tests without further evidence, we can recommend care improvements using current tests. This involves a more nuanced approach to UTI diagnosis, including assessing

symptoms and risk of consequences of a UTI, taking care in sample preparation, paying attention to microscopy including epithelial cell counts, and discussing symptomatic or high-risk women with microbiologists as different culture interpretations may apply.

Research findings will be disseminated via publication in peer-reviewed journals.

Thanks to this work I have secured ongoing funding for UTI in pregnancy research. I successfully competed for a post-doctoral lectureship (University of London Chadburn Lectureship). This supports ongoing diverse research in the field of UTI in pregnancy. I have been awarded a UCL Precision AMR (Antimicrobial Resistance) Seed Project Award to investigate bacteriuria in pregnancy in first trimester using a data science approach.

Having established a lack of consensus about outcomes for UTI in pregnancy research I have supervised a systematic review of outcomes in UTI in pregnancy research, and registered a protocol for development of a core outcome set for UTI in pregnancy research and am developing this with stakeholders. I am working with patient representatives to discuss future patient and public involvement (PPI) work and have been awarded a UCLH BRC PPI bursary.

1.5 Acknowledgements

I would like to thank the inspirational Professor Anna David, for championing me, for making supervisions enjoyable and productive, and for relentless enthusiasm. I thank Professor James Malone-Lee for sharing his infinite wisdom and experience on research in chronic UTI, and giving me his time even in retirement. Thank you to Dr Catherine James for conceiving of this collaboration, for setting up the CLIMB study, and for nurturing my research skills.

I could not have completed this work without the guidance and energy of my laboratory companions and mentors. I thank them for cell wizardry, microbiological mastery, and brightening lab days: in particular, Dr Harry Horsley, Dr Sanchutha Sathiananthamoorthy, Dr Sheela Swamy, and Dr Jennifer Rohn.

I thank my MSc student, Dr Natasha Liou, whose boundless enthusiasm was contagious and who conducted excellent work on the MSU-CSU study.

I am grateful to those who helped with recruitment at UCLH, including Frida Forya, and the clinical and administrative teams on the antenatal floor at UCLH. In particular I thank Kris McCann, ward clerk extraordinaire, for welcoming me at the front desk in MFAU and facilitating recruitment.

Thanks to Wellbeing of Women, and the British Maternal and Fetal Medicine Society scholarship, which supported this research financially. Thanks to the EGA Charity, for funding our microscope.

I sincerely thank my amazing family and friends, who have tolerated my absences, and my talking about urine in pregnancy, for more than six years. In particular my Dad Colin, who would have been very proud, and my Mum Sheila, who has always been my biggest champion. This would not have been possible without the tolerance and love of my husband Edouard and our children, and I cannot thank them enough.

Finally, but not least, I thank the women who participated in this study.

Dedication

To Nathan and Camille, the sunshine of my days.

1.6 Specific contributions to this work

I carried out the literature review, analysis of data, discussion and conclusion. I developed changes in existing methods including designing clean catch MSU kits, validating a commercial IL-8 assay for urine, and validating the 'count to 20' method for bacteria-associated urothelial cell counting.

For the "Abdominal pain study", I designed the study, recruited patients, collected samples, carried out laboratory analysis, input results, and analysed data.

For the "PTB study", Dr Catherine James originally conceived the study and ran a pilot with medical students before I started. These data were used to guide study design and were not originally included in this thesis; they have been added following the PhD viva to section 8.8. I recruited patients and collected samples (initially with Dr Arvinder Athwal for one month), carried out laboratory analysis, input results and analysed data. I designed an extension to the scope of the original study to perform a matched analysis against the Abdominal pain study, specifically additionally analysing a fuller range of urinary cytokines and identifying microbial isolates to species level.

For the "MSU-CSU study", I conceived the study and designed it to mirror the preceding two studies. I supervised Dr Natasha Liou, an MSc student, to perform the study where she recruited patients undergoing catheterisation for elective caesarean and cervical cerclage, collected samples, and carried out part of the laboratory analysis (fresh unspun microscopy, enhanced sediment culture to genus level, fluorescent microscopy of clue cells and uroplakin-3 stained epithelial cells). She submitted her findings for the caesarean patients for her MSc at UCL, and this was published (Liou, Currie et al. 2017). I additionally analysed microbial isolates to species level, and carried out ELISA for urinary IL-6, IL-8, lactoferrin and protein for all the samples. I completed additional clinical data collection including standard microbiology throughout the pregnancies. I combined the data with the cervical cerclage patients and re-analysed all the data before presenting it here.

For the pooled data, I designed and conducted the analysis independently.

While I acknowledge the contributions made by others above, I take responsibility for all interpretation presented here including any errors.

1.7 Publications arising from this work

Published abstracts:

- Currie J, James CP, Rohn J, Malone-Lee J, David A. Alternative tests for urinary tract infection in women at increased risk of preterm birth: a prospective case control study. BJOG; 126 (S1): 45-45 Presented as poster at BMFMS Annual Scientific Meeting, Edinburgh, March 2019 (EP.082)
- Currie J, James CP, Malone-Lee J, David AL. Do pregnant women with abdominal pain have urinary pathology not detected by standard tests? A prospective study (poster number PM.078). In Maternal Medicine Poster Abstracts. BJOG; 125 (S2): 57-86. Work presented as poster at BMFMS Annual Scientific Meeting, Brighton April 2018 and RCOG Annual Academic Meeting, February 2018.
- Currie J, James CP, Malone-Lee J, David AL. Urine of pregnant women is not sterile - use of an enhanced culture technique to explore bacteriuria in pregnancy (poster number P.MM.109). In Maternal Medicine Poster Abstracts. BJOG; 124: 82–121. Work presented as poster at BMFMS Annual Scientific Meeting, Amsterdam March 2017 and RCOG Annual Academic Meeting, March 2017.
- Currie J, James CP, Malone-Lee J, David AL. Pregnant women with abdominal pain report more urinary symptoms than controls but have a similar rate of positive urine culture (oral presentation number PM.62) BJOG: An International Journal of Obstetrics and Gynaecology. 2016; 123:35

Peer-reviewed publication:

Liou N, Currie J, James C, Malone-Lee J, David AL (2017) Urothelial cells may indicate underlying bacteriuria in pregnancy at term: a comparative study. BMC Pregnancy Childbirth 17: 414

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1.11 Glossary of abbreviations

%CV	Percentage coefficient of variation
AMPs	Anti-microbial peptides
ATP	Adenosine-5'-triphosphate
χ^2	Chi square
CCMSU	Clean-catch mid-stream urine
CFU	Colony-forming unit
CI	Confidence Interval
CLIMB	Cervical Length, InflaMmation and Bacterial species and preterm birth (CLIMB) study
CS	Caesarean section
CSU	Catheter specimen of urine
ELISA	Enzyme-linked immunosorbent assay
EPC	Epithelial cell(s)
FE	Fisher's Exact
GBS	Group B Streptococcus (<i>Streptococcus aeruginosa</i>)
IL-6	Interleukin-6
IL-8	Interleukin-8 / CXCL-8
LUTS	Lower urinary tract symptoms
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometer
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSU	Mid-stream urine
PBS	Phosphate buffered saline
PHE	Public Health England
PPROM	Preterm prelabour rupture of membranes
PROM	Prelabour rupture of membranes (at term)

PTB	Preterm birth
RBC	Red blood cell(s)
RCT	Randomised controlled trial
Sig.	Significance
SVD	Spontaneous vertex (vaginal) delivery
UP3	Uroplakin-3
UTI	Urinary tract infection
VAS	Visual analogue scale
WBC	White blood cell(s)
WHO	World Health Organisation
WOCA	Weekly oral cyclic antibiotic therapy
WSR	Wilcoxon signed rank

Study group names

LR	Low risk (PTB study)
HR-PTB	High risk with previous preterm birth (PTB study)
HR-non-PTB	High risk without previous preterm birth (PTB study)

1.12 Overview

When I tell clinicians, particularly fellow obstetricians, that I am researching urinary pathology in pregnancy, specifically the diagnosis of urinary tract infection (UTI) in pregnancy, many look puzzled. Most feel confident to diagnose a urinary tract infection, whether that is in pregnancy or not. This encapsulates one of the challenges of research in this field. Assumptions about how to diagnose urinary tract infection are deeply entrenched in the belief systems of patients and the clinical reasoning processes of clinicians. These range from the lay concept that normal urine is sterile, to more clinical assumptions such as the central importance of urine culture in making a diagnosis, or epithelial cells in urine being a marker of a contaminated specimen.

My perspective is as a doctor in Obstetrics and Gynaecology, currently a subspecialty trainee in Maternal and Fetal Medicine. This work has been a collaboration with three supervisors. Professor David, a subspecialist in Maternal and Fetal Medicine, leads a research-focussed Preterm Birth Clinic, looking after women who are at high risk for preterm birth. Professor Malone-Lee, a physician, leads a research-focussed service for male and female patients with chronic lower urinary tract symptoms (LUTS), many of whom have been unsuccessfully treated in the past and who often find relief through the reframing of their symptoms as chronic urinary tract infection. Dr James, an academic clinician with a research interest in infection and inflammation in Obstetrics, conceived the collaboration between these two uncommonly linked fields.

My research methods are indebted to techniques from microbiology, immunology and cell biology which have been new to me as an obstetrician. I do not pretend to be an expert and fully accept responsibility for any misunderstandings and gaps in broader knowledge. My original contribution to knowledge has been to link ideas from these fields and to apply them to problems in obstetrics in a way that either has not been done previously or has not integrated different sources of information.

In the first part of the background (see chapter 2) I critically review the literature about urinary pathology in pregnancy. After supplying basic definitions, I will outline the prevalence of urinary pathology in pregnancy, before critically reviewing evidence for the implications for both mother, and the baby, including preterm birth (PTB). I will specifically discuss asymptomatic bacteriuria as an important concept in obstetrics, and discuss issues with antibiotics in pregnancy. I will also give a brief background about the specific problems of abdominal pain in pregnancy.

In the second part of the background (see chapter 3) I shall evaluate the evidence underpinning current diagnostic methods for UTI. I shall take a series of UTI assumptions in turn, and question the evidence underpinning them. Where possible I will consider evidence from studies involving pregnant participants.

In the third part of the background (see chapter 4) I will introduce alternative diagnostic methods that will underpin this research. Some of these represent a 'back to basics' approach, such as analysis of lower urinary tract symptoms (LUTS) and fresh unspun urine microscopy, conducted in the clinical area. Some involve a modification of existing methods, such as enhanced urine culture using urinary sediment from larger volumes of urine. Some are more exploratory, such as near-patient ATP (Adenosine-5'-triphosphate), urinary cytokines, or the use of fluorescent microscopy to examine the cellular components of the urine.

In chapter 5 I will summarise the themes that emerge from the literature review and the subsequent research aims. I will then discuss some specific research contexts, also arising from the literature review and requiring further investigation, that led to the three main studies in this work. These are: women with abdominal pain in pregnancy ("Abdominal pain study"); women at risk of preterm birth ("PTB study"); and the role of catheter specimens versus voided samples ("MSU-CSU study"). I will outline the rationale and specific research questions for each of these studies.

In chapter 6 I will explain my methodological approach, followed by detailed methods for each study. Methods overlap between the three research contexts

and so Abdominal pain study methods will be described in full, with the other study methods described where there are differences.

In chapters 7-9 I will describe and discuss results of the Abdominal pain study, the PTB study and the MSU-CSU study respectively. In chapter 10 I will describe and discuss results of pooled MSU data from the three studies.

In chapter 11 I will discuss the findings from this research, revisiting the structure of background chapters 2-4. In chapter 12 I will conclude by summarising how the studies address the research questions, and discuss weaknesses in the data. I will describe the implications of this research for practice, policy and further research.

It should be noted that this work spanned 2014-2020. Background chapters 2-4 include references up to 2015, to contextualise the work within the literature at the time the study was designed. Subsequent chapters include more recent references.

2 Background part 1 - The importance of urinary pathology in pregnancy

2.1 Introduction

Urinary pathology in pregnancy is common (Sheiner, Mazor-Drey et al. 2009) and UTI has been associated with maternal and fetal complications.

Asymptomatic bacteriuria is the presence of cultivable bacteria in the urine, above a standard threshold, without symptoms of UTI ((NICE) 2018). In the UK it is standard practice to treat asymptomatic bacteriuria in pregnancy, to prevent associated maternal and fetal complications ((NICE) 2018). This follows an experiment conducted by Edward Kass on pregnant women with significant bacteriuria according to criteria he developed (Kass 1960). He allocated them to antibiotic treatment for the duration of the pregnancy or to no treatment. He recorded a lower complication rate amongst the treatment group.

A conundrum arises from recent evidence of the poor sensitivity of the MSU culture (Khasriya, Sathiananthamoorthy et al. 2013). What of the infections missed by the Kass culture? There may be women who have a UTI that does not show up on standard hospital laboratory culture. Given that concern, abdominal pain in pregnancy which causes women to seek help becomes more significant. Part of the standard obstetric assessment of abdominal pain includes exclusion of UTI by culture.

2.2 Terms and definitions

I will use the term *urinary pathology* to describe infectious and inflammatory processes occurring in the urinary tract. Urinary tract infection (UTI) is associated with specific diagnostic criteria. I will use that term when describing studies that used that term.

There are several definitions that are apposite to this subject and I will adopt the NICE terminology used at the start of this body of research (National Institute for Health and Care Excellence 2014):

“Bacteriuria: bacteria are present in the urine.

Significant bacteriuria: the count of bacteria in the urine exceeds the threshold for diagnosing the presence of infection in the urine. The threshold depends on the age and sex of the person, and on the risk of contamination during collection of the urine sample

Asymptomatic bacteriuria (ASB): there is significant bacteriuria without symptoms and signs of infection.

Urinary tract infection (UTI): there is significant bacteriuria and characteristic symptoms and signs”

A commonly accepted threshold for significant bacteriuria in UK practice ((NICE) 2018) is the Kass criterion, a culture of a single urinary pathogen with a yield of $\geq 10^5$ colony-forming units per ml (CFU/ml) (Kass 1957).

Asymptomatic bacteriuria (ASB) features heavily in the obstetric literature about UTI.

2.3 Prevalence and economic burden

UTI in women of child-bearing age is common, with 10.8% of women aged 18 and over reporting one physician-diagnosed UTI in the previous 12 months (Foxman, Barlow et al. 2000).

UTI in pregnancy is also common (Sheiner, Mazor-Drey et al. 2009). A US national database survey found that UTI was responsible for 10% of antenatal admissions (Bacak, Callaghan et al. 2005) and this may be the commonest admission cause (Waters and Bailit 2012). Asymptomatic bacteriuria complicates 2-10% pregnancies although rates may be higher in some developing countries (Smaill and Vazquez 2007, Smaill and Vazquez 2015). In the UK, screening for asymptomatic bacteriuria occurs at the booking visit ((NICE) 2008), however there is evidence that screening before 20 weeks misses more than half of cases of asymptomatic bacteriuria (McIsaac, Carroll et al. 2005).

Non-pregnancy comparisons are impossible because pregnancy sees much more screening.

Economic burden of UTI has been estimated outside pregnancy: In 2003 US cost of community-acquired UTI was estimated as \$1.6 billion (Foxman 2003).

The economic burden of screening for asymptomatic bacteriuria in pregnancy is substantial. In 2018 there were 664,370 live births in England and Wales (ONS 2019). Based on those data and a price of £7.71 per MSU test (National Institute for Health and Care Excellence 2019), at least £4.9 million must be spent on asymptomatic bacteriuria testing annually. The additional cost of symptomatic UTI may be substantial.

2.4 Urinary pathology in pregnancy and maternal conditions

2.4.1 Pyelonephritis

In the 1950s, pyelonephritis was a common finding at autopsy (Kass 1957) and was the commonest complication of pregnancy (Kass 1960).

Kass studied the natural history of asymptomatic bacteriuria in pregnancy (Kass 1960). Examination of urine between 2-7 months gestation showed that asymptomatic bacteriuria was acquired before eight weeks, with rates of 5-7%. Kass noted that rates increased with age and parity. His intervention study involved 48 women with asymptomatic bacteriuria being given placebo, and 40 women given antibiotics. This was 0.5g sulfamethoxypyridazine daily, initially planned for 1-2 weeks, but this was extended for the duration of pregnancy because of the persistence of positive cultures.

Of the placebo group, 20/48 (41.7%) developed pyelonephritis in the last trimester or first three months post-partum, compared with 0/40 (0%) in the treatment group. Kass predicted that "the way is thus clear for the virtual elimination of pyelonephritis of pregnancy" (Kass 1960). The relative risk of pyelonephritis with treatment compared to placebo was 0 (95% confidence interval 0 to 0.5). It has been suggested that pyelonephritis in pregnancy is so preventable that it can be used as a marker of the quality of care provided (Korst, Reyes et al. 2006).

It is worth considering some of Kass' related observations. He thought that bacteriuria was critical to health / disease:

"There is now clear evidence that bacteriuria is one of the commonest human infections, that it may be chronic and persistent, that it may influence structure and function outside of the urinary tract, and that it plays an important role in disease from the cradle to the grave – from prematurity to hypertension and renal failure." ((Kass 1962), p52)

He viewed asymptomatic bacteriuria as a chronic disease (Kass 1962).

He suggested that UTI may alter function in non-urinary organs:

"Whether similar more distant effects associated with chronic urinary tract infections can be discerned in other disease states is an important area for future exploration." ((Kass 1962), p51)

Kass separated the bacteriuria of pregnancy from anatomic changes by noting that:

"bacteriuria appears before the anatomic changes in the urinary tract that are characteristic of pregnancy have had time to occur" ((Kass 1960), p195).

Savage, working with Kass, subsequently carried out a placebo-controlled trial of treating bacteriuria in pregnancy (Savage, Hajj et al. 1967). In this study consecutive patients, between 1956-1960, attending a Boston clinic were recruited. 92% of all patients who delivered at the hospital during the study period registered for prenatal care.

The treatment consisted of, in the first instance, 0.5g/day of sulfamethoxypyridazine, for a week with a review of repeat cultures. Women who still had positive cultures were given nitrofurantoin 100mg three times per day. This regime led to negative cultures in all but three patients. Nevertheless, patients continued treatment until delivery.

Pyelonephritis was more common amongst untreated women with bacteriuria, with a rate of 26% antenatally, compared with none in the treated group. The background pyelonephritis rate in the study was 1.4%.

Modern estimates of the prevalence of pyelonephritis in pregnancy range from 2.1/1000 in a US national database study (Dotters-Katz, Heine et al. 2013) to 14/1000 deliveries in a large retrospective US study (Hill, Sheffield et al. 2005).

Globally, antenatal screening and treatment for asymptomatic bacteriuria in pregnancy is almost universal (Kazemier, Koningstein et al. 2015). A 2015 Cochrane review noted that a reduction in pyelonephritis rates in pregnancy seems to have occurred since the introduction of screening for asymptomatic bacteriuria, which is supportive evidence of its efficacy (Smaill and Vazquez 2015). This review, while acknowledging the poor quality of evidence, estimated that a third of women with asymptomatic bacteriuria develop pyelonephritis if untreated (Smaill and Vazquez 2015).

A recent two-year retrospective study of all women with pyelonephritis in a single hospital (n=440) identified risk factors for this occurrence as nulliparity, young age and having a known medical risk factor for pyelonephritis, and found acute pyelonephritis to occur most commonly in the second trimester (53%) (Hill, Sheffield et al. 2005).

Pyelonephritis in pregnancy has been associated with death and severe maternal morbidity including sepsis and acute respiratory, renal and cardiac failure (Jolley, Kim et al. 2012, Dotters-Katz, Heine et al. 2013).

2.4.2 Severe maternal morbidity and mortality including sepsis

Sepsis is one of the biggest causes of maternal mortality in the UK (Knight, Kenyon et al. 2014). In the 2009-2012 confidential enquiry into maternal deaths, the maternal mortality rate was 10.63/100,000 maternities (252 deaths), with 83 deaths due to sepsis. Of these, most were genital tract sepsis (20 women) and one woman died from sepsis following urinary tract infection.

The death rate from genital tract sepsis decreased from 1.13 to 0.50 deaths per 100,000 maternities between the confidential enquiries of 2006-2008 and 2009-2012 (Beveridge, Harris et al. 1967, Herbert, Beveridge et al. 2004, Knight, Kenyon et al. 2014). However, when infective causes beyond the genital tract, including UTI, were included, the figure rose to 2.04/100,000 maternities.

A national case-control study of maternal sepsis in the UK, from 2011-2012, examined all causes of sepsis (Acosta, Kurinczuk et al. 2014). They reported that for every death from all sepsis there were 100 women with severe

morbidity. This study identified that UTI and genital tract infections were the predominant sources of infection, and the largest proportion of cases of severe sepsis were caused by *E. coli*. 72/365 (19.7%) cases of sepsis originated from urinary tract infection, and of the antepartum cases, 45 (33.6%) originated from UTI, compared with 27 (11.7%) of postnatal cases. In women with a UTI diagnosis, over 40% of the causative organisms were *E. coli*, however *Group B streptococcus*, other streptococci, staphylococcus, and other organisms were also identified, and in some cases no organism was identified.

In summary, bacteriuria is associated with maternal pyelonephritis and UTI and is an important cause of maternal sepsis.

2.4.3 Pre-eclampsia

A population-based case control study (Minassian, Thomas et al. 2013) investigating the risk of pre-eclampsia following infection, or antibiotic use in pregnancy found increased odds of pre-eclampsia in women who were prescribed antibiotics and in women with UTI. This was consistent with a 2008 meta-analysis of the relationship between all infections and pre-eclampsia (Conde-Agudelo, Villar et al. 2008), which identified that pregnant women with UTI had an increased risk of pre-eclampsia. One hypothesis is that some cases of pre-eclampsia may be a form of nephrotic syndrome in a kidney previously damaged by recurrent UTI, particularly if it occurred in childhood (Ozlu, Alcelik et al. 2012).

2.5 Urinary pathology in pregnancy and fetal/neonatal conditions

2.5.1 Aetiology of preterm birth

Preterm birth (PTB), that is birth before 37 weeks gestation, is associated with neonatal mortality and severe morbidity, and can leave children with long-term health and developmental problems (Saigal and Doyle 2008).

Preterm birth can arise from spontaneous preterm labour, preterm prelabour rupture of membranes (PPROM), or medically indicated birth because of maternal or fetal problems (Goldenberg, Culhane et al. 2008).

Pre-pregnancy clinical risk factors for spontaneous preterm birth (which includes births arising from spontaneous preterm labour and PPRM) include: previous spontaneous preterm birth or late miscarriage; cervical surgery such as large loop excision of the transformation zone (LLETZ) or cone biopsy; and uterine malformations such as bicornuate uterus. In recent years an association with previous caesarean section, particularly full dilatation caesarean section, has emerged (Carlisle, Glazewska-Hallin et al. 2020). There are also epidemiological associations including ethnicity, smoking and socio-economic deprivation and body mass index (Goldenberg, Culhane et al. 2008). Clinical risk factors within the current pregnancy include: antepartum haemorrhage; multiple pregnancy; extremes of amniotic fluid volume such as oligohydramnios or polyhydramnios; and infection or inflammation. Infections include the intra-uterine infection chorioamnionitis, genital infections such as bacterial vaginosis, and non-genital tract infections such as urinary tract infection.

Spontaneous preterm birth is likely to be a syndrome with multiple phenotypes, where different aetiologies lead to a final common pathway. Infection has an important relationship with preterm birth, with multiple routes proposed for access of micro-organisms to the amniotic cavity, which appears to be sterile in normal circumstances even using sequencing methods, although there remains controversy (Blaser, Devkota et al. 2021). These routes include retrograde from abdominal cavity, haematogenous spread through placenta, direct inoculation via invasive procedures, or ascending from vagina (Goldenberg, Culhane et al. 2008).

Intrauterine infection is thought to lead to preterm labour via activation of the innate immune system (Romero, Espinoza et al. 2006). Pattern-recognition receptors, such as toll-like receptors, recognise microorganisms, which leads to release of inflammatory cytokines and chemokines, like interleukin 1-beta, interleukin 8, and tumour necrosis factor alpha. A combination of pro-inflammatory cytokines and microbial endotoxins leads to production of prostaglandins, and matrix-degrading enzymes. It is thought that prostaglandins stimulate uterine contractility, while degradation of extracellular matrix in fetal membranes leads to PPRM (Goldenberg, Hauth et al. 2000).

2.5.2 UTI and preterm birth

UTI in pregnancy has been linked with preterm birth since the time of Kass' work in the mid-twentieth century (Kass 1962). He compared bacteriuric pregnant women who either had placebo (n=95) or treatment with antibiotics (n=84). He found premature births in 26/95 (27.4%) of deliveries for the placebo group and 6/84 (7.1%) for the treated group. Although statistics were not cited, this was significantly different (Fisher's Exact, $p=0.0004$). The relative risk of premature birth in the treated group was 0.26 with a 95% confidence interval of 0.11 to 0.58 (my statistical analysis). The background rate of preterm birth in non-bacteriuric patients was 88/1000 (8.8%).

There are some pathophysiological explanations for why this association may occur. As described above, there is evidence that labour and preterm labour are inflammatory processes (Gomez-Lopez, StLouis et al. 2014), and that chemokines are involved in term and premature rupture of the amniotic membranes (Gomez-Lopez, Laresgoiti-Servitje et al. 2010). It could be hypothesised that inflammation arising from UTI could predispose to preterm labour or ruptured membranes.

However, the relationship has never been clear. If we return to the Savage study from 1967 (Savage, Hajj et al. 1967), treating women for bacteriuria did not reduce the preterm birth rate compared with placebo.

In a more recent example, a Taiwanese nationwide population-based study of women with UTI in pregnancy found no increased risk of adverse pregnancy outcomes (Chen, Chen et al. 2010, Chen, Chen et al. 2010). Using health insurance data of women treated for UTI, 42,742 women with UTI were compared with 42,742 randomly selected controls. The authors acknowledge that bacteriuria was aggressively treated, so there was little potential for finding a difference. This illustrates a key difficulty; the historic association of asymptomatic bacteriuria with preterm birth means that screening and treating asymptomatic bacteriuria in pregnancy is standard practice in many countries (Kazemier, Schneeberger et al. 2012).

The UK National Screening Committee (UK NSC) altered its advice in 2012 (Committee 2012) to claim that asymptomatic bacteriuria screening in

pregnancy was to prevent only pyelonephritis, rather than preterm birth. This was based on two systematic reviews. The first evaluated asymptomatic bacteriuria screening (Honest, Forbes et al. 2009). Asymptomatic bacteriuria performed poorly as a predictor for preterm birth but showed a significant reduction in preterm birth associated with antibiotics. The second (Smaill and Vazquez 2007), included 14 studies, from the 1960s to 1970s. They were poor quality. Only three of the studies examined preterm birth defined by gestational age rather than birthweight; they found no difference between antibiotic use and none. Both reviews spoke of the poor quality of the studies.

The Cochrane review was updated in 2015 (Smaill and Vazquez 2015) having redefined preterm birth as birth below 37 weeks, so that just two studies were included. They reported less preterm birth when antibiotics were used but the data were weak.

Since asymptomatic bacteriuria treatment is routine in antenatal care globally, it is ethically difficult to research its effectiveness. However, it is not routine care in the Netherlands. A multicentre prospective cohort study with an embedded randomised controlled trial (RCT) was conducted in the Netherlands (Kazemier, Koningstein et al. 2015). Women with a singleton pregnancy were screened at 16-22 weeks' gestation for asymptomatic bacteriuria using dipslide and two culture media. A positive culture was either 10^5 CFU/ml of a single organism or two different colonies where at least one was above 10^5 CFU/ml. The RCT compared nitrofurantoin 100mg twice a day for five days with placebo. The primary endpoint was pyelonephritis with or without preterm birth below 34 weeks. There were 686 dipslides thought to be contaminated out of 5132 eligible women (13.4%) and these women were excluded from the study.

Of 4238 participants eligible for inclusion, 248 (5.9%) had asymptomatic bacteriuria. Of these, 40 were randomised to nitrofurantoin, 45 to placebo and 163 followed without treatment. There was no difference in pyelonephritis between groups. A pooled analysis of asymptomatic bacteriuria-positive women compared with asymptomatic bacteriuria-negative women showed that if not given antibiotics 5/208 (2.4%) developed pyelonephritis versus 24/4035 (0.6%). The authors concluded that asymptomatic bacteriuria was associated

with pyelonephritis, albeit with a lower incidence than previously found, but not with preterm birth. The authors criticised routine screening for asymptomatic bacteriuria in pregnancy, but the data were insufficient to support this.

Their study had not scrutinised the women who are the focus of international guidelines, which advocate screening earlier in the first trimester (National Institute for Health and Care Excellence 2019, 2020), whereas the Dutch did this at 16-22 weeks' gestation. They also excluded women who had been treated with antibiotics earlier, which may have included those with asymptomatic bacteriuria during the first trimester.

Those women who were asymptomatic bacteriuria positive, and not treated with antibiotics, were more likely to be prescribed antibiotics for UTI later during pregnancy (20.2% versus 7.9%) and recurrence of UTI was higher (8.7% versus 2.6%). These later treatments may have reduced the primary outcome of pyelonephritis.

The authors also excluded from the study women at increased risk of UTI or preterm birth. This may have explained the unexpectedly low rate of pyelonephritis. The findings are therefore only applicable to women who are low risk for UTI or preterm birth.

Prematurity was defined as below 34 weeks. Birth between 34-37 weeks carries increased risks compared with term birth (Ananth, Friedman et al. 2013); if asymptomatic bacteriuria were associated with late preterm birth then this association could have been missed.

The study used a different culture method, dipslides, instead of the midstream urine culture which is more universally accepted (Mignini, Carroli et al. 2009).

Whilst the relationship between asymptomatic bacteriuria and preterm birth is unresolved, particularly in low-risk women, we should remember that asymptomatic bacteriuria is at one end of a spectrum; symptomatic infection may have a different influence on pregnancy outcomes.

We do know that antenatal pyelonephritis is independently associated with preterm birth (Farkash, Weintraub et al. 2012), particularly between 33-36 weeks' gestation (Wing, Fassett et al. 2014).

2.5.3 Low birthweight

Low birthweight research is afflicted by terminology perplexity with low birthweight, small-for-gestational-age and fetal growth restriction having different implications. These conditions are associated with increased neonatal morbidity and mortality and potential long-term health implications for offspring (Gynaecologists 2013).

The previously-discussed systematic review of antibiotic treatment for asymptomatic bacteriuria (Smaill and Vazquez 2007) also studied the effect on low birthweight. Of the 14 studies included in the meta-analysis, only seven studies, involving 1502 women, reported on low birthweight, defined as less than 2500g. This showed a reduction in birth weight with a risk ratio of 0.66 (95%CI 0.49-0.89). Since only one of the seven studies reported on preterm birth as an outcome, it is difficult to untangle low birthweight from preterm birth.

There is animal evidence of a physiological link between UTI and fetal growth restriction. In a murine model of uropathogenic *E. Coli* (Bolton, Horvath et al. 2012), bacterial cystitis was induced in pregnant mice at 14 days. Pups were smaller in the UTI group with no change in maternal size, suggesting a direct link between UTI and fetal growth restriction.

2.5.4 Neonatal / childhood impact

The effect on children of maternal UTI and related antibiotics exposure is difficult to know. One population-based cohort study found that genito-urinary infections with antibiotic use were associated with increased risk of cerebral palsy (Miller, Pedersen et al. 2013). The paper accepts the problem of separating the infection and the antibiotic use. There is an association between cytokine levels in amniotic fluid and periventricular leukomalacia, a predictive risk factor for the development of cerebral palsy (Yoon, Park et al. 2003).

Another population database study found an association between maternal admission for UTI and childhood asthma (adjusted odds ratio 1.49, 95%CI 1.23-1.79) (Algert, Bowen et al. 2011).

A systematic review and meta-analysis of the use of antibiotics for preterm labour with intact membranes (Flenady, Hawley et al. 2013) described increased risks of short and long term harm to offspring.

Although the associations are difficult to untangle, this is an aspect which would benefit from good prospective studies on UTI in pregnancy including long-term infant outcomes.

2.6 The special problem of asymptomatic bacteriuria in pregnancy

It could be argued that the near-universal treatment of asymptomatic bacteriuria in pregnancy (Kazemier, Koningstein et al. 2015) regardless of symptoms, reduces attention to LUTS in obstetrics by encouraging too much test reliance. If a positive culture must be treated it does not follow that symptoms with a negative culture does not reflect a UTI. I have not been able to find an obstetric inventory for UTI symptoms in the literature.

The original title of this thesis was “Do some pregnant women with abdominal pain and negative culture have subclinical UTI?” The phrase subclinical UTI reflects the prevalent belief that, without a positive culture, there is no UTI. Semantics may influence clinical reasoning.

2.7 Non-UTI urinary pathology in pregnancy

The prevalence of lower urinary tract symptoms in pregnancy, such as incontinence, urgency and frequency, will be discussed later (see 3.2.1).

Stone formation (urolithiasis) has the same prevalence as the general population although pregnant women are more likely to form calcium phosphate stones rather than calcium oxalate, thought to be due to renal excretory changes and a higher urine pH in pregnancy (Meher, Gibbons et al. 2014).

2.8 Issues with antibiotics

Antibiotics for UTI are one of the commonest medications taken during pregnancy worldwide (Lupattelli, Spigset et al. 2014). The potential fetal and neonatal consequences of antibiotics have already been discussed.

As regards the mother; a 2011 systematic review of antibiotics for symptomatic UTI in pregnancy (Vazquez and Abalos 2011) found no consensus on preferred regimes, antibiotics were effective and complications rare. There is a risk of anaphylaxis and antimicrobial resistance (AMR).

Compliance may be a problem, particularly amongst the asymptomatic seeking to minimise adversity (Matsui 2012).

2.9 Pathophysiology of UTI in pregnancy

The pathophysiology of UTI in pregnancy may differ from other situations. Nowicki et al reviewed this (Nowicki, Sledzinska et al. 2011). Uterine compression causing partial ureteric and bladder obstruction, physiological changes such as progestogenic effects, increased glomerular filtration rate, increased glycosuria are suspected. Immunological changes in pregnancy include a downregulation of cellular immunity and promotion of humoral immunity.

Some UTI in pregnancy research has examined virulence factors using molecular methods (Nowicki, Sledzinska et al. 2011). A multinational study (Ramos, Sekikubo et al. 2012) reported lower-virulence species featuring in UTI of pregnancy. Differences in immune response and pathogen virulence in pregnancy will be discussed in greater detail later.

I think that UTI in pregnancy should be understood as a specific pathology. Norms for UTI in non-pregnant people may not apply, to symptomatology, microbiological thresholds or molecular studies. Ideas about pathophysiology of UTI should be explored in an obstetric population.

2.10 Abdominal pain in pregnancy

There are many causes of abdominal pain in pregnancy (Cappell and Friedel 2003). Some specific, such as threatened miscarriage, or placental abruption; some non-obstetric from abdominal or pelvic organ systems. The assessment starts with analysis of pain location and qualities (Bates C. M. 2013). The abdominal location can be pathognomonic of the pathology, even in pregnancy, despite a belief that anatomical changes alter locations (Mourad, Elliott et al. 2000).

Initially, pain may be unexplained. A 1989 retrospective study of antenatal admissions for pain in one hospital in one year identified 140 in which no cause was found (Baker, Madeley et al. 1989); of these, 129 had negative MSU culture. These patients, compared with 280 controls matched for age and parity, had similar outcomes.

A 1992 study of antenatal presentations to one hospital, during a two-month study of 1040 acute presentations, identified 148 with positive urine cultures (MacDermott 1994). UTI was defined by Kass culture threshold plus pyuria in immediate uncentrifuged urine microscopy. 24/148 (16.2%) patients had significant pyuria and so were treated with antibiotics. Of those, 4/24 (16.7%) had a significant urine culture, with no relationship between the degree of pyuria and culture result. 4/24 (16.7%) had no significant growth but antibacterial substances were detected in their urine.

The relative risk of dysuria for UTI was 26.6 (95%CI 3.0-239.9), and for loin pain was 12.5 (95%CI 1.9-80.1). There was no association between a positive culture and urinary frequency, lower abdominal pain, back pain, preterm contractions or pyrexia. Symptoms or signs not documented in the notes were assumed absent which is problematic and the UTI criteria were unusual. There was no mention of obstetric outcomes.

A 1995 retrospective study of antenatal admissions to a single hospital in one year identified 69 patients admitted for abdominal pain, out of 3500 deliveries (Impey L 1995). Of these patients, 50/69 (72.4%) had no cause identified. Day assessment units in obstetrics may have changed management of abdominal pain (Dowswell, Middleton et al. 2009), although some cases require inpatient assessment.

A pregnancy symptoms inventory for normal pregnancies established 34 items (Foxcroft, Callaway et al. 2013). Whilst it found that back pain, and “hip pelvic pain”, were common symptoms of pregnancy, abdominal pain did not emerge.

It is likely that abdominal pain consumes resources and causes anxiety despite negative investigations. Avoiding over- or underdiagnosis of UTI, and avoiding missing other diagnoses, would be positive steps.

Unexplained acute abdominal pain is common outside pregnancy. In a 1995 US emergency department study (Powers et al 1994) it was found that abdominal pain could not be diagnosed in 24.9% of a series of 1000 cases presenting for acute care.

2.11 Conclusions about the importance of urinary pathology in pregnancy

Urinary pathology in pregnancy is common. The term encompasses a spectrum of issues, from asymptomatic bacteriuria, which if untreated is associated with pyelonephritis and may be associated with preterm birth, to symptomatic UTI, to pyelonephritis, which is associated with preterm birth and may cause life-threatening sepsis. Much of the evidence underpinning current practice is relatively old, with microbiological criteria derived from the 1950s when pyelonephritis was the commonest complication of pregnancy and was often identified at autopsy, and epidemiological or case control studies from the 1970s-1990s of poor methodological quality. More recently, universal screening and treatment of asymptomatic bacteriuria in pregnancy has been questioned. Whilst it is important to reduce bothersome symptoms, and prevent maternal and fetal complications, it is also important to avoid the unnecessary use of antibiotics, for maternal, fetal and population concerns. It is not clear from the evidence here that our understanding of urinary pathology in pregnancy is sufficient to be certain that our use or non-use of antibiotics is always correct. The role of urinary pathology in preterm birth, and unexplained abdominal pain in pregnancy, are specific issues where urinary pathology could be better understood.

In this chapter I have focussed on urinary pathology prevalence and risks. In the next chapter I will critically evaluate current methods for the diagnosis of urinary pathology in pregnancy, including assumptions that may be used in everyday clinical reasoning.

3 Background part 2 - Diagnosis of urinary pathology in pregnancy

3.1 Introduction

The diagnosis of UTI in pregnancy involves several assumptions. In the first part of this chapter (3.2) I will appraise the evidence. The assumptions are as follows: (1) altered urinary symptoms in pregnancy may be attributed to normal pregnancy; (2) normal urine is sterile; (3) urine dipsticks help to diagnose UTI; (4) mixed growth of a urine culture indicates contamination; (5) significant bacteriuria has a threshold of 10^5 CFU/ml; (6) epithelial cells in the urine are a marker of contamination; (7) cultured organisms can be categorised as uropathogens or contaminants; (8) catheter specimens avoid the problem of contamination.

In the second part of this chapter (1.1.1) I will consider the difference between dichotomy and spectrum disorders and the influence of language on diagnosis. I will introduce the damage-response framework, and a diagnostic approach derived from outcomes. I will briefly consider the statistical definitions and tools used in diagnostic test studies.

In the third part of this chapter (3.4) I will attempt to explore whether current diagnostic approaches correctly discriminate between patients who do or do not need treatment, by looking for evidence of harm caused by over-treatment or under-treatment.

3.2 Challenging assumptions in the diagnosis of UTI in pregnancy

3.2.1 Assumption 1: Altered urinary symptoms in pregnancy may be attributed to normal pregnancy

Many studies have attempted to describe normal urinary symptoms in pregnancy (summarised in Table 3-1).

A 1980 longitudinal study found daytime frequency at booking affected above 40% of nulliparous and multiparous women (Stanton, Kerr-Wilson et al. 1980). This increased over pregnancy with 95% nulliparous women and 76% multiparous women reporting this at term. Stress incontinence symptoms increased with gestation, along with urge incontinence and hesitancy, albeit with a lesser gestation effect. Patients with UTI, and those who delivered preterm, were excluded.

A subsequent study, taking account of confounders from previous birth, was a prospective study of primigravid women (Viktrup 1993). Both stress incontinence and urge incontinence were more frequent towards late gestation and then decreased post-partum, with new onset of these approaching term. No information was given about urine analysis.

Another study interviewed nulliparous women after 34 weeks and postnatally (Chaliha, Kalia et al. 1999). Women with current UTI or previous recurrent UTI were excluded. Increased urinary symptoms were reported during pregnancy compared with before and after.

More recent studies show similarly high rates of LUTS in patients in whom UTI has been excluded, with increases through gestation (Sharma, Aggarwal et al. 2009, Liang, Chang et al. 2012, Lin, Shen et al. 2014). Assertions about pre-pregnancy and early pregnancy symptoms in all these studies were retrospective and vulnerable to recall bias.

In a 1991 first trimester study (Cutner, Cardozo et al. 1991), excluding women with current or past UTI, LUTS were common. The commonest symptom was frequency (40%) but nocturia, urgency, incontinence and voiding symptoms were also noted.

A 2013 systematic review of studies of urinary incontinence in pregnant women (Sangsawang and Sangsawang 2013) found stress urinary incontinence had an average 41% prevalence (range 18.6% to 60%), urgency urinary incontinence of 2% to 35%, and mixed incontinence of 3.8% to 13.1%, with prevalence increasing with gestational age. A 2017 questionnaire study of urinary incontinence during pregnancy in 750 women found that previous UTI was a risk factor (OR = 3.8, 95%CI 1.5-9.3) (Dinc 2017).

It may be possible to discriminate pathological symptoms on bothersomeness. A standardised tool, the ICIQ-FLUTS, which assesses symptoms and how bothersome they are, was used in pregnancy (Adaji, Shittu et al. 2011). They found high prevalence of LUTS correlating with gestation, but the majority were rated as 'not bothered' or 'mildly bothered'. There was no microbiological data to compare with the findings.

All the above studies were on cohorts of pregnant women, without comparison with microbiological results. One study comparing LUTS with urine analysis recruited 1000 antenatal patients, all of whom underwent questioning and urine microscopy (Hanif 2006). Patients reporting symptoms (426/1000, 42.6%) also had a urine culture. Of the symptomatic women, 104/426 (25%) had pyuria and 37/426 (8.7%) had positive cultures. Because only symptomatic women had cultures, comparisons with normal were impossible.

Back pain may be an atypical symptom of UTI in pregnancy. In a study investigating the prevalence, 73% of pregnant women reported back pain, (Madeira, Garcia et al. 2013). 20% with low back pain reported UTI, and those had a higher pain score.

A primary care study of LUTS in non-pregnant women found four symptoms predicted UTI: cloudy urine; smelly urine; severe dysuria; and severe nocturia (Little, Turner et al. 2009) but the discriminating power was weak.

In conclusion, there is evidence that LUTS increase in pregnancy. Most studies have not included urinalysis or pregnancy outcomes. Prospective studies are missing, and we do not understand the information conveyed by symptoms.

Table 3-1: Summary of studies of urinary symptoms in pregnancy

Reference	Participants & study design	Symptoms identified	Microbiological data or pregnancy outcomes considered?
Stanton, Kerr-Wilson et al. 1980	Pregnant women, longitudinal study	Daytime frequency at booking >40% nulliparous and multiparous women versus 95% nulliparous and 76% multiparous women at term. Stress incontinence symptoms, urge incontinence, hesitancy increased with gestation	Excluded women with UTI and those who delivered preterm
Viktrup 1993	Primiparous women, prospective study	Stress incontinence and urge incontinence more frequent towards late gestation and decreased post-partum	No information about urine analysis
Chaliha, Kalia et al. 1999	Nulliparous women, interview after 34 weeks and postnatally	Increased symptoms during pregnancy compared with before and after	Excluded current UTI or previous recurrent UTI
(Sharma, Aggarwal et al. 2009, Liang, Chang et al. 2012, Lin, Shen et al. 2014)	Retrospective analysis of pre-pregnancy and early pregnancy symptoms	High rates of LUTS with increase with gestation	UTI excluded
Cutner, Cardozo et al. 1991	1 st trimester	LUTS common Frequency most common symptom (40%, nocturia, urgency, incontinence & voiding symptoms noted)	Excluded current or past UTI
(Sangsawang and Sangsawang 2013)	Urinary incontinence in pregnancy, systematic review	Stress urinary incontinence 41% prevalence (range 18.6-60%) Urgency incontinence 2-35% Mixed incontinence 3.8-13.1% Prevalence increasing with gestational age	No
Dinc 2017	Urinary incontinence in pregnancy, questionnaire	UTI a risk factor for urinary incontinence (OR 3.8%, 95% CI 1.5-9.3)	No
Adaji, Shittu et al. 2011	Pregnancy, use of ICIQ-FLUTS	High prevalence of LUTS, correlating with gestation Majority rated 'not bothered' or 'mildly bothered'	No microbiological data
Hanif 2006	Pregnancy, LUTS versus urine analysis	426/1000 symptomatic antenatally Of these, 104/426 (25%) had pyuria and 37/426 (8.7%) had positive cultures. Asymptomatic women did not have cultures so ASB rate not calculated	Symptomatic women had cultures tested
Madeira, Garcia et al. 2013	Back pain in pregnancy, questionnaire	73% women reported back pain. 20% with low back pain reported UTI, and had a higher pain score	Self-report UTI

3.2.2 Assumption 2: Normal urine is sterile

The belief that healthy urine is sterile is at least 100 years old (Hort 1914).

Enhanced culture techniques reveal an extensive normal bladder microbiome. (Khasriya, Sathiananthamoorthy et al. 2013). These researchers cultured a spun urinary sediment. Whilst routine cultures were negative, the sediment culture was positive. LUTS patients and controls had different bacterial profiles.

Molecular sequencing has shown similar findings. In subjects not known to have UTI, using voided samples, transurethral collection and suprapubic sampling, uncultivated bacteria was found in all three sample types using 16S r-RNA gene sequencing (Wolfe, Toh et al. 2012). The voided samples contained a mixture of urinary and genital tract bacteria. A subsequent study by that group, using enhanced cultures, demonstrated that many of the organisms identified by sequencing could be cultivated in both patients with LUTS and asymptomatic controls (Hilt, McKinley et al. 2014).

Normal urine, therefore, is not sterile. Enhanced culture methods may help to understand more about bacteria in the bladder in pregnancy.

3.2.3 Assumption 3: Urine dipsticks help to diagnose UTI in pregnancy

3.2.3.1 Introduction

Urine dipsticks are widely used in obstetric practice. Compared with cultures for detecting UTI, dipsticks are fast, cheap, and require minimal training. Clinicians interpreting dipsticks, particularly for UTI, should understand how they perform in pregnancy.

Dipsticks measure enzymatic reactions that indirectly indicate cellular or biochemical elements of the urine, and produce a colour matched to a scale. Analytes include leucocyte esterase, nitrite, protein, blood and glucose. Manufacturers specify minimum and maximum read times; false negatives can be obtained from reading the stick too quickly. Automated machines reduce the subjectivity from visual analysis (Penders, Fiers et al. 2002).

NICE guidelines specify that dipstick should not be used to screen for asymptomatic bacteriuria owing to inadequate sensitivity (National Institute for Health and Care Excellence 2014). Dipsticks are not mentioned as part of the diagnosis of symptomatic UTI in pregnancy.

3.2.3.2 Use of dipsticks to diagnose UTI

Dipsticks are inadequate to diagnose UTI in non-pregnant patients with symptoms of UTI, using Kass criteria (Hurlbut and Littenberg 1991). In a study of patients with painless LUTS, test performance for dipsticks was poor (Khasriya, Khan et al. 2010).

A meta-analysis of studies of dipsticks for detecting UTI included 74 papers published between 1990 and 1999 (Deville, Yzermans et al. 2004). Of these, 10 studies conducted in pregnant women reported nitrite sensitivity was 0.46 (95%CI 0.38–0.56) and specificity 0.98 (95%CI 0.79–1.00). Five studies examined leucocyte esterase and/or nitrite, with sensitivity 0.68 (95%CI 0.58–0.78) and specificity 0.87 (95%CI 0.81–0.92). Leucocyte esterase alone was not tested. No differentiation was made between asymptomatic bacteriuria and symptomatic UTI.

One study in non-pregnant patients with UTI found that while nitrite sensitivity was around 49.7% for gram negative organisms, it was 5.5% for gram positive

organisms (Al Majid 2010). This is consistent with not all bacteria reducing nitrates to nitrite.

When sequencing methods were used to demonstrate bacteriuria, dipsticks performed poorly (Lewis, Brown et al. 2013). A double-blind randomised controlled trial for non-pregnant women with acute symptoms of UTI and negative urine dipstick (Richards, Toop et al. 2005) showed that trimethoprim shortened the duration of dysuria from five days to three days compared with placebo (a significant difference with $p=0.002$). While the dipstick accurately predicted the MC&S result, it did not predict response to treatment.

A Cochrane review attempted to evaluate screening with urinary dipsticks for reducing morbidity and mortality (Krogsboll, Jorgensen et al. 2015), including in pregnancy. The authors were not able to identify any studies measuring test performance against outcomes that fit inclusion criteria.

3.2.3.3 Performance of urine dipsticks in pregnancy – pilot systematic review and meta-analysis

At the start of this study period (2014) there was a gap in the literature for a systematic review and meta-analysis of the performance of dipsticks in pregnancy for screening for asymptomatic bacteriuria and diagnosing UTI.

I carried out a pilot study and the findings are included here as a quasi-systematic review and meta-analysis.

Aim: To determine the sensitivity and specificity of urine dipstick, leucocyte esterase, nitrite and any combination of the two, in the diagnosis of asymptomatic bacteriuria and symptomatic UTI in pregnancy.

Methods: A search via Pubmed using the search terms 'urine' AND 'dipstick' AND 'pregnancy' revealed 128 papers which were scrutinised for relevance. Of these, 29 were relevant. Hand searching revealed a further 4 papers. Studies were scrutinised by a single researcher (myself) for a range of pre-specified features including location, methodology, asymptomatic or symptomatic UTI (or other outcome), ethical approval, reference criteria for diagnosis of UTI, aim of study, and specific tests included. Raw data for constructing 2x2 tables

were extracted or re-constructed if findings had been presented only as derived variables. These data were analysed.

Results: In total 10117 pregnant patient samples were included from 16 papers (Robertson and Duff 1988, Hagay, Levy et al. 1996, Millar, DeBuque et al. 2000, Shelton, Boggess et al. 2001, Abdullah and Al-Moslih 2005, Kacmaz, Cakir et al. 2006, Eigbefoh, Isabu et al. 2008, Jayalakshmi and Jayaram 2008, Kovavisarach, Vichaipruck et al. 2008, Kodikara, Seneviratne et al. 2009, McGready, Wuthiekanun et al. 2010, Tan, King et al. 2012, Thakre, Dhakne et al. 2012, Mokube, Atashili et al. 2013, Jido 2014, Okusanya, Aigere et al. 2014).

There was an overall prevalence of bacteriuria of 11.4% (95% confidence interval (CI) 10.8% to 12.0%). For nitrite, sensitivity was 39.8% (95% CI 35.6% to 44.2%), and specificity was 97.6% (95% CI 97.1% to 98.0%). For leucocyte esterase, sensitivity was 62.6% (95% CI 58.0% to 66.9%) and specificity was 81.5% (95% CI 80.3% to 82.7%).

Nine papers examined asymptomatic bacteriuria, including 7698 patient samples. For patients with asymptomatic bacteriuria, the prevalence of bacteriuria was 9.9% (95% CI 9.2% to 10.6%). For nitrite, sensitivity was 38.5% (95% CI 33.2% to 44.2%), specificity was 98.6% (95% CI 98.1% to 99.0%). For leucocytes, sensitivity was 58.8% (95% CI 53.2% to 64.2%), specificity was 88.0% (95% CI 86.8% to 89.0%).

Discussion: The pilot systematic review and meta-analysis included bacteriuria by all definitions even though slightly different thresholds may have been used, although most studies adopted the Kass criteria rather than other definitions of UTI or outcome measures. No formal inclusion or exclusion criteria were set, this was assessment by only one person, and no quality assessment was carried out as per QUADAS2 guidelines (Whiting, Rutjes et al. 2011).

Systematic reviews usually involve at least two reviewers. Although this is resource-intensive, there is evidence that some studies may be missed, particularly by inexperienced reviewers, and that this may influence the review conclusions ((Waffenschmidt, Knelangen et al. 2019)). However, this pilot study aimed simply to help develop a protocol and practise systematic review

methodology for an inexperienced reviewer, and to establish the need for a formal systematic review conducted according to standard guidelines.

This pilot study suggested a need for a formal systematic review and meta-analysis following Cochrane collaboration protocols for Diagnostic Test Accuracy (DTA) studies (Group 2015). However, Cochrane specifies that, owing to the complexity of DTA systematic reviews, they require a co-researcher with specific expertise and working within a Cochrane group. This was too expensive for the current project to pursue. Cochrane's recommended approach is PPPICPTR, which stands for patients, presentation, prior tests, index test, (comparator test), purpose, target disorder, and reference standard. In addition, on discussion with the local collaborating group, we agreed that the doubts about the usefulness of the gold standard reference test in particular did not justify investing time and funding in pursuing this question.

3.2.3.4 Conclusion

The dipsticks are most inaccurate and yet they are used widely in obstetrics. We know nothing about the performance of dipsticks in symptomatic pregnant patients. There is no data on dipstick performance in relation to pregnancy outcome.

3.2.4 Assumption 4: Mixed growth of a urine culture indicates contamination

3.2.4.1 Introduction

Culture of a single species was specified by Kass (Kass 1957) as critical to UTI diagnosis, and remains a diagnostic criteria (Network 2012). It is assumed that mixed growth represents contamination rather than pathology.

3.2.4.2 Polymicrobial cultures in non-pregnant patients.

Some studies have shown that mixed growth may be pathological, worthy of antibiotic therapy and repeat sample analysis (Bartlett and Treiber 1984). Enhanced culture studies, as described above, have described polymicrobial growth and shown how it relates to pathology (Khasriya, Sathiananthamoorthy et al. 2013). Genomic methods have alerted us to the existence of polymicrobial infections (Short, Murdoch et al. 2014).

A 1994 study compared features of monomicrobial and polymicrobial patients with a match between urine and blood cultures of at least one organism (Siegman-Igra, Kulka et al. 1994). Polymicrobial infection was more frequently associated with hospital-acquired infection and catheter use, and mortality was higher.

In one study in elderly patients, mixed growth samples yielded more invasive *E. coli* than monomicrobial samples (Croxall, Weston et al. 2011). It was proposed that the co-existence of *E. coli* and *Enterococcus* might increase pathogenicity.

A 2014 study compared genotypic analysis of UTI, diagnosed according to culture and presence of white cells, with standard culture (Willner, Low et al. 2014). Both the genus and strain of the dominant organism using sequencing mapped onto the standard culture organism. It was claimed that despite polymicrobial colonisation the dominant organism was most significant.

Sequencing studies, aiming to understand the urinary microbiome, have looked for differences between different patient groups and controls.

A sequencing study demonstrated differences in urinary microbiota between participants with and without urgency urinary incontinence, suggesting the role

of the microbiome in pathological processes (Pearce, Hilt et al. 2014). Others have demonstrated that the urinary microbiome may be affected by age and gender (Lewis, Brown et al. 2013). Another study compared healthy controls with patients with neuropathic bladder due to spinal cord injury, presumed at risk of asymptomatic bacteriuria, and found a different pattern of microbiome in the neuropathic bladder patients (Fouts, Pieper et al. 2012).

3.2.4.3 Polymicrobial growth in pregnancy

A retrospective cohort study compared pregnancy outcomes of 380 women with polymicrobial cultures at booking with 375 women with negative cultures, powered to detect differences in pyelonephritis and preterm birth (Naresh and Simhan 2011). Polymicrobial culture was defined as either four or more potentially pathogenic bacteria, each greater than 10^5 CFU/ml, or “any urine culture growing predominantly non-pathogenic bacteria such as lactobacillus and diphtheroids”. Lower levels of bacteriuria were not included. The women were not significantly different in demographic or medical risk factors, except the polymicrobial culture group was significantly more likely to have chronic hypertension, maternal obesity and a previous history of preterm birth. There were no differences in pregnancy outcomes including pyelonephritis and preterm delivery. The strength of the findings is limited by the retrospective use of coding, with the authors unable to establish whether patients received antibiotics.

In an unpublished local study (Nwokoro 2013) of 1240 women with risk factors for preterm birth, 40.3% of 3025 antenatal MSU samples were reported as having microorganisms present but not meeting criteria for significant bacteriuria. Women who were culture negative at all MSU assessments in pregnancy were more likely to have a term birth than a preterm birth compared with women who had any non-negative culture.

It is not known in what way the urinary microbiome is influenced by pregnancy. Microbiome research is becoming increasingly important in the field of preterm birth research (Prince, Chu et al. 2015).

3.2.4.4 Conclusion

Polymicrobial growth may be associated with increased harms. The relationship between standard culture results and enhanced culture or sequencing findings requires clarification, while standard culture continues to be practiced universally by laboratories. The relevance of mixed growth or polymicrobial cultures needs to be better understood. It cannot be assumed that mixed growth represents contamination rather than pathology.

3.2.5 Assumption 5: Significant bacteriuria has a threshold of 10^5 CFU/ml

3.2.5.1 Introduction

Asymptomatic bacteriuria in pregnancy in the UK requires bacteriuria of $>10^5$ CFU/ml ((NICE) 2018).

A diagnostic threshold determines when treatment is given; is there evidence of harm caused by this threshold, either in terms of over- or under-treatment?

3.2.5.2 Evidence for the threshold for significant bacteriuria

The 10^5 CFU/ml diagnostic threshold is attributed to Kass, but his description addressed the differentiation of contamination from true bacteriuria (Kass 2002). Kass accepted there could be significant bacteriuria at lower counts (Clague 1996).

This threshold has been criticised. In acutely dysuric women, 10^2 CFU/ml may be sufficient to diagnose UTI (Stamm, Counts et al. 1982, Kunin, White et al. 1993, Hooton, Roberts et al. 2013).

3.2.5.3 Threshold for significant bacteriuria in pregnancy

The threshold of 10^2 CFU/ml, with acute dysuria, may also be sufficient in pregnancy (Calderon Jaimes 1989). A study of 208 patients with low colony counts of *Enterococcus* species (Colodner, Eliasberg et al. 2006), which included 78 pregnant patients, showed that urgency and hospitalisation were independent risk factors for pyuria plus a positive culture of 10^2 CFU/ml.

In pregnancy, Group B *Streptococcus* (GBS, also known as *Streptococcus agalactiae*) bacteriuria is a particular concern, owing to the association between maternal GBS colonisation and neonatal infection (Hughes RG 2017). A 1987 placebo-controlled study found that bacteriuria at any level should be treated to prevent preterm labour; there was no correlation with the degree of bacteriuria and preterm birth (Thomsen, Morup et al. 1987).

A retrospective registry study compared 122 women with GBS bacteriuria before 20 weeks against 183 women with negative cultures (Anderson, Simhan et al. 2007). Of the women with GBS bacteriuria 104 (85%) had colony counts below 10^5 CFU/ml, and 61 (50%) had not received antenatal treatment,

as there was no guidance about treating lower counts. Untreated early GBS at lower colony counts was associated with chorioamnionitis. The histological grade of chorioamnionitis correlated with the bacteriuria colony count.

Whilst screening for asymptomatic bacteriuria aims to prevent pyelonephritis, not all cases of pyelonephritis arise in patients with asymptomatic bacteriuria using current criteria. In a review of the asymptomatic bacteriuria policy, it was estimated that of 10,000 women, 200 would develop pyelonephritis in pregnancy, of whom 125 would be screen-positive for asymptomatic bacteriuria, and 75 screen-negative. Treating those screen-positive would avert 109/125 cases, leaving 75 cases not prevented (Meads 2011).

3.2.5.4 Conclusions

Studies of UTI that investigate diagnosis using non-standard thresholds are often small, and rare in pregnancy. Knowledge about pregnancy outcomes with bacteriuria at lower colony counts is lacking.

3.2.6 Assumption 6: Epithelial cells in the urine are a marker of contamination

3.2.6.1 Introduction

Public Health England's (PHE) publication *UK Standards for Microbiology Investigations* states that squamous epithelial cells are "a useful indicator of the degree of contamination from the perineal region" (The Standards Unit 2014), p18. While no reference is given, this assertion was present in Kass' work:

"In voided specimens artefacts consisting of epithelial cells with adherent bacteria may appear and do not indicate true bacteriuria" (Kass 1957)

However, the original source is difficult to locate.

3.2.6.2 Epithelial cells in non-pregnant patients

In a prospective comparison of MSUs and catheter specimens of urine (CSU) in symptomatic non-pregnant women, epithelial cells were identified in 96% of MSUs and 94% of CSUs (Walter, Gibly et al. 1998). This was not related to contamination, defined by the researchers as low-count growth ($<10^4$ CFU/ml) or more than two commensals such as coryneforms or lactobacilli. It was concluded that squamous cells were common but did not indicate contamination.

In a laboratory-based study, epithelial counts positively correlated with the number of organisms cultured but did not predict mixed growth (Smith, Morris et al. 2003).

In more recent research, a study using epi-fluorescent microscopy showed that the majority of epithelial cells in patients with LUTS were of urothelial origin (Horsley, Malone-Lee et al. 2013). Epithelial cells in the urinary sediment of 22 non-pregnant patients with LUTS were stained with uroplakin-III (UP3), which is expressed solely by urothelial cells. These were compared with epithelial cells from vaginal swab samples from 22 age-matched patients with LUTS. The median proportion of UP3-positive cells in the MSU samples was 75% (IQR 68-78.5) compared with 25% (IQR 19-32) in the vaginal samples

($p < 0.001$). This confirmed that epithelial cells in the urine were largely urothelial. The degree of epithelial cell shedding correlated with the degree of pyuria in the sample, suggesting that epithelial cell counts corresponded to the severity of infection. There was no difference between those with positive and negative cultures.

The same study demonstrated that bacteria were closely adherent with epithelial cells in patients with LUTS and the proportion of these could be measured. Such cells were called “clue cells”. 75% of LUTS patients had specimens with clue cells compared with 17% of healthy controls.

The presence of urothelial cells, and clue cells, in the urine of LUTS patients has a pathophysiological significance. The urothelium is made up of umbrella cells, intermediate and basal cell layers (Khandelwal, Abraham et al. 2009). Layers of umbrella cells slough off in response to bacterial infection. The urothelium plays an active role in the defence of the urinary tract (Khandelwal, Abraham et al. 2009).

A bladder biopsy study of 33 patients with chronic or recurrent UTI found that the urothelium was disrupted in these patients, and that this was associated with symptoms (Elliott, Reed et al. 1985). Even in those 16 patients who had sterile urine cultures there was evidence of bacteria on biopsy in 8/16, and bacteria associated with epithelial cells in 14/16.

Exfoliated urothelial cells, with associated bacteria, are often found in the urine of patients with UTIs; this is considered part of the defence mechanism to UTI (Mulvey, Schilling et al. 2000). Normal turnover of urothelium is slow (Hicks 1975) until there is an insult. Mouse models have shown that the rate and degree of urothelial cell shedding is dependent on the genetic background of the host (Mulvey, Schilling et al. 2000).

Much research on the pathophysiology of UTI has used murine models and *Escherichia coli* (*E. coli*), and *E. coli* has been found to invade mouse urothelium to form intracellular colonies (Hannan, Totsika et al. 2012). *Enterococcus faecalis* has been found inside human urothelial cells (Horsley, Malone-Lee et al. 2013). *E. coli* has been found to form biofilm-like communities in humans (Rosen, Hooton et al. 2007). This may enable bacteria

to evade defence mechanisms and antibiotics, increasing the risk of chronic or recurrent UTI that is difficult to treat.

One issue is distinguishing between urothelial cells of urethral versus bladder origin. White cell counts from urethral samples (first part of void) are higher than vesicular samples (later part of void) (Moore, Hira et al. 1965), so this may also be the case for epithelial cells. However, bacterial colonisation of the urethra may still relate to intra-bladder pathology. Colonisation of, and adherence to, vaginal epithelial cells is of relevance in recurrent UTI (Schaeffer, Jones et al. 1981) and in pyelonephritis, including in pregnancy (Al-Mayahie 2013). Epithelial cells in the urine may be significant, even if they originate outside the bladder.

3.2.6.3 Epithelial cells in pregnant patients

A contamination-based study in 113 pregnant women compared three sampling methods (midstream, first-morning, and clean-catch) (Schneeberger, van den Heuvel et al. 2013). Contamination was defined as gram positive rods on urine gram stain, mixed growth or skin flora on culture, or more epithelial cells than leucocytes. There were minimal differences between the sampling methods, with epithelial cells present in 58.9% of midstream samples, 50.4% morning samples, and 56.8% clean-catch samples. No conclusion was drawn about the significance of the presence of epithelial cells other than that it indicated possible contamination.

3.2.6.4 Conclusion

Evidence does not support the idea that epithelial cells are a marker of contamination. Rejecting samples with high epithelial cell counts because of concern about contamination may risk missing pathology. The urothelial origin of urinary epithelial cells in pregnancy has not been tested.

3.2.7 Assumption 7: Micro-organisms can be categorised as uropathogens or contaminants

3.2.7.1 Introduction

Traditionally, micro-organisms cultured in the urine are categorised as urinary pathogens, such as *E. Coli* (Ronald 2002), or contaminants, such as lactobacilli or coryneforms (Schneeberger, van den Heuvel et al. 2013). This is incorporated into the definition of UTI with the stipulation of a known uropathogen. The assumptions about urinary pathogenicity may be inaccurate.

3.2.7.2 Urinary organisms that do not behave according to the uropathogen-contaminant dichotomy

Some but not all *E. coli* strains are uropathogenic. Non-uropathogenic *E. coli* have been studied as protective agents for the bladder (Sunden, Hakansson et al. 2010).

Gram positive organisms are often assumed to be contaminants (Schneeberger, van den Heuvel et al. 2013). But, some coryneform species, such as *C. urelyticum*, are associated with stone formation (Bernard 2012). Genomic analysis shows a higher preponderance of coryneforms in healthy bladders compared with neuropathic bladders (Fouts, Pieper et al. 2012). *Lactobacillus spp.*, a vaginal commensal, has been reported as a rare cause of UTI and pyelonephritis (Duprey, McCrea et al. 2012).

Next-Generation Sequencing (NGS) applied to non-pregnant patients and controls found that species of urinary lactobacillus correlated with phenotypes of urinary symptoms (Pearce, Hilt et al. 2014). *L. gasseri* was more common in patients with urgency urinary incontinence, whilst *L. crispatus* was more common in controls. The key differences between patients and controls revealed by NGS involve species dispersion but with considerable overlap (Hilt, McKinley et al. 2014).

3.2.7.3 Conclusions

Some species may behave both as uropathogens and as mutualists, depending on different circumstances. This needs further exploration in pregnancy.

3.2.8 Assumption 8: Catheter specimens avoid the problem of contamination

3.2.8.1 Introduction

It is thought that urine specimens may be contaminated from external surfaces. Mid-stream urine samples (MSU) are thought to reduce this risk compared with a full void. However, an emergency-department based study found that many patients used the wrong collection technique (Frazee, Frausto et al. 2012). A catheter specimen (CSU) may avoid this issue. However, catheterisation is a maternal risk, difficult, and expensive.

3.2.8.2 How do catheter specimens compare with voided specimens in terms of contamination?

In a study of non-pregnant patients with chronic LUTS, CSU gave similar results to a well-conducted clean catch midstream sample (Collins, Sathiananthamoorthy et al. 2020)

Another study of non-pregnant women with chronic LUTS, found lactobacillus, a vaginal microbe, more commonly in CSU compared MSU (Khasriya, Sathiananthamoorthy et al. 2013). Other isolates were similarly present regardless of method.

3.2.8.3 How do catheter specimens compare with voided specimens in terms of detection of urinary pathology?

Patients with acute cystitis were used to compare the MSU to CSU (Hooton, Roberts et al. 2013). With a high pre-test probability of UTI, MSU performed similarly to CSU. Again, vaginal commensals were detected in CSU samples.

A 1988 study of pregnant women found that suprapubic puncture samples were equivalent to MSU in terms of culture findings (Retzke, Waitz et al. 1988).

3.2.8.4 Conclusions

In non-pregnant patients CSU has not proven superior to MSU, for contamination, or diagnosis. We have no such data for pregnancy.

3.3 What does it mean to diagnose UTI?

3.3.1 Introduction

We have doubts about standard means of detecting UTI. Alternative tests will be discussed in chapter 4. Here I shall deal with some concerns.

3.3.2 Dichotomy or spectrum?

UTI is currently viewed as a dichotomy; positive or negative culture, which is reference standard, for new tests.

UTI may be a spectrum disorder, distributed from asymptomatic bacteriuria, through to severe sepsis. The *likelihood* of a UTI, may be a better option than a yes/no dichotomy.

3.3.3 Semantics of diagnosis

A semantic analysis of medical of medical textbooks, dictionaries and Medline articles, found that terms bacteriuria, UTI, and urethral syndrome, were inconsistently defined (Liss, Aspevall et al. 2003), which is a worry.

3.3.4 Damage-response framework

I find it helpful to consider the pathophysiology of microbial infection as a synthesis of the interaction between host and microbe, the damage that ensues and the response of the host to those events. This was proposed as a “Damage-response framework” by Pirofski and Casadevall in 1999 ((Casadevall and Pirofski 1999) (Casadevall and Pirofski 2003)). This outlook is inclusive of the disparate disease manifestations and seems an improvement on the basic culture-positive appreciation of events. Thus, the same infection harms some and not others, and consequences are equally disparate.

3.3.5 Statistical tools for diagnostic tests

Statistical analyses of diagnostic tests are pointless if the focus of analysis is ill-conceived. Furthermore, clinicians vary in their interpretation of diagnostic test information (Whiting, Davenport et al. 2015).

We view tests outcomes as dichotomous; positive or negative, but this ignores two other conditions - the false positive and false negative. Sensitivity and specificity are used to accommodate those properties but without considering the base-rate, or prior probability, of the disease. Thus, when we talk about the positive and negative predictive values of a test, we tend to extrapolate these derived values, from the sample that was studied, to the wider population.

Bayes' theorem introduces a consideration that changes the way we think about test statistics (Goodman 1999).

*The probability of **disease** if test **positive** $\propto$ sensitivity $\times$ prevalence*

The converse, in relation to a negative test is:

*The probability of **no disease** if test **negative**
 $\propto$ specificity $\times$ (1 – prevalence)*

The “prevalence” is that which is most applicable to the sub-set of the population that best represents that patient you are addressing. There is substantial difference between someone with no symptoms and signs, where a negative test is probably a true negative, compared to someone with a typical history of index symptoms and signs, when a negative test is probably a false-negative. In other words, the history, symptoms and clinical examination change the legitimate interpretation of a positive or negative test out of all recognition. There is evidence that clinical reasoning uses Bayesian principles (Gill, Sabin et al. 2005). With the advent of computer power, Bayes' theorem has moved from a niche interest to mainstream inference and is displacing frequentists methods more and more (Pearl and Mackenzie 2018).

Worse, diagnostic thresholds can be manipulated to maximise both sensitivity and specificity using ROC curves (receiver operating characteristic curve) but that assumes a common prevalence (Halligan, Altman et al. 2015).

The clinical applicability of a test and its relative cost also need consideration (Altman and Bland 1994). What might be useful for population based epidemiological surveys may be wholly inappropriate for the individual patient.

Another statistical problem in preterm birth research is exposure. Thus, women who experience pre-term birth have less time exposed to UTI risk than

controls. Therefore they provide less of a time-frame for detecting UTI than do the controls (Hertz-Picciotto, Pastore et al. 1996). This creates a bias that has been found to underestimate the contribution of UTI to preterm birth (M, Hertz-Picciotto et al. 2003).

There is no good reason to view UTI as dichotomous. The Kass criterion of $\geq 10^5$ CFUml⁻¹ is an arbitrary point estimate (see Figure 3-1). This threshold was not validated nor were similar alternatives. If we do this, we introduce a spectrum bias (Goehring, Perrier et al. 2004) which can cause much error.

Figure 3-1: A biological continuum applied to urinary culture results associated with UTI

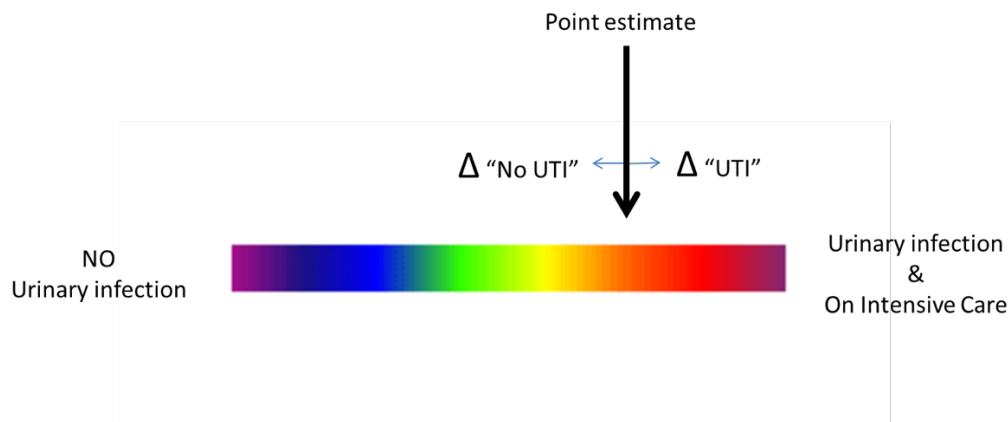
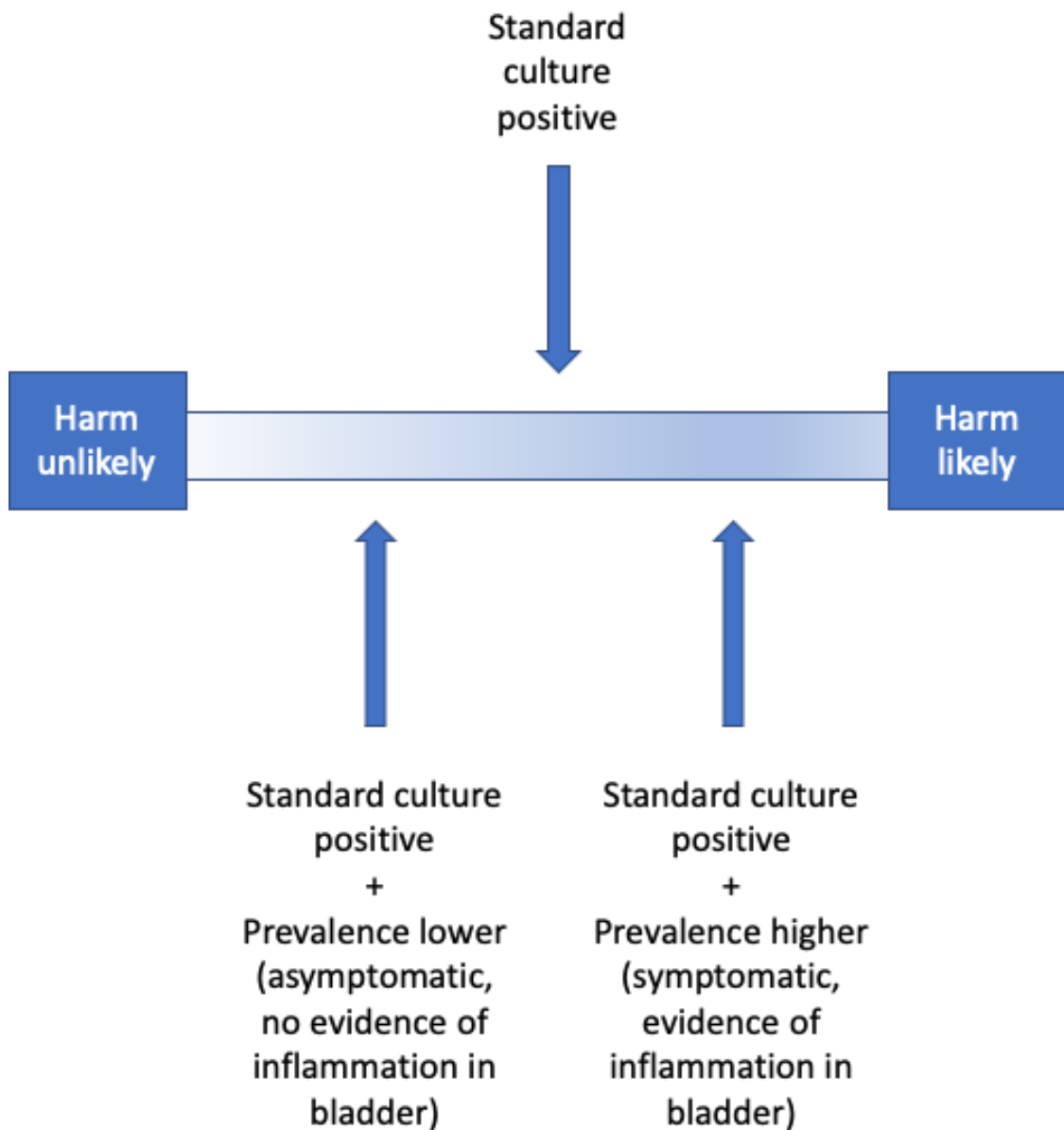


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If we were to combine the concepts that UTI in pregnancy is a spectrum, with a Bayesian approach, we might imagine a situation where we think about a UTI diagnostic threshold as indicating a 'risk of harm' from harm unlikely to harm likely (see Figure 3-2). Harm here refers to negative outcomes of UTI in pregnancy, such as pyelonephritis or preterm birth. The prevalence, which may be indicated by symptoms or markers of inflammation in the urine, would alter the risk of harm. This could also be thought of from the clinician's point of view as a spectrum of need for antibiotics from 'less likely to benefit' to 'more likely to benefit'.

Figure 3-2: A risk of harm continuum applied to urinary culture results associated with UTI in pregnancy



3.3.6 Conclusion

It may be better to view UTI as distributed along a continuum without dichotomous categorisation. The focus on a culture result ignores other important elements of the condition such as host immune response and patient experience. The analysis of test performance must take note of Bayes and particularly a disease prevalence relevant to the patient. By including this prior

probability, interpretation of a test result will vary with circumstance. We should be most cautious about predictions based only on test data.

3.4 Do current tests correctly identify patients who need antibiotics?

3.4.1.1 Introduction

I have used theory to criticise test performance but that must be followed up by the provision of empirical evidence

3.4.1.2 Is there evidence of overtreatment for UTI in pregnancy?

In a study of antibiotic use for symptomatic UTI in pregnancy in Uganda, symptomatic women given antibiotics, were compared with asymptomatic women (Sekikubo et al 2017). The symptomatic group showed 4% positive cultures, the asymptomatic group, 2%. So, 96% of symptomatic women were treated when culture negative. Clinical outcomes were not reported.

The 2015 Netherlands study found no difference in preterm birth regardless of asymptomatic bacteriuria or antibiotic treatment. The only difference was higher rates of pyelonephritis or symptomatic UTI in the asymptomatic bacteriuria group (Kazemier, Koningstein et al. 2015). It was proposed that asymptomatic bacteriuria screening was unnecessary and potentially harmful.

3.4.1.3 Is there evidence of undertreatment for UTI in pregnancy?

Women in a specialist preterm birth clinic with non-significant or mixed growth were more likely to have a preterm than term birth compared with women with all negative cultures (Nwokoro 2013). These authors proposed UTI was undertreated, in a high-risk population. Group B Streptococcus bacteriuria, regardless of colony counts, were more likely to have chorioamnionitis than controls (Anderson, Simhan et al. 2007).

3.4.1.4 Conclusion

The data from these publications is weak. Few pregnancy UTI studies have included obstetric outcomes. There is insufficient evidence to comment on the veracity of current protocols.

3.5 Conclusions

In the first part of this chapter, I discussed doubts about eight assumptions on UTI in pregnancy. We do not understand the significance of urinary symptoms in pregnancy. The normal bladder is not sterile, pregnancy or no. Urine dipsticks have been discredited. Mixed growth of a urine culture may not indicate contamination. The implications of polymicrobial cultures are poorly understood. A diagnostic threshold of 10^5 CFU/ml may be inappropriate. Urinary epithelial cells may not represent contamination. We do not know whether cultured isolates are pathogens or mutualists. Catheter specimens do not improve on specimen quality.

I argued that UTI exists as a continuum. Semantics confuse our understanding. Culture-focus excludes other disease elements. The statistical analyses of UTI tests have ignored the base-rate.

Current diagnostic approaches may or may not identify patients at risk of outcomes such as preterm birth, we just do not know. In the next chapter I introduce alternative methods for investigating urinary pathology in pregnancy.

4 Background part 3 - Alternative methods for investigating urinary pathology in pregnancy

4.1 Introduction

There are less well known but informative methods for investigating urinary pathology, many of which have been researched and validated in patients with chronic UTI:

- (1) Assessment of lower urinary tract symptoms by validated methods
- (2) Quality of life assessment using a validated instrument
- (3) Fresh, unstained, unspun microscopy for cell counts
- (4) Urinary ATP measurement
- (5) Enhanced, urinary spun sediment culture
- (6) Urinary cytokine/chemokine analysis, specifically IL-6, IL-8 and lactoferrin
- (7) Analysis of epithelial cells using immunofluorescence staining.

They have not been properly studied in pregnant women.

4.2 Standardised assessment of lower urinary tract symptoms (LUTS)

There is a lower urinary tract symptoms measure (Artemis LUTS inventory), created for chronic UTI, that has been found to correlate with a number of independent markers of UTI and which has been found to plot the course of infection and its recovery. This is a questionnaire assessment of urinary frequency, nocturia, incontinence, and 40 other LUTS, which are grouped into four symptom groups of stress incontinence symptoms (7 symptoms), urgency symptoms (12), voiding symptoms (8) and pain symptoms (13) (questionnaire in appendix, see 13.1).

The Artemis LUTS inventory was developed from spontaneous patient-described symptoms. It uses binary yes-no answers without qualifying bothersome-ness scales. Its most obvious competitor, ICIQ-FLUTS (Brookes, Donovan et al. 2004), was developed for primary incontinence symptoms in patients who had had culture-positive UTI excluded. Whilst other generic symptom scales exist, using the Artemis LUTS inventory would permit comparison with other research from within the local research group.

The Artemis LUTS inventory has been extensively validated (Khasriya, Barcella et al. 2018). Whilst used clinically in pregnant patients, its performance in this context has been examined only in one pilot study (Arasaretnam 2013). 32 women were recruited from a preterm birth clinic, of whom 12 (38%) had a history of preterm birth. Using the Artemis LUTS inventory, all women reported urinary symptoms of some kind. Whilst a small, limited study, the questionnaire described a burden of urinary symptoms in women at risk of preterm birth and thus motivated further investigation.

4.3 Quality of life assessment

Quality of life assessment is an important research tool (Ghoniem, Stanford et al. 2008) because pain and urinary problems have far-reaching consequences. It is hard to measure, and validated, reliable patient reported outcome measures may be an important tool that help put patients at the centre of care (Devlin 2010). A systematic review (Bermingham and Ashe 2012) found that only twelve studies of the many on UTI measured quality of life.

There are different quality of life measures available. The EuroQol EQ-5D-5L, a standardised measure of health status (EuroQol 1990), was identified by the Department of Health as the generic assessment tool of choice (Royal College of Obstetricians and Gynaecologists 2012)

The EQ-5D-3L (the predecessor to the EQ-5D-5L) was compared with another instrument, the SF-6D, in postnatal women (Petrou, Morrell et al. 2009).

Although it performed inferiorly to the SF-6D for discriminating between other maternal health indicators such as the Edinburgh Postnatal Depression Scale, it discriminated well between other self-reported measures of health status.

This was the only study of pregnancy-specific quality of life measures identified in a systematic review of quality of life measures in pregnancy (Mogos, August et al. 2013).

Thus, this project adopted EuroQol.

4.4 Fresh unspun urine microscopy

Microscopy is a standard of urinalysis (Standards Unit 2017). Pyuria can be a marker of infection (Khasriya, Khan et al. 2010, Kupelian, Horsley et al. 2013, Khasriya, Barcella et al. 2017). Red blood cells are less focused and reflect calculi, infection, inflammation, cancer, nephritis, or contaminate from the genital tract. Epithelial cells correlate with UTI and are less markers of contamination than assumed (Horsley et al. 2013). Nowadays microscopy is not routine, rarely at the bedside and omitted when screening for bacteriuria in asymptomatic pregnancy (Standards Unit 2017).

Modern microscopy occurs at some distance and time from sample collection. The sample is sent to a central laboratory for automated processing (Ince, Ellidag et al. 2016). The time delay limits accuracy (Delanghe and Speeckaert 2014). A pyuria signal disappeared in 35% of samples after three hours (Kierkegaard, Feldt-Rasmussen et al. 1980). A recent study found a 60% count loss within two hours of collection (Kupelian, Horsley et al. 2013). This is important clinically because pyuria may apparently be absent on microscopy if white cells lyse during transport to the laboratory.

Pyuria may be attributed to increased physiological vaginal discharge in pregnancy. In a study investigating low count bacteriuria in young women, pyuria was compared with vaginal white cells (leucorrhoea). Of 18/67 with pyuria (defined as >20 leucocytes / mm^3), 17/18 had leucorrhoea whereas in the 49/67 without pyuria, 22/49 had leucorrhoea (Kunin, White et al. 1993). This showed that pyuria may be negative in the presence of leucorrhoea.

White cell counts may be higher in pregnancy (Nelson, Cunningham et al. 2015). A study from 1965 found that the leucocyte excretion rate per hour in 50 non-bacteriuric pregnant women (using the Kass criteria) was 83,000 (standard deviation (SD) = 91,000), compared with 30,600 (SD = 28,000) in 32 male and female control students, and 15,000,000 (SD = 18,000,000) in 120 bacteriuric pregnant women (Kincaid-Smith and Bullen 1965). Leucocyte excretion rates (also known as an Addis count), whilst accurate, are difficult and rarely used now. A 1967 study, comparing 54 normotensive pregnant women with 61 hypertensive pregnant women, found that leucocyte excretion

rates were abnormal ($>200,000$ leucocytes/hour) in 31/54 (57.4%) normotensive women and 48/61 (78.7%) hypertensive women, without symptoms and negative culture (Chadd, Humphreys et al. 1967).

The significance of epithelial cells in the urine in chronic UTI has been discussed above. It is not known how urinary epithelial cells should be interpreted in pregnancy.

Pregnancy is a state of high oestrogen and high progesterone. Oestrogen induces proliferation of urothelial cells, at least partially mediated by oestrogen receptors (ER), of which both ER α and ER β are expressed in urothelial cells, and nerve growth factor (NGF) (Teng, Wang et al. 2002). Applying oestrogen in both menstruating and menopausal women modulates two epithelial defence mechanisms: promoting the expression of antimicrobial peptides and reduction of epithelial exfoliation (Luthje, Brauner et al. 2013). Progesterone also induces proliferation of urothelial cells, through progesterone receptor, and this is mediated by epidermal growth factor (EGF) but not NGF (Teng, Wang et al. 2003). Thus, there is good reason to evaluate urinary cell counts in pregnancy.

4.5 Urinary ATP measurement

Urinary adenosine-5'-triphosphate (ATP) has been suggested as a biomarker for UTI. ATP is released by inflammatory cells throughout the body (van der Weyden, Conigrave et al. 2000) and by microbes. In cell culture experiments, ATP was released from urothelial cells infected with uropathogenic *E. coli* as well as interleukin-8 (IL-8) (Save and Persson 2010). Porcine experiments have suggested that ATP is released by urothelium in response to stretch (Kumar, Chapple et al. 2004).

Compared against Kass criteria bacteriuria, ATP luminometry had a sensitivity of 92% and a specificity of 88% (Thore, Lundin et al. 1983). Using an assay method, urinary ATP has been studied as a rapid point of care test in women with LUTS (Lundin, Hallander et al. 1989). ATP has been shown to quantify the bacterial load in urine (Ivancic, Mastali et al. 2008) (Hanberger, Nilsson et al. 1990).

ATP can be measured through the light emitted when the firefly enzyme luciferase converts the ATP into adenosine monophosphate (AMP). This bioluminescence is quantified by a luminometer in relative light units (RLU). The results have been correlated with quantitative broth culture (Omidbakhsh, Ahmadpour et al. 2014).

ATP swab tests were used to study urine specimens from 53 renal transplant recipients (Kelley, Courtneidge et al. 2014) and showed promise as a marker of UTI in a group that was 78% culture negative but of whom 56% had evidence of intracellular bacteria in the urine.

Urinary ATP was higher in voided samples of patients with overactive bladder symptoms in whom UTI had been excluded (by standard criteria) compared with normal controls (Silva-Ramos, Silva et al. 2013).

Gill et al (Gill, Horsley et al. 2015) compared controls without urinary symptoms with chronic LUTS patients and found that urinary ATP was not useful diagnostically. Urinary ATP was found to degrade quickly, with implications for test timing.

To our knowledge the only exploration of urinary ATP in pregnancy is an unpublished pilot study from our group (Muse 2013). Urinary ATP was studied

in 50 women at high risk for preterm birth compared with 48 women at low risk for preterm birth, and found to be higher in the high-risk group ($p=0.033$). The groups were unmatched for gestation or parity. Data on symptoms, microbiological analyses and outcomes were not collected. There is a chance that urinary ATP may have a role in pregnancy, but validation is necessary.

4.6 Enhanced culture

4.6.1 Introduction

An enhanced culture method developed by this group, known as sediment culture, was introduced above (3.2.2). Importantly, sediment culture has demonstrated bacteria in the urine when routine culture has failed to do so. The method found quantitative differences between non-pregnant patients with LUTS and normal controls whilst discovering a microbiome in the normal bladder (Khasriya, Sathiananthamoorthy et al. 2013).

4.6.2 Sediment culture – background

The method will be described in detail later (see 6.16.5). In brief, 5ml of urine is centrifuged and the supernatant removed. The sediment is resuspended in phosphate-buffered saline (PBS) and serial dilutions performed to 10^{-3} . The dilutions are plated on a chromogenic agar, which favours common uropathogens including *E. coli* and *Enterococcus faecalis*, and on which different organisms grow in different colours (Perry and Freydiere 2007). Genus identification is performed using colour, morphology, and bench-top tests, with species identification carried out later from frozen isolates using Analytical Profile Index (API®) strip testing (bioMérieux).

Standard culture typically uses 1µL - 10µL of mixed urine to inoculate CLED (cystine lactose electrolyte deficient) or chromogenic agar. Compared with standard culture, sediment culture grows organisms associated with the cells in the urine, including shed epithelial cells. This may be an appropriate test given the fact that urinary bacteria parasitise to the urothelial umbrella cells, and are shed as part of the bladder response to infection (Mulvey, Schilling et al. 2000).

If the cellular content of urine contains vaginal discharge or perineal cells, then these may contribute to the organisms grown. It is traditional to aim to exclude such contamination using clean catch midstream urine.

Sediment culture identifies aerobic organisms that grow well in chromogenic agar at 37°C. It will therefore not detect fastidious organisms, entirely

anaerobic organisms, or those that require different nutrients or temperatures for growth.

API® testing, for identification of isolates to species level, involves suspension of a pure culture in a medium, addition of the resulting suspension to microtubes containing substrates for biochemical tests, incubation, and identification of colour changes which are scored and translated to a numerical database.

Whilst API® testing is generally reliable, there are some potential drawbacks. The databases rely on being updated to include newer taxa and amendments to descriptions of species; the identification panels favour species that grow rapidly, are reactive to the substrates tested, and do not require nutritional supplementation (Bernard 2005). It has been found to be inadequate for lactobacillus identification, owing to a high level of phenotypic variability in lactobacillus species and a limited database for such species; genomic methods might be more effective (Boyd, Antonio et al. 2005).

Whilst API® testing was considered the 'gold standard' in microbiology until the 1990s, it is commonly replaced now by automated methods of biochemical identification (O'Hara C 2005), including the MAST Uri® system adopted by UCLH, or other methods of identification such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Seng, Abat et al. 2013). The advantages of API® for the current study are that it was cost-effective on a small scale, and simple to learn.

4.6.3 Findings from sediment culture studies

165 patients with LUTS were compared with 47 controls (Khasriya, Sathiananthamoorthy et al. 2013). While pyuria (40% vs 0%) and positive routine culture (17% vs 2%) were both more frequent in the LUTS group, this did not correlate with symptoms. There was a mean of 1.36 (95% CI 1.2 – 2.1) different isolates in both groups, with no difference between groups. Total colony counts of either the dominant genera or total colonies were not different. Positive routine cultures and pyuria predicted higher colony counts. There was a difference in the distribution of genera identified in the two

groups, in terms of both the dominant genera and total genera ($p=0.001$), and while common acute UTI pathogens occurred in both groups (*E. coli*, *Enterococcus spp*, *Staphylococcus spp*) there were other organisms that only occurred in one group.

To our knowledge, the only research application of this sediment culture technique in pregnant women prior to this study was an unpublished pilot study (Kang, Malone-Lee et al. 2013). Women were recruited who were at low risk for preterm birth ($n=48$) or at high risk ($n=50$), and matched with 38 non-pregnant women without UTIs. Of these, 1/48 (2%) low risk women, 2/50 (4%) high risk women, and 0/38 (0%) non-pregnant women had positive cultures by standard testing. Using sediment culture, analysed to genus level, there was bacterial growth in 46/48 (95.8%) low risk women, 49/50 (98%) high risk women, and 26/37 (70.2%) non-pregnant women. Total colony counts were lower in non-pregnant women with no difference between the pregnant groups. There was a greater diversity, at a genus level, of organisms grown, in the pregnancy groups. Bacterial growth was detected in the majority of pregnant women despite negative routine cultures. There was no matching for parity and high-risk women included multiple different preterm birth risk factors. Obstetric outcome data was not collected.

4.6.4 A consideration of microbiome analysis

With the recent accessibility of molecular sequencing methods, detailed study of microbiota has become widespread. The urinary microbiome has become a topic of research outside pregnancy (Pearce, Zilliox et al. 2015). At the time of starting this study, maternal urinary microbiome was not well researched. It is not known how enhanced culture relates to microbiome.

4.6.5 Conclusion

Enhanced culture methods in pregnancy have not been well characterised and given the results from studies of non-pregnant women it was thought sensible to include this method in this study.

4.7 Cytokines

4.7.1 Introduction

Cytokines, chemokines and anti-microbial peptides form part of the host immune response. Particular interest in UTI research has focussed on urinary IL-6 and urinary IL-8. Urinary lactoferrin has also shown promise as a marker of UTI (Arao, Matsuura et al. 1999). Although IL-6, IL-8 and lactoferrin may respectively be described as cytokine, chemokine and anti-microbial peptide, when discussing them as a group I will refer to them as cytokines for simplicity.

4.7.2 IL-6 and UTI

Urinary IL-6 was secreted non-continuously in response to intravesical inoculation with *E. coli* (Hedges, Anderson et al. 1991). Levels were dependent on the colony count, with 10^5 CFU/ml needed to stimulate IL-6 secretion maximally. There was no difference depending on adherent or non-adherent strains. Serum levels did not change.

Urinary IL-6 has been found to be elevated in asymptomatic bacteriuria and pyelonephritis compared with healthy controls with negative cultures, but serum levels raised only in pyelonephritis (Hedges, Stenqvist et al. 1992). In a paediatric study, urinary IL-6 was reported to be able to differentiate lower from upper UTI (Rodriguez, Robles et al. 2008). It also became undetectable following treatment

4.7.3 IL-8 and UTI.

Urinary IL-8 was secreted in response to intravesical *E. coli* installation, with a correlation with urinary neutrophil counts (Agace, Hedges et al. 1993). Serum IL-8 was not detected. Urinary IL-8 was elevated in children with pyuria and bacteriuria compared with children with pyuria without bacteriuria or healthy controls (Zaki 2008).

Urinary IL-8 was associated with neutrophil migration into the urinary tract and elevated in patients with upper or lower UTI compared with controls (Ko, Mukaida et al. 1993). IL-8 was induced by a variety of microbes, not just gram negatives. Similarly, urinary IL-8 was elevated in patients with UTI (defined as

Kass criteria culture plus pyuria) compared with controls, correlated with white cell counts, and was associated with microbes including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Klebsiella oxytoca* and *Enterococcus faecalis* as well as *E. coli* (Oregioni, Delaunay et al. 2005). In this study, urinary IL-8 levels were stable for up to 24 hours, and in different storage conditions (37°C, 4°C, -20°C).

4.7.4 How do urinary cytokines relate to serum levels?

In healthy women aged 18-30, there was no correlation between urine and plasma levels of IL-6 and IL-8 (Nobles, Bertone-Johnson et al. 2015). In a study examining the response of cytokines to exercise, plasma IL-6 concentrations trended towards increasing with exercise, however urinary IL-6 rose significantly 1 hour after exercise (Suzuki, Nakaji et al. 2002). It was concluded that IL-6 was released systemically during exercise but eliminated rapidly into the urine.

4.7.5 Impact of female hormones on urinary cytokines

Urinary cytokines in reproductive age women vary according to stage in the menstrual cycle, suggesting they are under hormonal influence (Whitcomb, Mumford et al. 2014). In non-pregnant healthy women, oestradiol was inversely associated with IL-6 and IL-8, as was progesterone and LH. FSH was positively associated with IL-6.

4.7.6 Impact of catheters on urinary cytokines

An in-vitro study of the impact of catheterisation and bacterial instillation on cytokine response (urinary IL-6 and IL-8 and LDH) found that catheterisation caused immediate LDH release, while bacteria caused cytokine release after 12 hours (Barford, Hu et al. 2008). In catheterised post-operative patients with and without UTI, urinary IL-8 was a better marker for early host response to UTI than urinary IL-6 (Olszyna, Vermeulen et al. 2001).

4.7.7 Urinary Lactoferrin

Lactoferrin is an iron-binding glycoprotein found in exocrine secretions including urine, as well as amniotic fluid and the secondary granules of neutrophils (Sinha, Kaushik et al. 2013). Native lactoferrin has antimicrobial activity via sequestration of iron and is a source of anti-microbial peptides (Sinha, Kaushik et al. 2013).

In a study using kidneys that had been removed because of small tumours or rejected following transplant, it was found that lactoferrin is secreted in the collecting tubules and reabsorbed in the distal tubule with relatively low urinary levels (Abrink, Larsson et al. 2000). Six people were tested with an average (sic) level of 75ng/ml urine (range 14-145), which was considered low compared with moderate levels in plasma and serum and high levels in tears and saliva.

Urinary lactoferrin has shown some promise as a biomarker for UTI.

Lactoferrin has been studied in non-pregnant patients with UTI (Arao, Matsuura et al. 1999); it was able to distinguish patients with LUTS from controls, and correlated with pyuria (Aly, El-Zawawy et al. 2005, Gill K 2012).

Urinary lactoferrin showed a dose-response relationship with white cell counts measured by microscopy, in patients with spinal cord injury and so at high risk of UTI (Pan, Sonn et al. 2010). In a follow-up to this study, urinary lactoferrin was also found to correlate with bacterial concentration (Mohan, Mach et al. 2011).

Urinary lactoferrin was higher in patients undergoing prolapse or incontinence surgery who had UTI (positive urine cultures plus symptoms or haematuria) on day of surgery compared with those who developed positive cultures up to six weeks post-operatively or had negative cultures within six weeks post-operatively (Nienhouse, Gao et al. 2014).

Urinary lactoferrin has not previously been studied in pregnancy.

4.7.8 Cytokines in urological disorders

Urinary cytokines have been studied as potential biomarkers in bladder pain syndromes (BPS) or interstitial cystitis (IC). Urinary IL-6 was higher in BPS/IC

patients and correlated with pain scores (Erickson, Belchis et al. 1997), it was detected in those with severe BPS/IC but not those with mild disease (Lotz, Villiger et al. 1994), and it had some value to predict BPS/IC (Lamale, Lutgendorf et al. 2006). Contradictory findings affect urinary IL-8: one study reported lower expression of IL-8 associated with BPS/IC (Tseng-Rogenski and Liebert 2009) while another noted IL-8 and IL-6 to be elevated in BPS/IC (Peters, Diokno et al. 1999).

Urinary IL-6 is reduced in overactive bladder compared with controls; urinary IL-8 is increased in patients with UTI compared with overactive bladder patients (Ghoniem, Faruqi et al. 2011).

4.7.9 Cytokines in pregnancy

Urinary cytokines may be different in pregnancy compared with non-pregnant women.

The immune system in pregnancy is a balance between a maternal immune system that tolerates the fetus (expressing paternal genes) and one that defends against overwhelming infection (Sykes, MacIntyre et al. 2012). This is supported by a hormonally driven shift in the balance from T helper 1 (Th1) profile (CD4+ cells producing inflammatory cytokines) to T helper 2 (Th2) profile (CD4+ cells producing anti-inflammatory cytokines). IL-6 and IL-8 can be described as non-Th1:Th2 as their behaviour is more complex (Sykes, MacIntyre et al. 2012).

In healthy pregnant women with normal pregnancy outcomes, plasma IL-6 was increased with gestation and remained elevated post-partum (Palm, Axelsson et al. 2013). In another longitudinal study, plasma IL-6 was found to decline until approximately 20 weeks gestation before increasing towards term. It was associated with spontaneous preterm birth (Ferguson, McElrath et al. 2014).

IL-6 in amniotic fluid is associated with preterm birth in the context of prelabour rupture of membranes (PPROM) (Romero, Yoon et al. 1993). Both IL-6 and IL-8 are higher in amniotic fluid of women who deliver preterm (regardless of mode of delivery) and in women who spontaneously labour at term, compared with caesareans without labour (Keelan, Marvin et al. 1999). IL-8 expression is

increased in preterm labour, particularly with evidence of infection (as summarised in (Marvin, Keelan et al. 2002)).

A systematic review including 6270 pregnant women from seventeen studies of inflammatory cytokines and preterm birth reported that mid-trimester cervico-vaginal IL-6 was associated with spontaneous preterm birth (Wei, Fraser et al. 2010). Plasma IL-6 was not associated with preterm birth.

4.7.10 Urinary cytokines in pregnancy

Given the association between UTI in pregnancy and preterm labour, and inflammatory cytokines and preterm labour, the role of urinary cytokines in pregnancy is of interest. Reported values for urinary IL-6, IL-8 and lactoferrin in pregnancy are summarised in Table 4-1.

Table 4-1: Published values for urinary cytokines in pregnancy

Reference	Urinary cytokine	Setting	Values	Normalised
(Dimitrakova and Kostov 2011)	IL-6	Pregnant women with pyelonephritis vs. pregnant controls	Not stated	Not stated
(Petersson, Hedges et al. 1994)	IL-6	Pregnant women with pyelonephritis vs. non-pregnant women with pyelonephritis	Not stated	Not stated
(Wing, Rumney et al. 2010)	IL-6	Pregnant women ingesting cranberry vs. pregnant women ingesting placebo	Median IL-6 in cranberry group of 3.16pg/ml (range 0.01-7.34) vs. placebo group of 9.32pg/ml (range 0.53-29.61) (p=0.038)	No
Shelton, Boggess et al. 2001)	IL-8	Pregnant women with ASB vs. pregnant women with mixed growth or negative culture	Median IL-8 356pg/ml (range 22-5620) in ASB vs. 125pg/ml (6-5365) for women without ASB (p<0.01, Mann Whitney U)	No
(Basso, Gimenez et al. 2005)	IL-6, IL-8	Pregnant women – controls vs. ASB vs. genital infections	IL-6 undetectable (no LLOD stated) IL-8: 1200.7 +/- 375 pg/ml and in the C group it was 40.2 +/- 17 pg/ml (p< 0.0001)	No
(Dulay, Buhimschi et al. 2015)	IL-6	Pregnant women with intra-amniotic infection with or without chorioamnionitis vs. preterm birth vs. SIRS	Urinary IL-6 had an optimal cut-off of 6pg/ml for diagnosis of intra-amniotic infection with AUROC 0.567, sensitivity 54.6, specificity 80, PPV 75, NPV 61.5 (paper gives 95% CIs).	No

Urinary IL-6 was lower in pyelonephritis in pregnancy compared with pyelonephritis in non-pregnant women (Petersson, Hedges et al. 1994). However, urinary IL-6 was higher in pregnant women with pyelonephritis than pregnant controls (Dimitrakova and Kostov 2011).

Urinary cytokines in pregnant women were studied in response to cranberry ingestion (Wing, Rumney et al. 2010). Urinary IL-6 was lower in the cranberry group than a placebo group; no patients had UTI.

Urinary IL-8 was studied in the context of asymptomatic bacteriuria in pregnancy, using Kass criteria (Shelton, Boggess et al. 2001). In 200 samples, 20 had asymptomatic bacteriuria, 144 mixed growth and 36 negative culture. Asymptomatic bacteriuria was compared against non-asymptomatic bacteriuria (mixed growth and negative). Urinary IL-8 was higher in the asymptomatic bacteriuria group.

Urinary IL-8 increased in response to genitourinary infections in pregnancy (bacterial vaginosis, vaginitis, asymptomatic bacteriuria) compared with healthy controls, but urinary IL-6 was not detected (LLOD not stated) (Basso, Gimenez et al. 2005).

Urinary IL-6 was included in a study of IL-6 in different compartments (blood, cord blood, amniotic fluid and urine) in women with intra-amniotic infection, with or without chorioamnionitis or sepsis (Dulay, Buhimschi et al. 2015). Whilst amniotic fluid IL-6 was different in different situations, urinary IL-6 did not distinguish between them.

An unpublished pilot study, carried out by our group, compared urinary IL-6 in women at high and low risk of preterm birth, as described previously (Muse 2013). There was no difference in urinary IL-6, corrected for urinary protein, between women at high risk of preterm birth ($11.0 \pm 2.8 \text{ pg}/\mu\text{g}$) and women at low risk $7.9 \pm 1.9 \text{ pg}/\mu\text{g}$, $p=0.532$.

Urinary cytokines have been studied in pre-eclampsia. Urinary IL-6 and IL-8 were significantly higher in women with chronic hypertension than pregnant controls; they were non-significantly higher in women with pre-eclampsia (levels normalised for urinary creatinine) (Wang, Gu et al. 2015). Plasma IL-6

was higher in pregnant women with severe pre-eclampsia than controls, excluding women with positive urine cultures (Udenze, Amadi et al. 2015).

I was unable to find any research regarding urinary lactoferrin in pregnancy.

4.7.11 Conclusions

Urinary IL-6 and IL-8 have been extensively researched outside pregnancy, with some preliminary data in pregnancy, with studies mostly compared to the standard culture definition of UTI without reference to other outcomes. Urinary lactoferrin has shown some promise outside pregnancy but not been researched in pregnancy.

4.8 Fluorescent microscopy to analyse urothelial cells

4.8.1 Introduction

The potential significance of shed epithelial cells has been discussed above (3.2.6). A continuing issue is the concept that shed epithelial cells may represent contamination. Two fluorescent microscopy techniques have been previously developed by this group to (1) confirm whether shed epithelial cells arise from the urothelium and (2) explore the association of epithelial cells with bacteria. These techniques have not been explicitly studied in pregnancy, except for an unpublished pilot study described below (Dewar 2013).

4.8.2 Use of uroplakin-staining to assess proportion of urothelial cells

Morphologically, squamous epithelial and uroepithelial cells can be distinguished using light microscopy, however this relies on subjective assessments about the cells and is vulnerable to error; urine cytology in women is known to be particularly vulnerable to confusion between squamous and urothelial epithelial cells (Sullivan, Chan et al. 2010). As described above (3.2.6.2), an alternative method is to visualise the cells using epifluorescence and a stain unique to urothelial cells. Uroplakin-III (UP3) is a 47kDa protein, a component of the asymmetric unit membrane of the urothelial umbrella cells (Balsara and Li 2017), expressed solely by urothelial cells (Wu, Manabe et al. 1990, Wu, Lin et al. 1994). Using this method in patients with chronic LUTS, shed epithelial cells were largely urothelial.

4.8.3 Bacteria-associated epithelial cells (clue cell analysis)

Epi-fluorescent microscopy can be used to analyse the proportion of epithelial cells with closely-associated bacteria (known as clue cells); this proportion was higher with patients with LUTS than controls, as described above (Horsley, Malone-Lee et al. 2013). In this study, one LUTS patient with clue cells grew only *E. coli* and *E. faecalis*; examination with confocal scanning microscopy and 3-D image reconstruction confirmed that while *E. coli* externally colonised the urothelial cells, *E. faecalis* was intracellular, a finding which was confirmed by a cell culture model system in which *E. faecalis* invaded cells.

A subsequent use of a similar technique in 53 renal transplant recipient patients found that 41/53 (77%) of patients had shed urothelial cells and of those 23/41 (56%) had urothelial cells with associated bacteria (Kelley, Courtneidge et al. 2014). Of these, only one was culture positive by Kass criteria. It was noted that if bacteria were identified in one urothelial cell then they would also be identified in other shed urothelial cells.

As far as we know, the only use of this technique in pregnant women has been in a previously-described unpublished pilot study comparing proportion of clue cells in women at high risk of preterm birth versus women at low risk (Dewar 2013). There was no difference between the mean proportions (high risk mean proportion 0.10 versus low risk 0.05, $p=0.056$). This data is incompletely presented, however confirms the presence of clue cells in urine in pregnancy which warrants further study.

4.8.4 Conclusion

There is a paucity of pregnancy-specific data regarding both the urothelial origin of epithelial cells in urine, and analysis of clue cells.

4.9 Conclusion

While the alternative urinary tests outlined in this chapter have increased our understanding of urinary pathology in patients with chronic LUTS, and in some other circumstances of UTI, there is minimal evidence for these tests in pregnancy.

The current study employed these tests, in comparison with the routine tests of urine dipstick, and standard microscopy and culture, to address the aims of understanding more about urinary pathology in pregnancy, particularly in unexplained abdominal pain in pregnancy, and preterm birth. Given concern about contamination in voided samples, I also wanted to compare these tests in voided versus catheter-specimen samples. In the next chapter I will set out the research questions and hypotheses to be tested.

5 Research questions

5.1 Introduction

Several themes emerged from the literature reviewed in chapters 2, 3 and 4:

- UTI may be pathophysiologically different in pregnancy to outside pregnancy.
- Tests for UTI may behave differently in pregnancy, and in different contexts in pregnancy.
- The standard tests for UTI may be based on flawed assumptions.
- Pregnant women may have clinically relevant urinary pathology that is not correctly detected by current tests (risking overdiagnosis or under-detection).
- There is a need to compare tests with clinically important outcomes, not just against historical gold standards.
- Many potential alternative tests for UTI have not been researched in pregnancy.

5.2 Overall research aims

1. Compare the prevalence of urinary pathology in pregnant women in the following contexts;
 - a. abdominal pain,
 - b. increased risk of preterm birth
 - c. normal pregnancy
2. Compare alternative tests with standard diagnostic tests and clinical outcomes in pregnancy
3. Compare sampling methods using both standard and alternative diagnostic tests to understand their performance in pregnancy
4. Describe urinary pathology in pregnancy more comprehensively than simply by culture outcome

5.3 Research contexts

Clinical research is constrained by ethics, practicality and cost. I concentrated on three related contexts:

1. "Abdominal pain study" - women presenting acutely with abdominal pain in second or third trimester.
2. "PTB study" - women with additional risk factors for preterm birth at 13-17 weeks.
3. "MSU-CSU study" - women requiring catheterisation for medical reasons.

We wished to sample women with greater probability of UTI than the general population of pregnant women. Thus, we sampled women with acute abdominal pain in second or third trimester. This was an explicit conjecture, but such abdominal pain is under-researched.

If the assumed association between UTI and risk of preterm birth is true we should expect women with known additional risk factors for preterm birth to exhibit a higher incidence of UTI. This too was an explicit conjecture.

Because urinalysis plays a significant part in decisions on antibiotic intervention in pregnancy the properties of specimen collection methods merit scrutiny because relevant data are absent.

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5.4 Abdominal pain study research questions

Aims:

To compare the prevalence of urinary pathology in (1) women presenting with abdominal pain in pregnancy, (2) women presenting acutely with non-abdominal pain complaints, and (3) asymptomatic women.

Hypothesis:

Women presenting with abdominal pain in pregnancy may have urinary pathology missed by standard tests.

Research questions:

- (1) Compare the prevalence of urinary pathology in each group using standard and alternative methods:
 - a. Structured lower urinary tract symptoms assessment using the Artemis LUTS inventory
 - b. Quality of life assessment using Euro-Qol EQ-5D-5L
 - c. Clinician's diagnosis of UTI
 - d. Patient's belief of a UTI
 - e. Standard dipstick, microscopy and culture
 - f. Fresh unspun, unstained urine microscopy
 - g. Urinary ATP measurement
 - h. Sediment culture with colony counts and microbial species identification
 - i. Urinary cytokines – IL-6, IL-8, lactoferrin
 - j. Bacterial-associated clue cell counts.
- (2) Measure proportion of uroplakin-positive epithelial cells in a subset of samples.

5.5 PTB study research questions

Aims:

To compare the prevalence of urinary pathology in (1) women at increased risk of preterm birth (2) women at low risk of preterm birth.

Hypothesis:

Women at increased risk for preterm birth may have urinary pathology missed by standard tests.

Research questions:

(1) Compare the prevalence of urinary pathology using standard and alternative methods:

- a. Structured lower urinary tract symptoms assessment using Artemis LUTS inventory
- b. Standard dipstick, microscopy and culture
- c. Fresh unspun urine microscopy
- d. Urinary ATP measurement
- e. Sediment culture with counts and identification to species level of cultured organisms
- f. Urinary cytokines – IL-6, IL-8, lactoferrin
- g. Bacterial-associated clue cell proportions.

5.6 MSU-CSU study research questions

Aims:

A paired comparison of MSU and CSU sampling.

Hypothesis:

MSU versus CSU from pregnant women may differ in pathophysiological indicator content.

Research questions:

- (1) Describe the cohort in terms of likelihood of urinary pathology using the following methods:
 - a. Structured lower urinary tract symptoms assessment using Artemis LUTS inventory
 - b. Quality of life assessment using Euro-Qol EQ-5D-5L.
- (2) Compare the prevalence of urinary pathology in voided and catheter specimens using standard and alternative methods:
 - a. Standard dipstick, microscopy and culture
 - b. Fresh unspun urine microscopy
 - c. Urinary ATP measurement
 - d. Sediment culture with counts and identification to species level of cultured organisms
 - e. Urinary cytokines – IL-6, IL-8, lactoferrin
 - f. Bacterial-associated clue cell counts
 - g. Uroplakin-positive epithelial cell proportions.

5.7 Pooled data research questions

Aims:

To compare alternative tests with standard diagnostic tests, and clinical outcomes, to understand their performance in pregnancy.

Hypotheses:

Alternative urinalyses methods may be superior to standard tests.

Research questions:

- (1) Describe the relationship between standard and alternative tests.
- (2) Compare the performance of alternative methods against the gold standard culture.
- (3) Compare the performance of standard and alternative methods in warning of complications:
 - a. Pyelonephritis
 - b. Preterm birth below 37 weeks.

6 Methods

6.1 Core outcome sets and reporting checklists

To ensure that this research could contribute to future systematic review and meta-analysis, avoiding research waste, it is important to standardise presentation of findings including outcomes (Khan 2014). This calls for methodological checklists and core outcomes sets.

The studies in this thesis were observational. Observational studies have in the past been inconsistently reported with missing information, reducing applicability or comparability with other studies. This led to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) initiative, a checklist to improve the reporting of observational studies (Vandenbroucke, von Elm et al. 2014). The methods in these studies were designed using the STROBE checklist. The full checklist is in the appendix (13.2).

The numbers recruited in these studies may not be sufficient to detect a difference in outcomes, particularly given findings of the previously described Dutch asymptomatic bacteriuria study (Kazemier, Koningstein et al. 2015). There is currently no published core outcomes set for UTI in pregnancy research, however at the time of designing these studies, a minimum dataset for genetic epidemiology studies in preterm birth had been developed and endorsed by the International Preterm Birth Collaborative (PREBIC) (Pennell, Jacobsson et al. 2007). These were considered when designing the current studies.

6.2 Study designs

Abdominal pain study: A prospective case-control study comparing standard and alternative tests for urinary pathology in women presenting with abdominal pain in pregnancy ("Pain cases") against women presenting with other non-pain acute problems ("Acute controls") and women attending for routine antenatal care ("Normal controls").

PTB study: A prospective case-control study comparing standard and alternative tests for urinary pathology in women with previous preterm birth ("HR-PTB"), against women with other risk factors for preterm birth such as cervical surgery or uterine anomalies ("HR-non-PTB"), and low risk women with previous term births ("LR").

MSU-CSU study: A prospective paired samples study examining standard and alternative tests for urinary pathology in women having a urinary catheter for elective surgical indications (elective caesarean or cervical cerclage), comparing voided samples ("MSU") with catheter samples ("CSU").

These three studies all examined the performance of the same tests. Therefore, in the analysis we planned to scrutinise the pooled results ("synthesis"), to compare against the gold standard and relevant clinical outcomes.

6.3 Settings

The setting was the Elizabeth Garrett Anderson Wing at University College London Hospital (UCLH), which houses all the UCLH maternity services, providing care to around 6500 women per year (2019).

Pain cases and Acute controls for the Abdominal pain study were recruited in the Maternal Fetal Assessment Unit (MFAU). This is where women present with any acute problem, including normal maternity symptoms such as term labour, and abnormal symptoms such as vaginal bleeding, from 15 weeks gestation.

Cases for the PTB study were recruited in the Preterm Birth Clinic. This is a tertiary referral service for women with risk factors for preterm birth. Risk factors include previous preterm birth or second trimester miscarriage, uterine anomalies such as bicornuate uterus, cervical surgery such as cone biopsy and large loop excision of the transformation zone (LLETZ).

Controls for both Abdominal pain and PTB studies were recruited from midwife-led antenatal clinics (ANC), or the obstetric ultrasound department.

Participants for the MSU-CSU study were recruited prior to their elective theatre procedure.

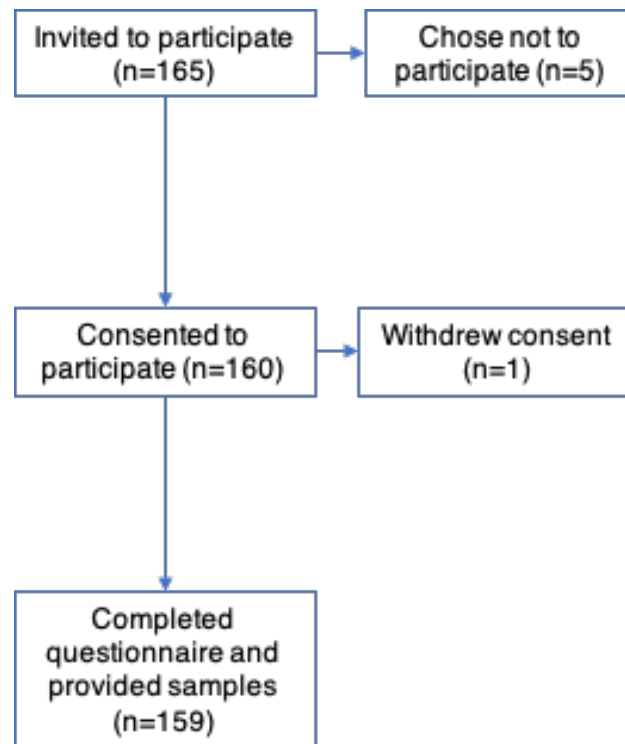
6.4 Recruitment

A version of the PTB study had been piloted previously (Arasaretnam 2013) (Muse 2013) (Kang, Malone-Lee et al. 2013) by others using different recruitment criteria. I recruited to the PTB study starting May 2014, until July 2015. I started with the PTB study first and used this to train in relevant laboratory techniques, and address recruitment processes and challenges, while I finalised the study protocol for the Abdominal pain study. Recruitment to Abdominal pain study ran from October 2014 to October 2015. Recruitment to the MSU-CSU study ran from July 2015 to August 2015.

Potential participants were provided with an information leaflet (see appendix 13.3).

Sessions during which patients were recruited and their data recorded were limited to three hours so that samples could be processed within four hours. Typically, 2-3 patients (range 0-7) were recruited per session.

I recruited consecutively during these sessions. A recruitment flowchart for the Abdominal pain study is shown in Figure 6-1. Data were not collected on eligible patients who were not recruited.

Figure 6-1: Recruitment flowchart for Abdominal pain study

Women were able to consider participation while waiting to be seen by a healthcare professional or for their procedure. If more time was required, they were invited to make another appointment to meet with a researcher. Those consenting patients had up-to-date Good Clinical Practice (GCP) certification.

6.5 Sampling method - clean-catch mid-stream urine

We collected a clean-catch mid-stream urine (MSU) specimen.

All women were given a study-specific MSU kit (see Figure 6-2). This was a paper bag containing a single-use sensitive (gentle) wipe, and a 250ml sterile sample container with a wide mouth. There were simple printed instructions detailing how to give a clean-catch mid-stream sample, attached to the bag closure, to encourage women to read them when opening the bag.

Figure 6-2: Study-specific MSU kit and instructions attached to the bag



How to give your urine specimen:

The most accurate way to collect a urine specimen is called a *clean mid-stream* sample.

Wash your hands.

Use the wipe provided to clean yourself (from front to back).

Catch the middle part of the urine stream in the container provided.

You will be asked for your urine sample when you see the midwife or doctor.

All participants had provided a standard MSU sample at clinic attendances prior to the study, which used a 25ml universal container without instructions. This was in contrast to the 250ml pot, accompanied by instructions and a wipe, used in the study.

A subset of participants (the first eight) was contacted two weeks after their visit and asked to complete an acceptability questionnaire about the study-specific MSU kit.

7/8 participants responded. The questions and responses are summarised in Table 6-1. These confirm that this was an acceptable method and women found it easier to use the 250ml pot.

Table 6-1: Acceptability of alternative clean catch MSU method: questionnaire and responses

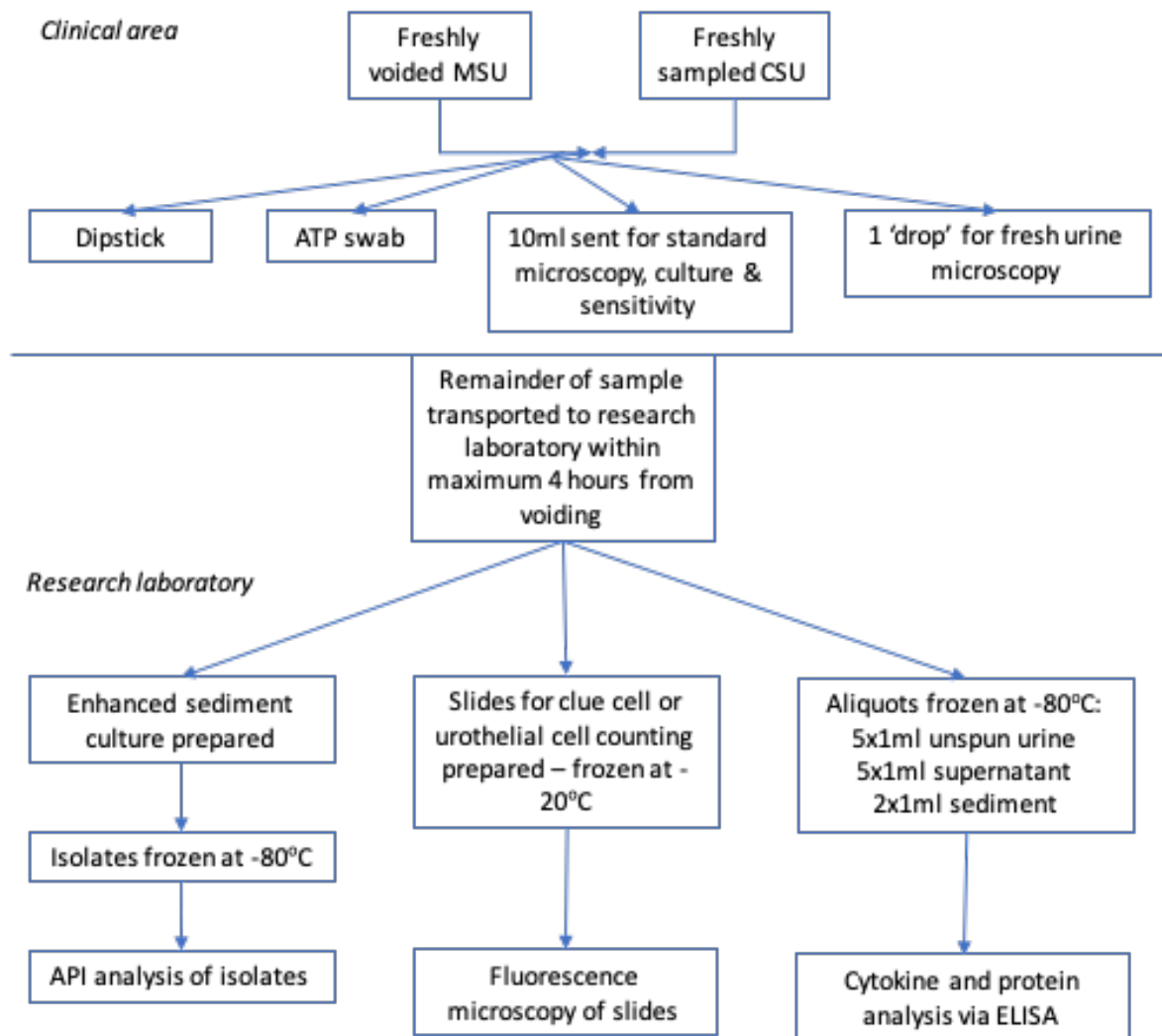
Statement	Responses (n=7)
I was happy to give a sample of my urine this way	Agree - 6 Strongly agree – 1
I understood the instructions on the bag	Agree – 7
I was happy to use the wipe provided	Agree – 7
I was happy to use the urine container provided	Agree – 7
I would recommend this test to other pregnant women	Agree – 7
I liked this test because ... (space for free text responses)	"Clearly explained" "Not particularly different to normal" "Nice to have the wipe and a large pot to use" "Easier to use than the small one" "Large pot much easier to use than the small one" "It was easier to use the large pot, liked the wipe" "Easier to use the large container when pregnant"
I disliked this test because ... (space for free text responses)	"Not particularly different to normal" "Was worried hadn't done enough as it was such a large pot"

Options for Likert scale responses were:

Strongly disagree / Disagree / Neither disagree nor agree / Agree / Strongly agree

6.6 Sample handling and processing

Figure 6-3 shows a flowchart summarising sample handling and processing. Note all laboratory methods will be described in full later in this chapter (6.15 and 6.16).

Figure 6-3: Flowchart of sample handling and processing

MSU or CSU samples underwent immediate dipstick and ATP analysis in the clinical area. Dipstick results were documented in patient notes. A portion (at least 10ml) was sent to the hospital laboratory for standard microscopy, culture and sensitivity (MC&S). This was either via a pathology sample pod transporter or a porter. All standard MC&S results were checked within 48 hours and patients informed by telephone of positive results.

A haemocytometer was loaded with 1µl of urine and the cells counted. The microscope was setup in the clinical areas.

The remainder of the sample was transferred to the research laboratory by the researcher as soon as possible, with a maximum of four hours from voiding to laboratory preparation. There were no delays in transport.

Enhanced sediment cultures were prepared, clue cell and urothelial cell slides were prepared, and aliquots of neat urine, supernatant and sediment were frozen at -80°C.

Following sediment culture, the subcultured isolates, bacterial and fungal, were frozen using Cryobank™ (Copan Diagnostics Inc.) bottles at -80°C. Prepared clue cell and urothelial cell slides were frozen at -20°C. Specimens were divided between two freezers in case of freezer failure. Repeated freeze-thaw cycles were avoided. Laboratory analysis of frozen samples continued until April 2018.

Training to carry out near-patient testing (fresh unspun urine microscopy, ATP testing), and research laboratory methods (sediment culture, preparation of fluorescent microscopy slides) was led by a trained member of the Chronic UTI laboratory team (Dr Sheela Swamy, Dr Harry Horsley, and Dr Sanchutha Sathiananthamoorthy).

Training on cytokine assay (ELISA) was provided by Dr Penny James, according to manufacturers' instructions. Training on API testing followed manufacturers' instructions with support by Dr Sanchutha Sathiananthamoorthy.

For each study, for most analyses, there was a single researcher (myself for Abdominal pain and Preterm birth, and all cytokine and API analyses, and Dr Natasha Liou for MSU-CSU recruitment and initial processing) with occasional cross-cover, and with additional input from Dr Arvinder Athwal for the first month of recruitment to the PTB study.

Samples were labelled by a research number, date of sample and name of study, with no other identifiers. Clinical data were collected after near-patient testing had been completed (dipstick, ATP and fresh urine microscopy counts), and stored separately from test results.

A separate identification key, linking patient identifiers with their research number, was maintained in a locked hospital cabinet and in a password-protected file saved on the hospital server, in line with Good Clinical Practice requirements.

I had planned to double read the microscopy, to check reproducibility of results. A second researcher was trained to analyse samples. Unfortunately, this failed because of their workload.

Cytokine analyses, API testing of isolates, and fluorescent cytology cell counts were all carried out in batches, and results stored separately. The researcher was always blinded.

Anonymised data (identified only by a research number) was stored in an Excel spreadsheet and later decanted into an SPSS table. Data analysis was carried out using Excel, SPSS version 26, with graphs created in Prism version 8.

6.7 Variables

Given the association between preterm birth and UTI, I formatted my data to be compatible with PREBIC minimum dataset (Pennell, Jacobsson et al. 2007), a standardised dataset for outcome measures in studies relevant to preterm birth so that my data could be included in future systematic review and meta-analysis. The data collection proforma were designed in line with PREBIC and used to collect data for the Abdominal pain and MSU-CSU studies (see Appendix 0). Whilst this was to be used at entry into the study, the need for timely arrival of samples in the lab meant that some data were obtained later from clinical records, and these were sometimes incomplete. Clinical and demographic data for the PTB study were collected by research midwives and nurses as part of a prospective preterm birth clinic database. The data set covered gestation at delivery, but omitted admission to neonatal care, mode of delivery or birth weight. Those data were extracted separately. Outcomes, exposures and predictors are listed in Table 6-2.

Table 6-2: Study outcomes, exposures and predictors

Outcome / exposure / predictor	Assessment method	Measurement
Assignment to study group (Abdominal pain study)	Patient self-report	Pain cases, Acute controls, Normal controls
Assignment to study group (PTB study)	Risk factors for PTB	High risk – PTB High risk – non-PTB Low risk - multiparous
Assignment to study group (MSU-CSU study)	Clinical reason for catheter	Cerclage cases CC MSU, CC CSU
	Sampling method	Caesarean cases CS MSU, CS CSU
Patient self-assessment of UTI (Abdominal pain study)	Questionnaire	Yes / No / Not sure
Documentation of clinical assessment of urinary symptoms at time of clinician review (Abdominal pain study only)	Clinical record	Documentation of urinary symptoms present / absent Nature of urinary symptoms noted including negative findings
Treatment for UTI	Clinical record	Any treatment for UTI at the time of recruitment
MSU at booking	Clinical record	Culture result (Kass criteria)
Other microbiological findings	Clinical record	Any positive MSU Any positive vaginal swabs Any other microbiological tests
Suspected maternal sepsis antepartum, intrapartum or postpartum	Clinical record	Documentation of suspected sepsis Blood cultures Use of antibiotics (excluding prophylactic)
Preterm birth below 37 weeks	Clinical record	Preterm birth below 37 weeks (spontaneous or iatrogenic) present / absent Obstetric outcomes (gestation at delivery, mode of delivery, birth weight)
Neonatal sepsis	Clinical record	Documentation of suspected neonatal sepsis Neonatal outcomes to describe group characteristics (stillbirth, neonatal death, admission to neonatology, Apgars at 1 and 5 minutes)
Cervical length	Data from PTB clinic	Cervical length scan done Shortest ever cervix Cervix < 15mm Cervix 15 – 25mm Presence of sludge / funnelling

Continued next page

Outcome / exposure / predictor	Assessment method	Measurement
Fetal fibronectin	Data from PTB clinic	FFN done FFN Positive / negative (Positive FFN taken to be >50 UNITS) FFN quantitative
Cervical cerclage insertion	Data from PTB clinic	Cerclage inserted Gestation at insertion Type of cerclage
Progesterone	Data from PTB clinic	Progesterone prescribed Gestation at progesterone
Previous cervical cerclage	Clinical records review	Previous cerclage
Dipstick	Dipstick – visual reading	Proportion positive (any of leucocyte, nitrite, protein) Leucocyte esterase, protein, blood (all range 0 to 4+), nitrite (negative or positive),
Standard urine microscopy, culture and sensitivity	Hospital laboratory measurement	Qualitative cell counts Urine culture result (negative, non-significant or mixed growth, positive) Positive urine culture defined as >10 ⁵ CFU/ml of a single uropathogen
Structured LUTS assessment	Artemis LUTS inventory	Total score Domain scores x 4 Individual item scores
Quality of life	EQ-5D-5L questionnaire	Health index (max 1) Health assessment (%)
Fresh unspun urine microscopy cell counts	Fresh unspun urine microscopy	Number of cells/mm ³ : White cell count Red cell count Epithelial cell count
Urine ATP	Luminometer	ATP measurement in relative light units (RLU)
Enhanced sediment culture of urine	Sediment culture Analytical profile index (API)	Total colony counts Dominant organism colony counts Number of organisms identified Species identified
Urinary IL-6, IL-8, lactoferrin, protein	ELISA Bradford Assay for protein	Concentration Concentration corrected for protein concentration
Clue cell proportions in urine	Epifluorescent microscopy	Proportion of epithelial cells with associated bacteria
Uroplakin-positive epithelial cell proportions in urine (subset of patients in Abdominal pain study; all patients in MSU-CSU study)	Epifluorescent microscopy	Proportion of epithelial cells staining positive for uroplakin

Confounders and effect modifiers are outlined in Table 6-3. These were determined from clinical records.

Table 6-3: Confounders and effect modifiers

Confounder	Measurement
Gestation at recruitment	Days
Time recruitment to delivery	Days
Maternal age	Age in years on day of recruitment
Body mass index	Weight / height ² (kg/m ²)
Parity	Nulliparous or parous
Preterm birth risk factors	Present or absent
UTI risk factors	Present or absent
Socio-economic markers	Index of Multiple Deprivation – top five or bottom five deciles (see note below)
Ethnicity	White versus non-white (see note below)
Smoking status	Non-smoker at time of recruitment or smoker
Singleton or multiple pregnancy	Singleton or multiple pregnancy

I obtained the socio-economic status from the Index of Multiple Deprivation 2015 (Government 2015). This ranks every small area in England from most deprived area (1) to least deprived area (32,844) (Government 2015). These are arranged as deciles, dichotomised for simplicity into top five and bottom five deciles.

Ethnicity was recorded using an eight-level categorisation (Snelgrove and Murphy 2015). The sample limited analysis to comparisons of white versus non-white status.

6.8 Statistical methods

SPSS version 26 was used for most descriptive and inferential statistical analysis, unless stated otherwise. Prism version 8 was used for some graphics.

Online calculators were used to calculate confidence intervals of proportions (<http://vassarstats.net/prop1.html>) according to the Newcombe method, using continuity correction (Newcombe 1998). Online calculators were used for ELISA calculations (*myassay.com*). Venn diagrams were constructed using a web-based tool (<http://www.interactivenn.net>) (Heberle, Meirelles et al. 2015).

Normality was assessed using the Kolmogorov-Smirnov test. From previous work it was anticipated that most variables would be best described by non-parametric distributions, and this was the case for all variables except age. Thus, non-parametric statistical methods were used throughout. All statistical tests were two-sided and at the 95% level of confidence.

For all studies, continuous variables were described using median, 95% confidence intervals of the median, and sometimes range. Categorical variables were described using proportion and 95% confidence intervals of the proportion. Some continuous variables were coerced into dichotomous categories, such as white cell count; $0/\text{mm}^3$ / $> 0/\text{mm}^3$. Some categorical variables were similarly treated. Log transformation was used for cell and microbial counts. Before log transformation zeros were changed to 1.

For the Abdominal pain and PTB studies, between-group differences were compared using the Kruskal-Wallis test and the 95% CI about the median were used. Categorical variables were compared using Chi Square (χ^2), or Fisher's Exact (FE) where assumptions were not met.

The MSU-CSU study involved paired samples, so comparisons used the Wilcoxon signed rank (WSR) matched pairs tests for continuous variables. For related samples, Macnemar test was used for categorical variables instead of Chi Square.

To compare microbial species growth between study groups, species and genus were plotted as a proportion of all isolated for each study group. Distributions were compared using Chi Square or Macnemar tests.

The between-group analysis of the pooled data used similar methods. Prior to pooling, I compared characteristics of the three studies and compared the spread of values across the studies. With only three studies to combine, it was not appropriate to use a mathematical assessment of heterogeneity that would

be used in meta-analysis, such as I^2 , as the small number of studies would make the I^2 statistic very imprecise, and exacerbate its inherent bias (von Hippel 2015).

A non-parametric method to assess correlation, Spearman's rank correlation, was used. The closer the value of Spearman's rho (r_s) is to one, the stronger the correlation. The description of the strength of correlation varies (Akoglu 2018). In this work, Spearman's rho (r_s) of $0.6 < r_s \leq 0.8$ was taken to represent a strong correlation, $0.4 < r_s \leq 0.6$ a moderate correlation, and $0.2 < r_s \leq 0.4$ a weak correlation.

Bland-Altman analysis was used to measure agreement (test described in detail in context in section 6.16.8). Multiple regression was used to assess the differential influence of the variables in the model.

I must declare that the final analysis differed from planned analysis because I developed a better understanding of the statistics and the subject matter (Plos Medicine Editors 2014).

The original Abdominal pain study protocol, as submitted in the funding application, contained the following hypothesis and objectives:

"Hypothesis:

We hypothesise that women presenting with abdominal symptoms in pregnancy may have urinary pathology that is undetected by conventional tests.

Objectives:

In women with abdominal pain in pregnancy, pregnant women presenting acutely without pain and asymptomatic pregnant women attending low risk antenatal clinics I will:

- (1) determine the prevalence of UTI using fresh microscopy and sediment cultures,
- (2) compare the performance of fresh microscopy and sediment cultures against urine dipstick and traditional laboratory microscopy, culture and sensitivity and
- (3) study the prevalence of lower urinary tract symptoms (LUTS) using a structured approach."

Development of the full study protocol led to a broadening of these objectives. This was to take into account a wider range of alternative tests, and to move away conceptually from a simple comparison with standard culture owing published work alerting me to the serious shortcomings of standard culture (see section 3.2). The original objectives were used to inform sample size calculation.

6.9 Qualitative analysis methods

Thematic analysis was planned for analysis of text, specifically documentation of UTI symptoms (Braun 2006). In the event, there was minimal documentation and responses were simply categorised and coded with minimal thematic analysis required.

6.10 Bias

The study situation meant that fresh unspun microscopy was conducted unblinded. Work demands precluded a second researcher from doing microscopy to protect blinding. Samples were minimally labelled to reduce risk of bias. Recruitment was strictly consecutive during a clinical session.

6.11 Sample size calculation

Abdominal pain study:

At the start of this work, the best biological marker for discriminating between people with UTI and no UTI was the white cell count from fresh unspun, microscopy, at least in the Chronic LUTS setting from where this work had emerged.

Using white cell count as the primary outcome, I calculated a sample size of 40 in each group. These sample sizes would have more than 80% power to detect a clinically significant effect, based on $\log_{10}$ WBC counts. In anticipation of using non-parametric methods, and loss to follow-up, I aimed to recruit 50 women presenting with abdominal pain, 50 presenting acutely without pain, and 50 low risk antenatal clinic attendees.

As with all emerging research, it is challenging to achieve a legitimate sample size calculation, because there are so many unknowns. Our centre previously used pyuria data to guide estimates in non-pregnant cohorts. I was concerned that we knew so little about pyuria in pregnancy that it might not be a safe option for making a reasonable estimate.

As a comparison, we ran an alternative sample size calculation, using LUTS scores as an alternative primary outcome. This was based on a database of over 13,000 questionnaires obtained from the chronic LUTS clinic, where the mean total symptom score was 6.68 with standard deviation of 5.8. In order to show a clinically significant difference between two independent groups, which would equate to at least 4 symptoms, a two-tailed t-test sample size calculation was carried out with 80% power to detect a difference of 4. This gave a sample size of 34 in each group.

MSU-CSU study:

Considering the active research in the group at the time this study started, polymicrobial abundance was starting to emerge as higher in patients compared to normal controls. Since our historical understanding of UTI in pregnancy has been based on quantitative urine culture we based our sample size estimates on urine culture data being collected from Dr Kiren Gill's PhD thesis which studied women with UTI (Gill, Kang et al. 2018). Her sample size estimate was based on pilot data showing that standard deviation of the $\log_{10}$ total bacterial count was 2 (SD=2) and the standardised mean difference in $\log_{10}$ bacterial counts was 0.5 ($f=0.5$). A sample size of 20 subjects in each group would provide 83% power to detect a significant difference in $\log_{10}$ bacterial growth at the 5% level ($\alpha=0.05$). As before, because of the issues with any clinical study, we chose sample groups of 30.

6.12 Ethical considerations

Ethical approval was obtained under the umbrella of the Cervical Length, InflaMmation and Bacterial species and preterm birth (CLIMB) study protocol (James CPJ 2015). Ethical approval for CLIMB was given by the joint UCL/UCLH Committees on the Ethics of Human Research (REC reference: 09/H0714/66). The CLIMB protocol was amended to include a specific consent

form and patient information sheet relevant to the Abdominal pain study, which was approved in January 2015.

Patient Information Sheets and consent forms are shown in appendices 13.3 and 13.5.

Recruitment was carried out in accordance with Good Clinical Practice (GCP). Potential participants were given written information about the study and associated risks. They were allowed time to consider participation, and signed a consent form covering risks and confidentiality. Study participants were advised they could withdraw consent at any time.

Outside of use in the LUTS clinic, alternative tests had not been approved for clinical care, and many were analysed much later from frozen samples, hence were not used to direct treatment. Where symptoms or standard urine tests suggested infection, or carriage of Group B Streptococcus (GBS), patients were treated according to standard guidelines.

6.13 Data protection and data storage

All records were anonymised at time of data entry in adherence with the Data Protection Act 1998. Only members of the research team had access to participants' personal data during the study.

Clinical records with patient identifiers were maintained on hospital premises in locked cabinets, or in password-protected files stored on hospital servers.

6.14 Financing

The study was funded by Wellbeing of Women via the British Maternal and Fetal Medicine Society Scholarship (£19,765). This paid for laboratory consumables, computing equipment and contributed towards conference attendance.

A microscope, for fresh urine microscopy, was purchased using a grant from the Elizabeth Garrett Anderson Charity (£2,928).

Consumables for the MSU-CSU study, and laboratory bench costs, were supported by a £2000 laboratory bursary associated with Dr Natasha Liou's MSc project at UCL's Institute for Women's Health.

6.15 Standard test methods

6.15.1 Urine dipstick

The urine dipsticks used were Multistix®8 SG reagent strips (Siemens, Munich, Germany). Colour was assessed by eye after two minutes, according to manufacturer's instructions. Results for leucocyte esterase, nitrite, protein, blood, glucose, pH and specific gravity were recorded. Individual results were summarised as positive if trace positive or more.

Midwifery practice is typically to send a urine sample for culture if any of these are trace positive or more: leucocytes, nitrite or protein; this is referred to as 'dipstick positive' in this study.

6.15.2 Standard culture

Urine samples were decanted into 25 ml sterile containers without boric acid and transferred to the laboratory via porter or pod system. At the time of the study, tubes containing boric acid were not routinely used in the hospital. Routine laboratory microscopy, culture and sensitivity (MC&S) was undertaken in the local NHS laboratory using the Mast Uri® robotic system.

Standard NHS microscopy cell count results were reported as not seen / very scanty / scanty / moderate / numerous. These were summarised for this study as not seen / present, with all other categories collapsed into present.

The NHS laboratory reported a positive culture as a single urinary pathogen of $\geq 10^5$ CFU/ml colony count. Other possible results reported were: screening culture negative; no significant growth; mixed growth of uncertain significance. Antimicrobial sensitivity results were not analysed in this study.

6.16 Alternative test methods

6.16.1 LUTS assessment – Artemis LUTS inventory

The Artemis LUTS inventory was used (Khasriya, Barcella et al. 2018) (introduced in 4.2). This was designed by, and routinely used in, the LUTS clinic. This is a questionnaire assessment of urinary frequency and nocturia, incontinence, and 34 other LUTS, grouped into four symptom domains of

stress incontinence (7), urgency (12), voiding (8) and pain (13). Symptoms were recorded as binary variables (present or absent). Patients were asked to record symptoms recalled from the preceding few days. Scores were summed for each of the four symptom groups. Threshold reference values have not been determined. Population estimates of mean and standard deviation are available based on previous research in non-pregnant people (Khasriya, Barcella et al. 2018). The Artemis LUTS inventory is shown in appendix 13.1.

6.16.2 Quality of life (QoL)

QoL assessment used the EQ-5D-5L questionnaire (introduced in 4.3) (EuroQol 1990). This has five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). It is divided into five levels of perceived problems (from no problems to extreme problems). The subject selects one level from each dimension. These selections are combined to create a health status value, which is converted to an index value for ease of comparison between subjects. The questionnaire also has a visual analogue score (VAS). The subject self-rates their health on a vertical visual scale from 0-100%, with the endpoints labelled 'the worst health you can imagine' (0%) and 'the best health you can imagine' (100%). Thus the outcome is an index value and a VAS measure (EuroQol 2013). The EQ-5D-5L tool is shown in appendix 13.6. Permission to use the tool was granted for research purposes.

6.16.3 Fresh unspun urine microscopy

A drop of freshly voided, unspun, urine was transferred to a Neubauer haemocytometer counting chamber using a disposable Pasteur pipette (Sigma-Aldrich, Gillingham, UK) according to standard technique (McGinley, Wong et al. 1992). Trained researchers counted white blood cells, red blood cells and epithelial cells, using an Olympus CX41 light microscope (x 400; Olympus). The microscope was set up in the clinic so that examination happened within 30 minutes of sample collection.

A Neubauer haemocytometer has a fixed volume (0.9 μ l) and gridlines creating 9 large squares. Cells in 5 squares were counted and doubled to give a count

per μl (μl^{-1}). Cells at boundaries were counted if they crossed the upper or left margin of the square.

All researchers were trained in the same way by the Chronic LUTS technicians. This involved being shown the counting technique and then blind counting the same slides as an experienced cell counter, until the trainer was satisfied with the level of agreement on cell counts.

In the Chronic LUTS clinic, normal white blood cell count (WCC) is 0 μl^{-1} with pyuria defined as $\geq 1 \mu\text{l}^{-1}$ (Barcella W 2015), with a further threshold at $\geq 10 \mu\text{l}^{-1}$ corresponding to standard reference values (Kupelian, Horsley et al. 2013).

6.16.4 ATP measurement

ATP was measured using the LuciPac pen system (Kikkoman). This consists of a cotton swab, which is removed from a pen-shaped tube, dipped into a fresh unspun urine sample for approximately 15 seconds, then reinserted into the tube and shaken. This is inserted in a portable luminometer (Lumitester PD-20; both pen and luminometer by Kikkoman) according to the manufacturer's instructions. The luminometer produces a digital reading, in relative light units (RLU).

ATP Lucipac pens were stored at 4°C as per manufacturer's instructions.

ATP was measured as soon as possible on unspun urine as levels have been shown to decrease with centrifugation and delay in testing (Gill, Horsley et al. 2015).

6.16.5 Enhanced sediment culture and identification of cultured micro-organisms

Sediment culture protocol was based on that developed by our laboratory team (Khasriya, Sathiananthamoorthy et al. 2013).

Samples were transported to the research laboratory for processing within 4 hours.

5ml unspun urine was removed and frozen at -80°C in 1ml aliquots.

Supernatant and sediment were frozen for later analysis. 10ml urine was centrifuged for 10 mins at relative centrifugal force (RCF) of 1500g, removing

8ml supernatant. Of this, 5 x 1ml supernatant aliquots were frozen at -80°C. The remaining 2ml centrifuged urine, containing sediment, was split between two cryovials and frozen at -80°C.

To prepare sediment cultures, 5ml urine was centrifuged at RCF of 1500g at room temperature for 10 minutes (Sigma 3-18KS Centrifuge). Supernatant was removed and frozen at -80°C in 1ml aliquots. The sediment was resuspended in 400µl phosphate buffered saline (PBS). 100µl resuspended sediment was added to 900µl PBS and serial dilutions performed to 10⁻³ dilution.

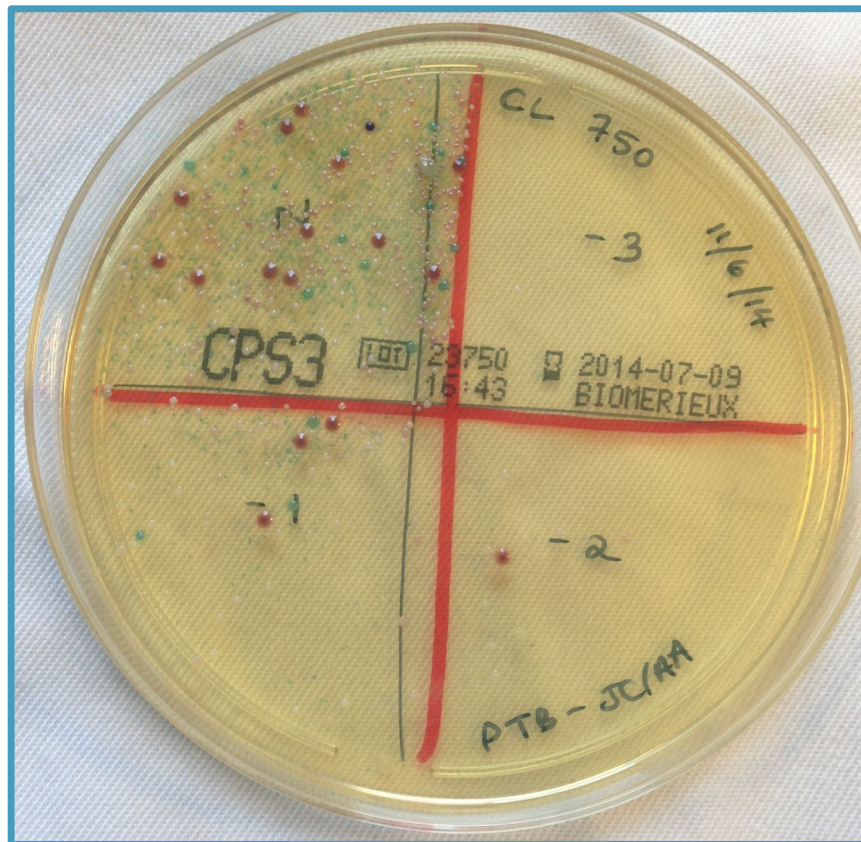
A chromogenic agar (CPS3, bioMérieux, France) plate was divided into four quadrants. 50µl quantities of the resuspended sediment and dilutions were pipetted into each quadrant, spread with a hockey stick spreader, and incubated in air at 37°C for 24 hours.

After incubation the different colonies on each plate were identified using the CPS3 colour chart (see Figure 6-4 and Figure 6-5). A colony count was performed for each type on each quadrant of the plate. Colony forming units per ml (CFU/ml) were calculated as follows:

$$\begin{aligned} \text{Mean count} &= \frac{(\text{Neat count}) + (\text{count in } -1) \times 10 + (\text{count in } -2) \times 100 + (\text{count in } -3) \times 1000}{4} \\ \text{CFU per ml} &= \frac{(\text{mean count}) (\text{dilution factor})}{\text{volume of urine used}} = \frac{(\text{mean count}) \frac{400\mu\text{l}}{50\mu\text{l}}}{5\text{ml}} = (\text{mean count}) \frac{8}{5} \end{aligned}$$

An example sediment culture plate (using CPS3) is shown in Figure 6-4. This represents a participant whose standard culture was reported as screening culture negative. This demonstrates six different isolates, including *E. coli* (large burgundy), *Enterococcus faecalis* (green) and *Streptococcus agalactiae* (tiny pink).

Figure 6-4: Sediment culture example plate



Organisms were identified to genus level using a series of bench tests: chromogenic agar colour and morphology identification (see Figure 6-5), Gram staining (see Figure 6-6), and catalase and indole tests (see Figure 6-7). These will be collectively referred to as bench tests to genus level.

Chromogenic agar, initially Chromid® CPS3 then Chromid® CPS® Elite (CPSE) contain specific substrates for the colour-based identification of common urine pathogens, such as *E. coli*, *Proteus spp*, and *Enterococcus spp*.

Figure 6-5: Chromogenic agar example photos

CHROMID[®] CPS[®] Elite

Isolation, enumeration and direct or presumptive identification of urinary tract infection organisms.



E. coli
P. mirabilis
E. faecalis



P. mirabilis

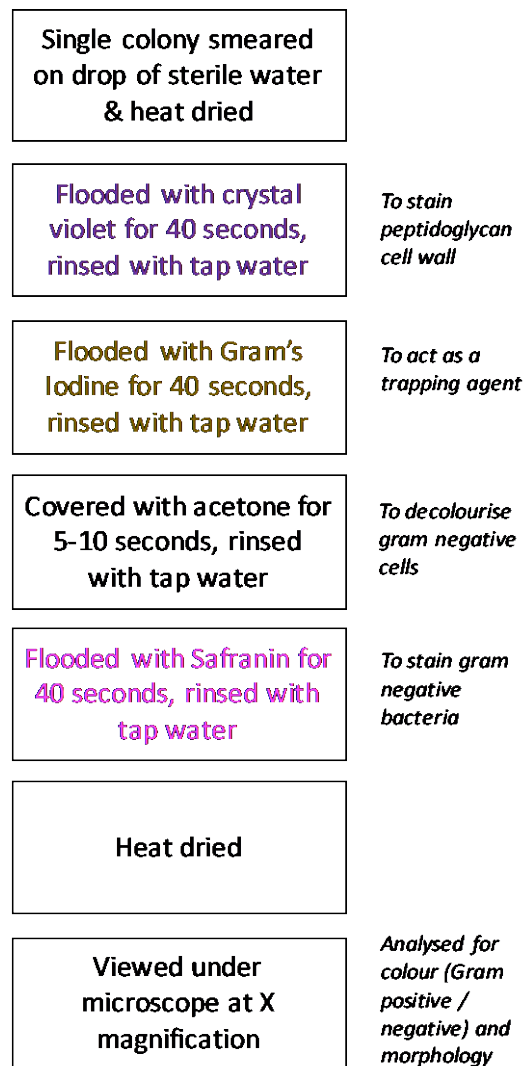


E. faecalis



E. coli

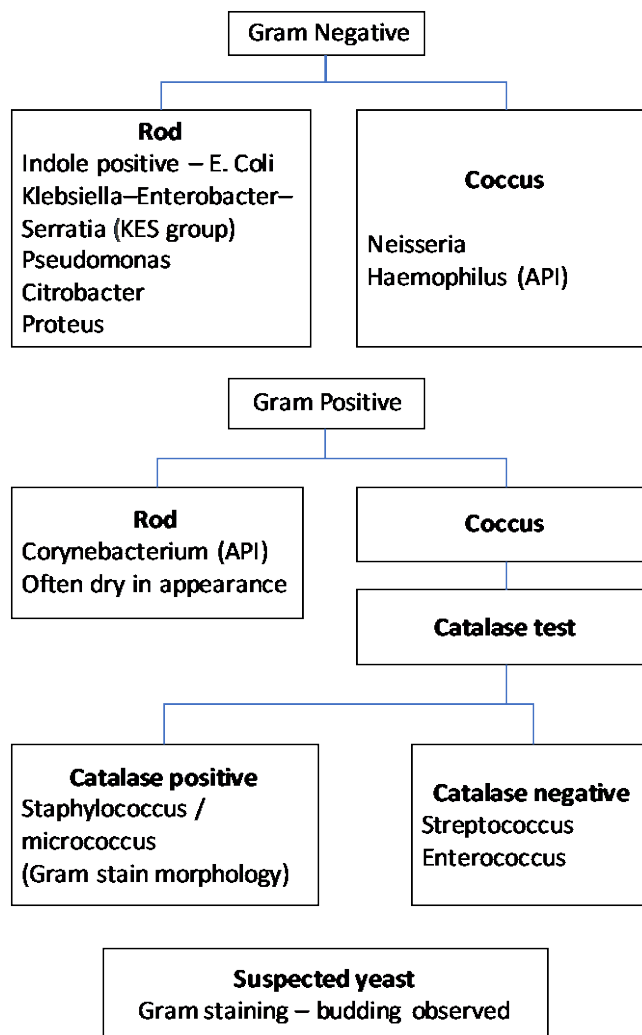
Reproduced from: <http://www.biomerieux-culturemedia.com/product/9-chromid-cps-elite> (last accessed 07/12/2020)

Figure 6-6: Gram stain procedure

Catalase: All Gram-positive white colonies underwent catalase testing. A single colony was added to a slide to which a drop of hydrogen peroxide had been applied. Bubbles of oxygen indicated a catalase positive organism.

Indole: All Gram-negative colonies underwent Indole testing. A drop of Indole spot reagent was added to filter paper, and a colony smeared on it. If colour changed to blue it was indole positive, if it was pink it was indole negative.

Indole positive indicates bacteria with the ability to produce the enzyme tryptophanase, that breaks down the amino acid tryptophan to indole.

Figure 6-7: Isolate identification flow chart

Reproduced with thanks to Dr Sanchutha Sathiananthamoorthy

Sub-cultures were taken from each identified isolate, plated on both CPS3 (to ensure pure growth of the isolate), and Columbia Blood Agar (CBA) (to increase yields). Each isolate was collected using a sterile loop and transferred to a Microbank™ long-term bacterial and fungal storage tube (Pro-lab Diagnostics, UK) for storage at -80°C, for species-level identification by Analytical Profile Index (API, bioMérieux, France) testing at a later date.

For API testing, isolates were re-cultured from Cryobeads on CPSE (CPS-Elite, bioMérieux, France) to ensure pure growth of a single organism, and CBA if specified in manufacturer's instructions. API testing was performed according to manufacturers' instructions. Based on genus-level identification, organisms underwent API testing using one of the following API®-

20E, API®-Staph, API®-Strep, API®-Candida and API®-Coryne (all bioMérieux, France), as described in Table 6-4.

Table 6-4: Analytical Profile Index (API) testing kits used

API test kit	Target organisms
API®-20E	Enterobacteriaceae and other non-fastidious gram-negative bacteria
API®-Staph	Clinical staphylococci and micrococci
API®-Strep	Streptococci and enterococci
API®-Candida	Yeasts, notably those most frequently encountered in clinical microbiology
API®-Coryne	Corynebacteria and coryne-like organisms

Each API kit followed similar principles. The re-cultured isolate was resuspended in a sterile culture medium, which was pipetted into a strip of tubules and cupules, either empty or containing enzymatic or biochemical reagents. Some were covered in mineral oil to make them anaerobic. These were then incubated in air at 37°C for a specified length of time (4-24 hours). If any further enzymatic or biochemical reagents were required they were added after this. Each colour was compared against a key and a score assigned, giving rise to a numerical code. This was then input into APIweb (<https://apiweb.biomerieux.com/>, last accessed 09/12/20), an online database which reported the likely organism with an estimate of the accuracy of this. If the identification was inadequate, the isolate underwent repeat bench tests to confirm the genus, with repeat API testing. The proportion of isolates not identified by API testing is reported along with results.

While *Lactobacillus spp* was easy to identify using chromogenic agar and morphology, it was difficult to regrow sufficiently as a subculture for frozen

storage and subsequent API testing. Therefore, no species-level identification was carried out.

API testing from frozen isolates was from October 2015 to January 2016 and again from January 2017 to March 2017.

There was an alteration to the enhanced culture protocol during the PTB study. Initial PTB study samples were centrifuged using a Denley BR401 centrifuge ($r_{max}=140\text{mm}$) at 2000rpm for 5 minutes, equivalent to 627g relative centrifugal force (RCF), according to the original local laboratory protocol. From 15/10/14 this was changed to 3000rpm (equivalent to RCF of 1411g) for 10 minutes, to maximise isolate yields according to local lab practices. When a new centrifuge was purchased (Sigma 3-18KS Centrifuge), programme settings were fixed to match these protocols. "Programme 2" provided 693g RCF for 5 minutes; "programme 3" provided 1500g for 10 minutes. Programme 3 was used for all Abdominal pain and MSU-CSU study samples.

Total colony counts for PTB study were compared on the original protocol ($n=57$) and the new ($n=23$) (see Figure 6-8). There was no difference in total colony counts (Mann Whitney U, $p=0.463$). Low risk and HR-PTB were not evenly distributed. Analysing only HR-non-PTB, with 17 before and 20 after, there was no difference (Mann Whitney U, $p=0.892$).

Table 6-5: Urinary cytokine ELISA kits

Urinary cytokine	Kit	Range	Sensitivity
IL-6	Human IL-6 Quantikine ELISA kit D6050 (R&D Systems)	3.1-300pg/ml	0.7pg/ml
IL-6 (high sensitivity)	Human IL-6 Quantikine HS ELISA kit HS600B (R&D Systems)	0.2-10pg/ml	0.11pg/ml
IL-8	Human IL-8/CXCL-8 Quantikine ELISA kit D800C (R&D Systems)	31.2-2000pg/ml	7.5pg/ml
IL-8 (high sensitivity)	Human IL-8/CXCL-8 Quantikine HS ELISA kit HS800 (R&D Systems)	1.0-64pg/ml	0.4pg/ml
Lactoferrin	Human Lactoferrin ELISA kit (E-80LF) (Immunology Consultants Laboratory Inc.)	3.125-100ng/ml	not stated
Protein	Coomassie (Bradford) Protein Assay Kit (23200) (Thermo Fisher Pierce™),	100-1500 µg/ml (standard microplate protocols) 1-25 µg/ml (micro microplate protocols)	Not stated

General processes

All assays were run in duplicate. A blank 96-well dummy plate was used to prepare initial standards and samples. Multichannel pipettes were used for uniformity. Thorough mixing was achieved using a vortex mixer (Scientific Industries, New York, USA). All optical densities were analysed using Biochrom EZ Read 400 microplate reader.

Assays were carried out from 10/2015-01/2016 and 01/2017-03/2017, using thawed frozen supernatant. Repeated freeze-thaw cycles were avoided. All cytokine analyses were performed within three years of initial sample collection.

A standard curve for each ELISA plate or protein assay was created using the commercial ELISA-analysis programme *myassay.com*, which calculates a

four-parameter logistic curve fit using standard data points and calculates unknown concentrations from this fit, including a background correction.

The output was analysed immediately and checked for quality. Results from each plate were accepted if $r^2 > 0.98$ for the standard curve. Individual results were accepted if within the standard curve and percentage coefficient of variation (%CV) was $< 20\%$ as per manufacturer's advice. 15/1227 (1.2%) results included in the final database (13xIL6, 1xIL8, 1xlactoferrin, no protein) had %CV of between 20.0 and 26.4, despite multiple repeats. A further 4/1227 (0.3%, all IL6) had %CV above this but all were very low concentrations, with values well below the lower limit of detection, making precision difficult. These results were accepted as below the lower limit of detection.

Samples with a result above the standard curve were diluted according to manufacturers' instructions. Samples below the standard curve were analysed using a high sensitivity kit if available (for IL-6 and IL-8) or high sensitivity protocol (for protein).

Results below the lower limit of detection (LLOD) are known as censored data, which refers to non-zero values which cannot be measured but are known to be below a known threshold (Hornung and Reed 1990). A statistical method of dealing with this is to substitute the LLOD with $\text{LLOD} / \sqrt{2}$ (Hornung and Reed 1990). Substituted values below the LLOD were: protein 0.71 $\mu\text{g/ml}$; IL-6 0.14pg/ml; IL-8 0.71pg/ml; lactoferrin 2.21ng/ml. Results below the LLOD were compared, to detect bias caused by insensitive tests.

IL-6

Samples were initially run using the standard kit. Where results were below the lower limit of detection (LLOD) they were repeated using the high sensitivity (HS) kit. Where results were above the upper limit of detection they were diluted and repeated.

The Human IL-6 Quantikine HS ELISA kit was validated by the manufacturers for use with urine. The kits used a quantitative sandwich enzyme immunoassay technique.

IL-6 - standard kit

Standards solutions were prepared by serial dilution according to instructions. A 96-well microplate had been pre-coated with monoclonal antibody specific for human IL-6. 100µl assay diluent RD1W was added to each well. 100µl standards and samples were pipetted into the wells in duplicate, covered and incubated for 2 hours at room temperature; any IL-6 present was bound by the antibody. Four washes with wash buffer were used to wash away unbound substances. 200µl human IL-6 conjugate (an enzyme-linked polyclonal antibody specific for human IL-6) was added to each well, covered and incubated for 2 hours at room temperature. There were four washes to remove unbound antibody-enzyme reagent. 200µl substrate solution was added, covered away from light, and incubated for 20 minutes at room temperature. Colour developed in proportion to the amount of IL-6 bound initially. 50µl stop solution was added to each well. Colour changed from blue to yellow. Optical density of each well was read within 30 minutes, at 450nm with wavelength correction of 570nm.

IL-6 – High sensitivity (HS) kit

Initial steps were as for the standard kit. After incubating 200µl Human IL-6 HS conjugate for 1 hour at room temperature on an orbital shaker (0.12" orbit set at 500+/- 50rpm), and washing four times, 200µl amplifier (Streptavidin Polymer-HRP (1X)) was added to each well, covered and incubated for 30 minutes at room temperature on the orbital shaker. This was washed four times before adding 200µl substrate solution to each well, and incubating covered without light for 30 minutes on the bench. 50 µl stop solution was added to each well. Colour changed from blue to yellow. Optical density was analysed within 30 minutes and read at 492nm with wavelength correction of 650nm.

IL-8 – standard kit and HS kit

IL-8 was measured using two commercial ELISA kits to cover a range of concentration where necessary, as per Table 6-5, and were performed according to manufacturer's instructions. These kits used the same quantitative sandwich enzyme immunoassay technique outlined for IL-6.

Urinary IL-8 – standard kit

Standards solutions were prepared by serial dilution according to instructions. A 96-well microplate had been pre-coated with monoclonal antibody specific for human IL-8. 100µl assay diluent RD1-85 was added to each well. 50µl standards and samples were pipetted into the wells in duplicate, covered and incubated for 2 hours at room temperature. Four washes with wash buffer were used to wash away unbound substances. 100µl Human IL-8 Conjugate was added to each well, covered and incubated for 1 hour at room temperature. There were four further washes to remove unbound antibody-enzyme reagent. 200µl substrate solution was added, covered away from light, and incubated for 30 minutes at room temperature. Colour developed in proportion to the amount of IL-8 bound initially. 50µl stop solution was added to each well. Colour changed from blue to yellow. Optical density of each well was read within 30 minutes, at 450nm with wavelength correction of 570nm.

Urinary IL-8 – HS kit

Initial steps were as for the standard kit. 100µl assay diluent RD1-85 was added to each well. 100µl standards and samples were pipetted into the wells in duplicate, covered and incubated for 2 hours at room temperature. Six washes with wash buffer were performed. 200µl Human IL-8 HS Conjugate was added to each well, covered and incubated for 1 hour at room temperature. There were a further six washes. 50µl substrate solution was added, covered away from light, and incubated for 1 hour at room temperature. 50µl amplifier solution was added to each well, initiating colour development. 50µl stop solution was added to each well. Optical density was read within 30 minutes, at 492nm with wavelength correction of 650nm.

Validation of IL-8 kits

The IL-8 kits had not been validated for urine by manufacturers. On manufacturer's advice and following their instructions, a spike-recovery assay and a linearity assay were performed to assess accuracy and rule out urine matrix effects from the diluents.

A sample, and a control consisting of calibrator diluent, were spiked with a known concentration, before running the ELISA and assessing the concentration, or recovery, of the spiked substance. Spike recovery range within 80-120% would suggest no matrix effects.

Spiking stock solution was prepared at 20ng/ml. Three aliquot tubes were prepared as follows: (1) unspiked: 1000µl urine sample (2) spiked: 980µl urine sample (3) control: 980µl reagent diluent. 20 µl of spiking stock solution (containing 400pg) was pipetted into each of the "control" and "spiked" samples. Serial dilutions of each, of 1:2, 1:4 and 1:8, were prepared. The ELISA was run as per manufacturer's instructions. Using calibration diluent RD5P diluted 1:5, there was adequate recovery and linearity. This was discussed with the manufacturer who confirmed there was no evidence of matrix effects.

Urinary lactoferrin - kit

Lactoferrin was measured using a commercial ELISA kit, as per Table 6-5, and performed according to manufacturer's instructions.

This was a double antibody sandwich ELISA. Standards were prepared in serial dilutions from 0ng/ml to 100ng/ml. Anti-lactoferrin antibodies had been adsorbed to polystyrene microtitre 96-well plates. 100µl of samples and standards were added and incubated, covered, for 30 minutes. After washing four times to remove unbound proteins, 100µl anti-lactoferrin antibodies conjugated with horseradish peroxidase (HRP) were added and incubated, covered and in the dark, for 30 minutes, forming complexes with previously bound lactoferrin. After washing four times again, 100µl of chromogenic substrate was added (3,3',5,5'-tetramethylbenzidine (TMB)) and incubated in the dark for 10 minutes. The quantity of bound enzyme varied directly with the concentration of lactoferrin. 100µl stop solution was added to each well and optical density (at 450nm) read within 30 minutes.

6.16.7 Normalisation of concentrations using protein (Bradford Assay)

Concentrations calculated using spot samples of urine are typically adjusted to account for variations in dilution. There are many different possible adjustment factors, including volume, specific gravity (SG), osmolality, creatinine and protein. Creatinine, while commonly used, can vary by age, sex, ethnicity, body mass index and time of day, and it is suggested that urinary analytes should be reported both with and without adjustment in multiple regression models (Barr, Wilder et al. 2005). Caution has been urged in the blanket use of creatinine adjustments (Boeniger, Lowry et al. 1993).

A previously described study comparing urinary antimicrobial peptides, including lactoferrin, in urogynaecological patients, used total protein for normalisation (Nienhouse, Gao et al. 2014).

Normalising with protein rather than creatinine has been justified on the basis that protein is less influenced by haematuria (Lokeshwar, Obek et al. 2000). However, a study compared no adjustment, creatinine, osmolality and protein as adjustment methods for urinary IL-6 and IL-8 in 120 patients with haematuria (Reid, Stevenson et al. 2012). There was similar test performance for no adjustment, osmolality and creatinine, while protein concentrations were linked with diagnosis. It was concluded that in haematuria, at least, adjustment was not necessary.

It has been suggested that urine biomarker studies should report both adjusted and unadjusted biomarker concentrations to facilitate detection of appropriate biomarkers and enable comparison between studies (Tang, Toh et al. 2015).

Consequently, I decided to present concentrations of IL6, IL8 and lactoferrin both unadjusted and corrected for protein.

The Bradford Assay for determining protein concentration was used.

This was found to be the most accurate assay method, compared with the modified Lowry assay and a bicinchoninic acid (BCA) assay, against an automated system, in the previously described study of urinary AMPs (Nienhouse, Gao et al. 2014). This is not without controversy. A study of pregnant patients with and without pre-eclampsia and non-pregnant patients, reported that the BCA assay was superior to the Bradford Assay (Mistry,

Bramham et al. 2013) when compared with the amino-acid assay. An earlier study involving pregnant patients found that the BCA assay and Bradford assay performed differently in pre-eclampsia, but did not establish superiority (Waugh, Bell et al. 2005).

The Bradford Assay was carried out using the Thermo Fisher Pierce™ Coomassie (Bradford) Protein Assay Kit (23200), according to the manufacturer's instructions. There were two protocols with different reference ranges which were non-overlapping: standard microplate protocols (working range 100-1500 µg/ml) and micro microplate protocols (working range 1-25 µg/ml). Samples falling between these ranges were diluted and the micro protocol used.

Samples were divided into aliquots on 96-well flat-bottomed plates in duplicate. Standards were known dilutions of bovine serum albumin. When mixed with a protein solution, the acidic coomassie-dye reagent changed colour from brown to blue in proportion to the concentration of protein. Absorbance was read at 595nm.

6.16.8 Fluorescent microscopy analysis of bacterial-associated epithelial cells (clue cells), urothelial cells, and combined stain

The protocol for epifluorescent microscopy for bacteria-associated epithelial cells (used in the Abdominal pain and PTB study) and uroplakin-3-associated epithelial cells (used for a subset of the Abdominal pain study) was originally developed and adapted by Harry Horsley (Horsley, Malone-Lee et al. 2013). A combined approach staining for both clue cells and urothelial cells was developed and adapted by Harry Horsley and Linda Collins (Collins, Sathiananthamoorthy et al. 2020) and used for the MSU-CSU study. Protocols are in full in appendix 13.7 and summarised here.

Labelling: slides were labelled only with the study number to blind the researcher to other findings.

Fixation: 80µl of specimen was added to a Shannon single funnel cuvette assembly containing a pre-labelled glass slide and a Shandon filter card (Fisher Scientific). This was centrifuged in a Shandon Cytospin 2

cytocentrifuge at 800rpm ($\approx 75g$ RCF) for 5 minutes, creating a visible disc of urinary particulate deposited on the slide. This was circumscribed with a hydrophobic barrier pen (ImmEdge pen, Vector Laboratories). Cells were fixed in 4% formaldehyde (Thermo Scientific, Fisher Scientific) in phosphate buffered saline (PBS, Sigma-Aldrich) for 20 minutes. The formaldehyde was aspirated, and the slide washed three times with PBS. All incubations took place at room temperature.

Clue cells: wheat germ agglutinin (WGA) conjugated to Alexa Fluor-488 (Invitrogen) was used to label the cell membrane to aid cellular identification and demarcation, and DAPI (4',6-diamidino-2-phenylindole) (Sigma-Aldrich) was used for fluorescent counterstaining of host and pathogen DNA. After fixation, the fixed cells were incubated for 20 minutes with 0.5 μ g/ml WGA and 1 μ g/ml DAPI in Hank's balanced salt solution minus phenol red (HBSS, Invitrogen). The WGA/DAPI stain was aspirated and the slide washed six times with PBS.

Uroplakin-3: cells were permeabilised with 0.2% Triton-X100 (Sigma-Aldrich) in PBS for 5 minutes and aspirated without washing. They were primed with 10% normal goat serum (Sigma-Aldrich) in PBS for 40 minutes prior to a 1-hour incubation with the primary antibody - a 1:10 dilution of primary anti-uroplakin-III mouse monoclonal antibody (Progen Bioteknik) in 1% normal goat serum. The slide was washed five times with PBS then incubated for 60 minutes with the secondary antibody with counterstain – a solution containing a 1:250 dilution of goat anti-mouse secondary antibody conjugated to Alexa Fluor-555 (Invitrogen), 1 μ g/ μ l of the DNA counterstain 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich), and 1% goat serum in PBS.

Combined approach: The slide was prepared as for clue cells and for uroplakin-3 up to the primary antibody step. After incubation with the primary, it was aspirated and washed three times with 50 μ l of 1% goat serum. The slide was then incubated with 50 μ l secondary antibody stain, containing 1:500 Alexa Fluor-555 goat anti-mouse IgG in 1% goat serum, for 60 minutes. This was aspirated and the slide washed 5 times with 50 μ l of 1% goat serum, aspirating all fluid at the end

Mounting: following staining, the slide was immediately mounted with FluorSave reagent (Calbiochem) and a coverslip fixed with nail varnish. Once dry, slides were stored at 4°C in a light protective box.

Cell counting: cells were counted using 100x magnification on a fluorescent microscope using appropriate fluorescent filters. Alexa fluor 488 (WGA) excites at a wavelength of 495nm and emits at 519nm, giving a green appearance to cell membranes under fluorescence. Alexa fluor 555 (Uroplakin-3) excites at a wavelength of 555nm and emits at 565nm, giving a red appearance to urothelial cell membranes. DAPI excites at 360nm and emits at 460nm giving a blue appearance to nuclei and bacteria.

Images: Images of example cells were captured at 100x magnification with the Infinity 3 Monochrome camera with Infinity Capture, and processed for presentation using Image J.

Counting methods: I counted slides for the Abdominal pain study first, before the slides for the PTB study. For the Abdominal pain study, total epithelial cells, bacteria-associated epithelial cells, and uroplakin-3 associated cells were counted up to a maximum of 100 epithelial cells, to enable proportions to be calculated. 100 was used as an acceptable number to facilitate counting and avoiding errors from counting slides with hundreds of cells; this was standard practice in the research laboratory for all the work produced by this team (Horsley, Malone-Lee et al. 2013). Whilst performing counts for this study, I hypothesised that counting to 20 would give similar results in terms of calculated proportions of clue cells. I discussed this observation with other lab team members, who agreed that this was plausible. I therefore recorded counts for the first 20 epithelial cells, as well as the first 100, to enable a comparison.

The proportion of cells obtained by counting to 20, and by counting to 100, was plotted to assess the relationship (Figure 6-9). There was a strong correlation between the two ($r_s=0.932$, $p<0.001$). Two methods that are designed to measure the same parameter should have good correlation. However, a high correlation may just reflect a widespread sample, and may not imply good agreement between the two methods (van Stralen, Dekker et al. 2012). For

example, if one measurement method records 1, 2, 3, 4, and 5 for 5 participants, and the second method records 10, 20, 30, 40, and 50 for the same participants, correlation would be perfect but there would be no agreement.

The Bland-Altman plot is a graph of the difference between two measurements (y axis) as a function of the average of the two measurements of each sample (x axis) (Bland and Altman 1999). The average of the two measurements is the best estimate of the true value. The bias is the mean difference, and is plotted as a straight horizontal line on the graph, with 95% limits of agreement (± 1.96 SD) plotted as straight horizontal lines as well. The 95% limits of agreement (mean difference ± 1.96 standard deviations of the differences) provide an interval within which 95% of differences between measurements by the two methods are expected to lie. The Bland-Altman plot allows identification of any systematic difference between the measurements (fixed bias) or possible outliers.

A Bland-Altman plot was performed to compare the two methods (Figure 6-10).

Figure 6-9: Scatter plot comparing clue cell counts obtained by counting to 100 epithelial cells versus counting to 20

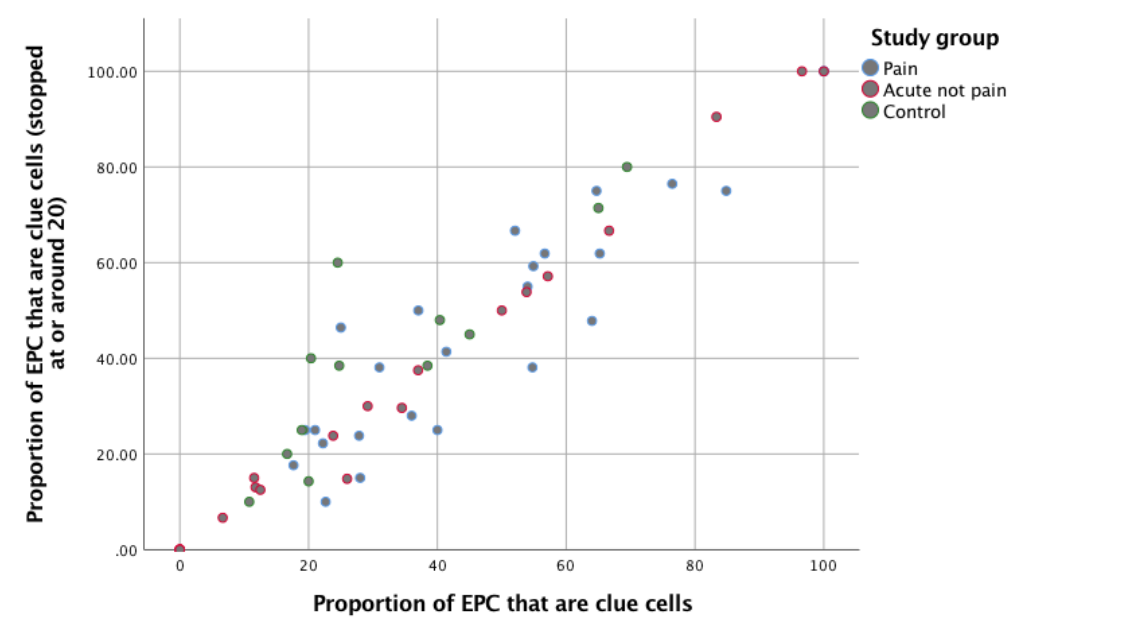
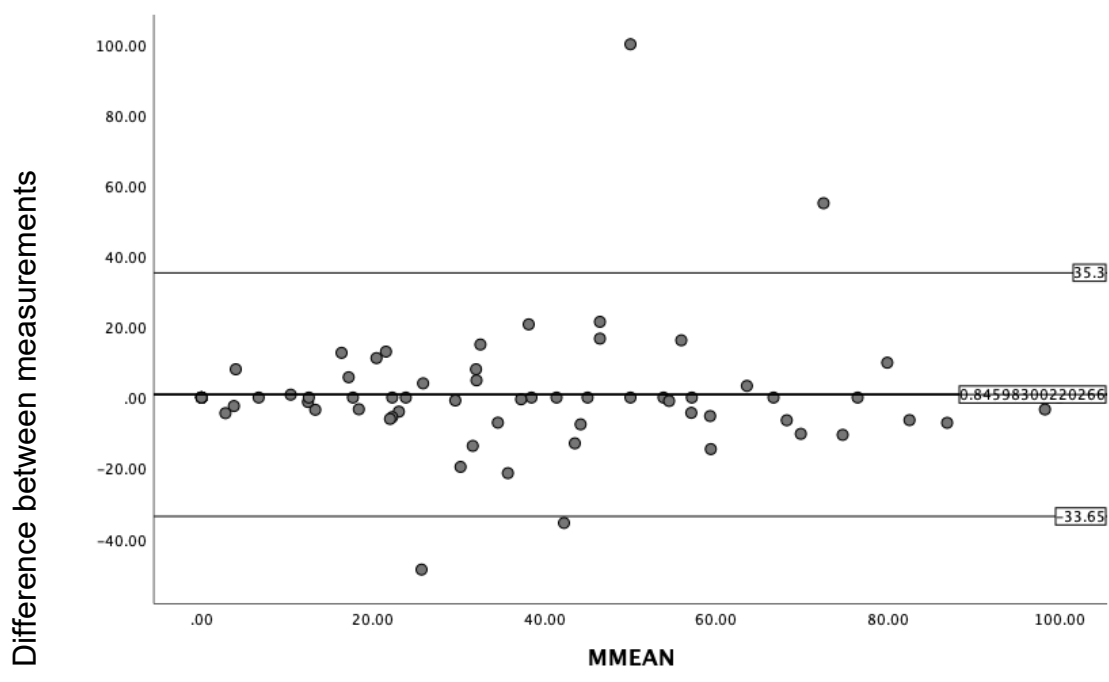


Figure 6-10: Bland-Altman plot comparing clue cell counts obtained by counting to 100 epithelial cells versus counting to 20



Average difference between measurements

The Bland-Altman plot can be interpreted as follows:

- (1) The bias should be close to zero and clinically should not be large enough to be important. Here the bias is close to zero.
- (2) The limits of agreement should be narrow; if too wide then they would be clinically ambiguous. Here the limits of agreement were within a difference of proportion of around 0.34; this clinically means that there may be variation in the proportion within ± 0.34 , however a difference between very few clue cells and nearly all clue cells would be valid with both measurements.
- (3) There is no trend with the differences increasing or decreasing as the average increases
- (4) The variability is consistent across the graph, with the scatter around the bias line not changing as the average gets higher.

This shows that there was good agreement between the two measurements methods. Therefore, for the subsequent PTB study counts, cells were counted up to 20 epithelial cells per slide ('count-to-20' approach) to calculate proportions. Note that all slides in the Abdominal pain study were counted using a count-to-100 approach and those are the results presented in 7.5.13.

For the MSU-CSU study, a different approach to cell counting was used, in that all cells on the slide were counted without a counting limit. This was to permit a comparison of absolute cell counts as well as proportions, consistent with the method used in an MSU-CSU comparison in non-pregnant patients with chronic UTI (Collins, Sathiananthamoorthy et al. 2020).

6.17 Summary of methodological contributions to knowledge

The principal novel development in terms of methods has been to combine a range of standard and alternative methods in a cross-sectional approach to UTI in pregnancy. Some of these methods have not been previously described in pregnancy, such as the Artemis LUTS inventory, sediment culture as a form of enhanced culture, urinary lactoferrin, urinary ATP, epithelial clue cells, and uroplakin-3 staining of epithelial cells. In addition, I have also studied tests that previously have been looked at in isolation in pregnancy, rather than in

comparison with standard culture, such as fresh unspun microscopy, urinary IL-6 and IL-8, and EuroQol quality of life assessment.

The mid-stream urine collection system I developed was intended to recreate the quality of the sampling technique used in the LUTS clinic, adapted to a very different maternity environment and patient care pathway. Participant feedback showed that this was well received by patients.

Although urinary cytokines were assayed using commercial packages, the IL-8 assay had not previously been validated for urine and so I validated it myself, as described.

The cell counting method for bacterial-associated epithelial cells (clue cells), counting to 100, was time intensive, so I developed an alternative method of counting to 20, and validated it, as described.

7 Urinary pathology in pregnant women presenting with abdominal pain, using standard and alternative tests; a prospective case-control study ('Abdominal pain study')

7.1 Introduction

Abdominal pain in pregnancy is a paradigm for exploring UTI in pregnancy, without assumptions about what defines urinary pathology. This presenting symptom would be expected to include some women with urinary pathology. In this study (the "Abdominal pain study") I prospectively compared urinary pathology in women presenting with abdominal pain ("Pain cases"), using both standard and alternative tests, against women presenting with another acute problem ("Acute controls"), and asymptomatic women ("Normal controls"). This allowed me to address two questions: firstly, do women with abdominal pain have urinary pathology not detected by standard tests, and secondly, how do alternative tests perform in pregnancy, compared against standard tests, and clinically relevant outcomes. The latter will be addressed in chapter 10.

7.2 Aims

(3) Compare urinary pathology in pregnant women with (1) abdominal pain ("pain cases"), (2) another acute problem ("acute controls") or (3) no current problem ("normal controls"), using the following standard and alternative methods:

Standard methods

- a. Standard dipstick
- b. Standard microscopy and culture

Alternative methods

- c. Structured lower urinary tract symptoms (LUTS) assessment (using the Artemis LUTS inventory)
- d. Quality of life assessment (using EQ-5S-5D)
- e. Clinician assessment of whether they have a UTI
- f. Patient assessment of whether they have a UTI

- g. Fresh unspun urine microscopy
- h. Urinary ATP measurement
- i. Urinary sediment culture with colony counts and identification to species level of cultured organisms
- j. Urinary cytokines – IL-6, IL-8, lactoferrin
- k. Bacteria-associated epithelial cells ('clue cell') proportions

The null hypothesis was that there would be no difference between groups using each method.

- (4) Measure proportion of uroplakin-positive epithelial cells in a subset of samples

7.3 Methods summary

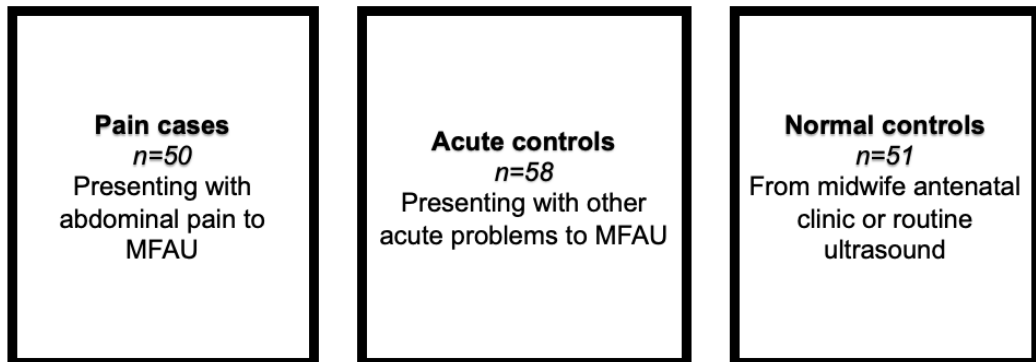
See chapter 6 for complete methods. Specific additional details for the Abdominal pain study are described here.

7.3.1 Study design

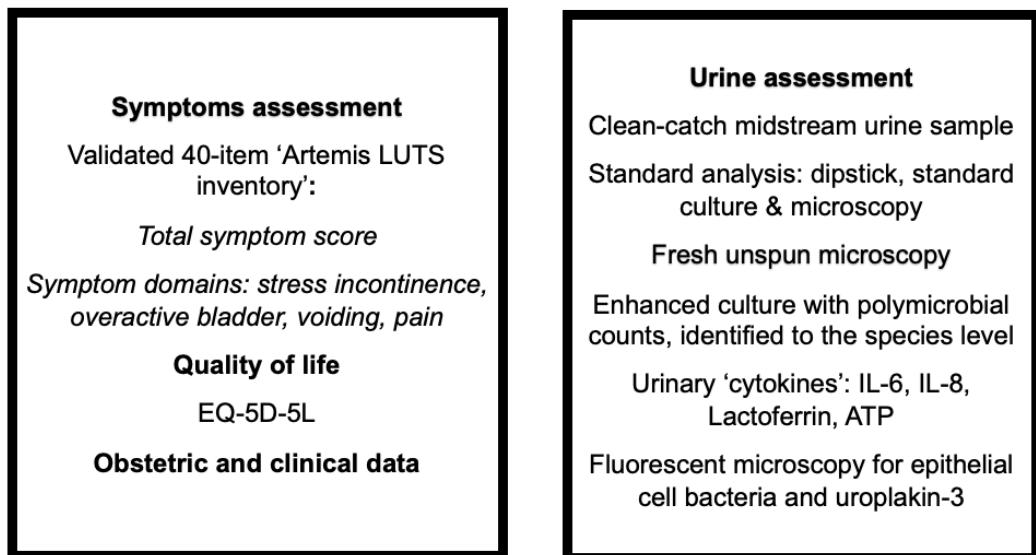
This study (the "Abdominal pain" study) was a prospective case-control study comparing urinary pathology in women presenting with abdominal pain in pregnancy ("Pain cases") against women presenting with other acute problems ("Acute controls") and in women attending for routine antenatal care ("Normal controls"). The study design is summarised in Figure 7-1.

Figure 7-1: Diagram to summarise study design and methods for the Abdominal pain study

Prospective recruitment of women > 14 weeks gestation in three groups:



Between-group comparisons of clinical data and urine tests:



7.3.2 Recruitment

Recruitment occurred in Elizabeth Garrett Anderson Wing, University College London Hospital (UCLH), from 23/10/2014 until 14/10/2015 inclusive.

Pain cases and acute controls were recruited in Maternal Fetal Assessment Unit (MFAU). Patients could attend MFAU from 14 weeks gestation. Normal controls were recruited in midwife antenatal clinics or obstetric ultrasound department.

Eligibility criteria: Any pregnant woman (≥ 18 years of age, ≥ 14 weeks gestation) presenting acutely to MFAU or attending a routine appointment.

Exclusion criteria: Women not booked for their pregnancy at UCLH, to reduce the risk of loss of follow-up of outcome data.

Methods of case ascertainment: Allocation as pain cases or acute controls was based on information patients provided on arrival, not on post-triage categorisations. This was to avoid preconceptions about the nature of pain in urinary pathology and recruit a broad group of patients.

Control selection: There was no exclusion for obstetric or medical complications other than no current problem requiring review in MFAU.

Matching: Acute controls and normal controls were gestation-matched to pain cases, plus or minus two weeks, aiming for one acute control and one normal control for each pain case.

Gestation-matching was chosen as gestation was a possible confounder. Demographic and risk factor data was subsequently compared between the three groups to assess for the risk of recruitment bias.

7.3.3 Data collection

Demographic, obstetric and medical data was collected. Prior to review by a clinician, participants were asked if they thought they had a urinary tract infection currently. Possible responses were no/unlikely, don't know/possible, and yes/likely.

Participants completed the Artemis LUTS questionnaire and EQ-5D-5L quality of life questionnaire. These were labelled only with a study ID for later analysis in batches.

A voided sample was collected using a clean catch mid-stream technique using the study-specific kit. Samples underwent standard analysis with dipstick and were sent to the hospital laboratory for routine microscopy and culture.

Urine samples underwent alternative analysis with point-of-care ATP test, fresh unspun microscopy, enhanced sediment culture, ELISA for IL-6, IL-8 and

lactoferrin, and epifluorescent microscopy for clue cells and, for a subset of samples, for uroplakin-3 staining.

Obstetric and neonatal outcome data and standard microbiological results for the whole pregnancy were collated later. Clinician diagnoses, and documentation of LUTS, were extracted following review of the notes.

7.4 Demographics, medical and obstetric risk factors, clinical outcomes and group characteristics

7.4.1 Demographics

Table 7-1 compares demographic features of participants in the Abdominal pain study.

159 patients were recruited: 50 pain cases, 58 acute controls, and 51 normal controls. Median gestation at recruitment was 191 days (range 98 - 287 days). Median age at recruitment was 33.5 years (range 19.5 – 43.6 years). There was no difference between groups for these parameters.

The three groups differed in the distribution of those from deprived areas ($p=0.029$ by Chi Square). 41/48 (85.4%) of pain cases lived in the most deprived top five deciles, compared with 38/57 (66.7%) of acute controls and 32/51 (62.7%) of normal controls.

There were no between group differences in relation to ethnicity, with 49/147 (33.3%, with 12 missing) of non-white participants ($p=0.260$).

Table 7-1: Demographic features of participants in Abdominal pain study

Demographic feature	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Gestation at recruitment in days (median) (95% CI)	183.0 (157-201)	212.0 (183-229)	180.0 (176-202)	p=0.119 Kruskal-Wallis	190.0 (180-206)
Range	103-270	98-284	103–287		98–287
Age (years) at recruitment (median) (95% CI)	32.2 (29.9-34.6)	34.0 (31.9-35.3)	33.5 (32.3-35.4)	p=0.104 Kruskal-Wallis	33.5 (32.3-34.3)
Range	19.5–43.6	22.6–42.5	23.1–43.5		19.5–43.6
Socio-economic status (Proportion of top 5 most deprived deciles) (%) (95% CI)	41/48 (85.4%) (72.8-92.8)	38/57 (66.7%) (53.7-77.5)	32/51 (62.7%) (49.0-74.7)	$\chi^2=7.03$ p=0.029	111/156 (71.2%) (63.6-77.7)
Ethnicity (Proportion of non-white ethnic groups) (%) (95% CI)	18/47 (38.3%) (25.8-52.6)	20/54 (37.0%) (25.4-50.4)	11/46 (23.9%) (13.1-39.1)		49/147 (33.3%) (25.9-41.6)

Sig.=Significance

Groups were well matched for gestation, age, and ethnicity. The socio-economic differences may be relevant. Poor psychosocial circumstances are linked to increased risk of preterm birth (Snelgrove and Murphy 2015).

Deprivation has been associated with increased preterm birth rates in low risk women, related to low maternal weight and smoking (Taylor-Robinson, Agarwal et al. 2011). Socio-economic factors have also been associated with UTI in pregnancy (Dimetry, El-Tokhy et al. 2007).

7.4.2 Medical and obstetric history and risk factors

Table 7-2 compares clinical features of participants in terms of their medical and obstetric history, and risk factors. Table 7-3 summarises UTI history, and Table 7-4 summarises medical history.

89/159 (56.0%) of participants were nulliparous. Median BMI was 23.5 (95%CI 22.8-24.3). There were no between groups differences in these parameters.

Preterm birth risk factors (excluding multiple pregnancy) were present in 14/159 (8.8%) with no difference between groups ($p=0.247$). These included previous preterm birth/late miscarriage ($n=8$), LLETZ/cone ($n=5$), and uterine anomaly ($n=1$).

A previous (patient-defined) history of UTI was present in 17/159 (10.7%), with no difference between groups ($p=0.139$). Table 7-3 summarises the nature of the UTI; six patients reported recurrent UTI outside pregnancy and one during pregnancy.

Additional medical history was present in 41/159 (25.8%), with no difference between groups ($p=0.358$).

Table 7-4 shows that this included a range of conditions including gynaecological and psychiatric diagnoses as well as medical.

There were 7/157 (4.5%) current smokers, with no difference between groups ($p=0.797$).

There were 5/159 (3.1%) twin pregnancies, with no difference between groups ($p=0.235$).

Table 7-2: Clinical features of participants

Demographic feature	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance	Overall (n=159)
Parity - nulliparous (%) (95% CI)	24/50 (48%) (33.9-62.4)	33/58 (56.9%) (43.3-69.6)	32/51 (62.7%) (48.1-75.5)	$\chi^2=2.259$ p=0.323	89/159 (56.0 %) (47.9-63.8)
Body mass index / kg/m ² Median (95% CI)	24.0 (22.8-25.1)	23.5 (22.6-25.0)	22.7 (21.2-24.0)	Kruskal-Wallis p=0.403	23.5 (22.8-24.3)
Preterm birth risk factors (%) (95% CI)	3/50 (6%) (1.6-17.5)	8/58 (13.8%) (6.6-25.9)	3/51 (5.9%) (1.5-17.2)	$\chi^2=2.830$ p=0.247	14/159 (8.8%) (5.1-14.6)
Nature of PTB risk factors present	PTB=2 LLETZ/cone=1	PTB=4 LLETZ/cone=3 Uterine=1	PTB=2 Cone=1		
Previous UTI history (%) (95% CI)	8/42 (19.1%) (9.1-34.6)	7/58 (12.1%) (5.4-23.9)	2/51 (3.9%) (0.7-14.6)	$\chi^2=4.039$ p=0.139	17/159 (10.7%) (6.5-16.8)
Medical history (%) (95% CI)	11/50 (22%) (12.0-36.3)	13/58 (22.4%) (12.9-35.6)	17/51 (33.3%) (21.1-48.0)	$\chi^2=2.237$ p=0.358	41/159 (25.8%) (19.3-33.4)
Current smoker (%) (95% CI)	3/50 (6%) (1.6-17.5)	2/56 (3.6%) (0.6-13.4)	2/51 (3.9%) (0.7-14.6)	Fisher's Exact=0.542 p=0.797	7/157 (4.5%) (2.0-9.3)
Multiple pregnancy (%) (95% CI)	3/50 (6%) (1.6-17.5)	2/58 (3.4%) (0.6-13.0)	0/51 (0%) (0-8.7)	Fisher's Exact=2.871 p=0.235	5/159 (3.1%) (1.2-7.6)
Spontaneous pregnancy (%) (95% CI)	43/47 (91.5%) (78.7-97.2)	50/57 (87.7%) (75.7-94.5)	49/51 (96.1%) (85.4-99.3)	Fisher's Exact=2.394 p=0.311	142/155 (91.6%) (85.8-95.3)

Table 7-3: Details of previous UTI history

	Pain cases (n=50) 8 cases (<i>including one duplicate</i>)	Acute controls (n=58) 7 cases	Normal controls (n=51) 2 cases
UTI in pregnancy	3	4	1
UTI outside pregnancy	3	0	0
Recurrent UTI in pregnancy	1	0	0
Recurrent UTI outside pregnancy	2	3	1

Table 7-4: Details of medical history

	Pain cases (n=50) 11 cases	Acute controls (n=58) 13 cases	Normal controls (n=51) 17 cases
Medical	5	8	14
Surgical	1	1	2
Gynaecological	1	6	0
Psychiatric	2	0	4
Other	2	0	0

The lack of differences in medical or obstetric risk factors helps between-group comparisons. Groups were heterogeneous in terms of risk factors for preterm birth, sepsis and UTI. This was consistent with the recruiting strategy.

Over 10% women reported previous UTI; this shows the importance of this topic. 7/159 (4.4%) reported recurrent UTI, including one normal control.

An American study of nearly 3 million pregnant women found a rate of recurrent symptomatic UTI of 3.1/1000 based on hospital coding records (Baer 2019). This study may represent a higher rate but is based on self-report so is unlikely to be comparable.

7.4.3 Obstetric and neonatal outcomes

Table 7-5 compares obstetric and neonatal outcomes for the three groups. Delivery details were missing for 3 participants (2 pain cases, 1 normal control).

Median gestational age at delivery was 276 days (95% CI 274-280), with no difference between groups ($p=0.067$). Median number of days from recruitment to delivery was 86 days (95% CI 69-97, no difference, $p=0.220$). There were 5/48 (10.4%) preterm births in the pain group, compared with 3/58 (5.2%) acute controls and 2/50 (4%) normal controls (no difference, $p=0.462$). One preterm birth could not be categorised as spontaneous or iatrogenic. Spontaneous preterm births occurred in 2/47 (4.3%) of pain cases, 2/58 (3.4%) acute controls and 1/50 (2.0%) normal controls (no difference, $p=0.864$).

There were no differences in rates of induction of labour, mode of delivery, infant sex, birthweight, or Apgar scores.

There were no admissions for antenatal sepsis or pyelonephritis. Intrapartum sepsis, or suspicion of sepsis, occurred in 10/154 (6.5%) (no difference, $p=0.402$, causes in Table 7-6). Postpartum sepsis, or suspicion of sepsis, occurred in 15/154 (9.7%) (no difference, $p=0.715$, causes in Table 7-7). Neonatal sepsis, or suspicion of sepsis, occurred in 16/150 (10.7%) (no difference, $p=0.094$, causes in Table 7-8). Babies were admitted to neonatology care in 17/154 cases (11.0%, no difference, $p=0.054$) (see Table 7-9 for details). This includes babies in transitional care, such as those having intravenous antibiotics. Note that Table 7-6 to Table 7-9 are intended simply to illustrate the data, without intention of statistical comparisons, given the small numbers involved.

Table 7-5: Obstetric and neonatal outcomes for Abdominal pain study

Outcome	Pain cases (n=48)	Acute controls (n=58)	Normal controls (n=50)	Significance	Overall (for all three groups, n=156)
Gestation at delivery in days Median (95% CI)	275.0 (271-279)	275.5 (272-282)	280.0 (276-283)	p=0.067 Kruskal- Wallis	276.0 (274-280)
PPROM (%) (95% CI)	0/47 (0%) (0-9.4)	1/58 (1.7%) (0.09-10.5)	1/50 (2%) (0.1-12.0)	Fisher's exact=3.034 p=0.649	2/155 (1.3%) (0.2-5.1)
Preterm birth (%) (95% CI)	5/48 (10.4%) (3.9-23.5)	3/58 (5.2%) (1.3-15.3)	2/50 (4%) (0.7-14.9)	Fisher's exact=1.755 p=0.462	10/156 (6.4%) (3.3-11.8)
Preterm birth – spontaneous (%) (95% CI)	2/47 (4.3%) (0.7-15.7)	2/58 (3.4%) (0.6-13.0)	1/50 (2.0%) (0.1-12.0)	Fisher's exact=0.592 p=0.864	5/155 (3.2%) (1.2-7.8)
Days from recruitment to delivery in days Median (95% CI)	94.0 (63-120)	62.5 (36-97)	94.0 (70-105)	p=0.220 Kruskal-Wallis	86.0 (69-97)
Induction of labour (%) (95% CI)	14/47 (29.8%) (17.8-45.1)	14/58 (24.1%) (14.3-37.5)	14/50 (28%) (16.8-42.7)	$\chi^2=0.4499$ p=0.799	42/155 (27.1%) (20.4-34.9)
Mode of delivery (%)*	SVD 21/47 (44.7%)	SVD 21/58 (36.2%)	SVD 21 /50 (42%)	$\chi^2=5.708$ p=0.224	SVD 63/155 (40.6%)
	Instrumental 5/47 (10.6%)	Instrumental 14/58 (24.1%)	Instrument al 14/50 (28%)		Instrumental 33/155 (21.3%)
	Caesarean 21/47 (44.7%)	Caesarean 23/58 (39.7%)	Caesarean 15/50 (30%)		Caesarean 59/155 (38.1%)
Sex of baby/babies Proportion male (% male) (95% CI)	35/50 (70%) (55.2-81.7)	32/60 (53.3%) (40.1-66.1)	26/50 (52%) (37.6-66.1)	$\chi^2=4.233$ p=0.120	93/160 (58.1%) (50.1-65.8)
Birthweight (g) Median (95% CI)	(n=50) 3400 (3190-3490)	(n=60) 3388 (3220-3550)	(n=50) 3445 (3250- 3540)	Kruskal-Wallis p=0.586*	(n=160) 3400 (3310-3460)
Apgars at 1 minute Median (95% CI)	(n=48) 9 (9-9)	(n=59) 9 (9-9)	(n=49) 9 (9-9)	Kruskal-Wallis p=0.536*	(n=156) 9 (9-9)
Apgars at 5 minutes Median (95% CI)	(n=48) 10 (10-10)	(n=59) 10 (9-10)	(n=49) 10 (9-10)	Kruskal-Wallis p=0.145*	(n=156) 10 (10-10)

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Outcome	Pain cases (n=48)	Acute controls (n=58)	Normal controls (n=50)	Significance	Overall (for all three groups, n=156)
Antenatal sepsis (possible or likely) (%) (95% CI)	0/48 (0%) (0-9.2)	0/58 (0%) (0-7.7)	0/50 (0%) (0-8.9)	n/a	0/156 (0%) (0-3)
Intrapartum sepsis (possible or likely) (%) (95% CI)	3/47 (6.4%) (1.7-18.6)	3/57 (5.3%) (13.7-15.5)	4/50 (8%) (2.6-20.1)	Fisher's Exact=3.769 p=0.402	10/154 (6.5%) (3.3-11.9)
Postnatal sepsis (possible or likely) (%) (95% CI)	4/47 (8.5%) (2.8-21.3)	7/57 (12.3%) (5.5-24.3)	4/50 (8%) (2.6-20.1)	$\chi^2=0.6715$ p=0.715	15/154 (9.7%) (5.7-15.8)
Neonatal sepsis (possible or likely) (%) (95% CI)	2/44 (4.5%) (7.9-16.7)	6/57 (10.5%) (4.4-22.2)	9/49 (18.4%) (9.2-32.5)	$\chi^2=4.467$ p=0.107	17/150 (11.3%) (6.9-17.8)
Admission under neonatal care (%) (95% CI)	3/47 (6.4%) (1.7-18.6)	4/57 (7.0%) (2.3-17.8)	10/50 (20%) (10.5-34.1)	$\chi^2=6.065$ p=0.054	17/154 (11.0%) (6.8-17.3)
Pyelonephritis (95% CI)	0/48 (0%) (0-9.2)	0/58 (0%) (0-7.7)	0/50 (0%) (0-8.9)	n/a	0/156 (0%) (0-3)

Table 7-6: Details of suspected intrapartum sepsis

Pain cases (3/47)	Acute controls (3/57)	Normal controls (4/50)
Maternal pyrexia intrapartum (2)	Maternal pyrexia intrapartum (2)	Maternal pyrexia intrapartum (2)
History of fever at home, presented with maternal and fetal tachycardia	PROM and pyrexia	PROM and pyrexia (2)

PROM = Prelabour rupture of membranes

Table 7-7: Details of suspected postpartum sepsis

Pain cases (4/47)	Acute controls (7/57)	Normal controls (4/50)
Discharged on antibiotics (no cause specified)	Discharged on antibiotics (no cause specified) (2)	Infected wound (2)
Intrapartum + postpartum pyrexia (source not known)	Maternal pyrexia (2)	Endometritis/UTI
Postnatal pyrexia (source not known) (2)	Postnatal pyrexia (source not known)	Intrapartum pyrexia (source not known)
	Infected wound / endometritis	
	UTI (1)	

Table 7-8: Details of suspected neonatal sepsis

	Pain cases (n=44)	Acute controls (n=57)	Normal controls (n=49)
n/a	42	51	40
Prophylaxis owing to risk factors / but negative CRP / septic screen – antibiotics stopped by 48 hours	0	2	7
Chorioamnionitis requiring intubation and NNU		1	
Postnatal signs of sepsis – 5 days antibiotics	2	3	2

Table 7-9: Details of admission under neonatology

The 17 admissions under neonatal care all received antibiotics. Additional issues included the following:

	Pain cases 3/47	Acute controls 4/47	Normal controls 10/50
PROM		1	2
Hypoglycaemia			2
Low Apgars			1
Grunting		2	
Acidosis			1
Hot cot for temperature control		1	

There were no between-group differences for any obstetric or neonatal outcomes. In particular, women presenting with pain were not more likely to have a preterm birth than controls. There were no cases of pyelonephritis documented in the study participants. In the Dutch study of asymptomatic bacteriuria (Kazemier, Koningstein et al. 2015), the rate of pyelonephritis was 29/4243 (0.7%), which is consistent with this study.

This does not imply that pain or acute problems are not associated with certain outcomes; even if they are, treatment may negate that association. The rate of preterm birth is similar to the national rate, which has remained between 7-8% between 2010-2019 (ONS 2020).

Given the small sample size, gross differences in outcomes would not be expected. In the Dutch study of asymptomatic bacteriuria (Kazemier, Koningstein et al. 2015), the outcomes were rare, and it was calculated that large sample sizes would have been required to detect a difference. The current study could however inform future meta-analysis.

7.4.4 Group characteristics

Table 7-10 and Figure 7-2 compare the presenting problems for Pain cases and Acute controls. All pain cases had pain as a presenting problem. In addition, 25/48 (52.1%) reported additional symptoms, the most common being contractions (6/48, 12.5%), vaginal bleeding (6/48, 12.5%), urinary symptoms (4/48, 8.3%) and back pain (4/48, 8.3%). The commonest presenting symptoms for acute controls were reduced fetal movements (17/54, 31.5%), possible ruptured membranes (11/54, 20.4%) and vaginal bleeding (11/54, 20.4%). Urinary symptoms accounted for 2/54 (3.7%). Some participants mentioned more than one symptom at presentation, therefore the total number of presenting symptoms is more than the number of participants.

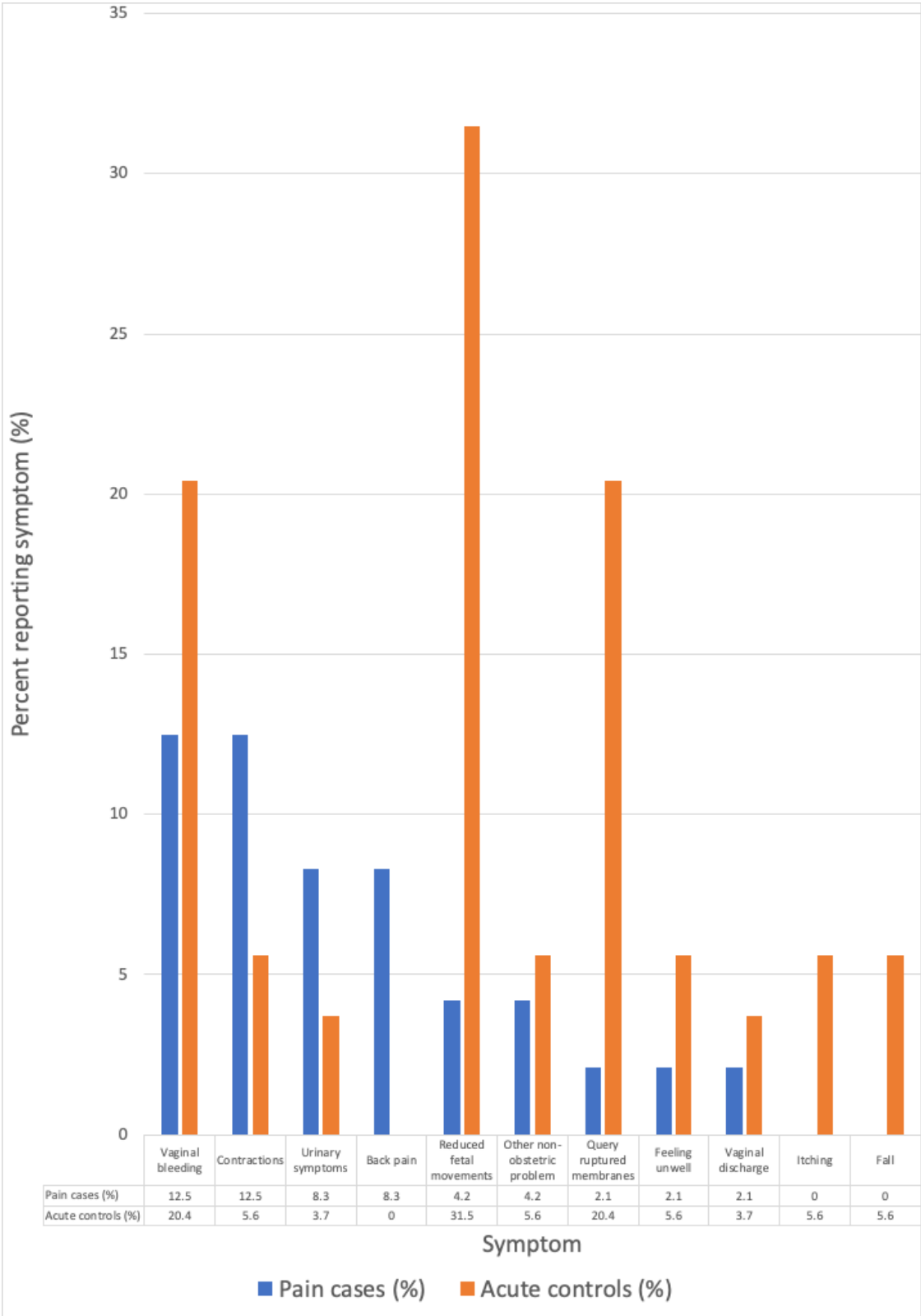
The only symptoms that differed between groups were suspected ruptured membranes, reduced fetal movements (both commoner in acute controls) and back pain (commoner in pain cases). Following a Bonferroni correction only reduced fetal movements remained significant ($p < 0.005$).

Table 7-10: Summary of presenting problems for pain cases and acute controls

	Pain cases (symptoms in addition to pain) (%)	Acute controls (%)	Fisher's Exact
No additional symptoms	25/48 (52.1%)	n/a	n/a
Vaginal bleeding	6/48 (12.5%)	11/54 (20.4%)	p=0.425
Contractions	6/48 (12.5%)	3/54 (5.6%)	p=0.300
Urinary symptoms	4/48 (8.3%)	2/54 (3.7%)	p=0.416
Back pain	4/48 (8.3%)	0/54 (0%)	p=0.046
Reduced fetal movements	2/48 (4.2%)	17/54 (31.5%)	p<0.001
Other non- obstetric problem	2/48 (4.2%)	3/54 (5.6%)	p=1.000
Query ruptured membranes	1/48 (2.1%)	11/54 (20.4%)	p=0.005
Feeling unwell	1/48 (2.1%)	3/54 (5.6%)	p=0.620
Vaginal discharge	1/48 (2.1%)	2/54 (3.7%)	p=1.000
Itching	0/48 (0%)	3/54 (5.6%)	p=0.245
Fall	0/48 (0%)	3/54 (5.6%)	p=0.245

Bonferroni correction for 11 tests: significance level (p) = 0.05/11 = 0.005

Figure 7-2: Bar chart comparing presenting symptoms for pain cases and acute controls (excluding abdominal pain)



There was heterogeneity of presenting symptoms. A small number in both groups mentioned urinary symptoms. Acute controls, in particular, represent a diverse range of potential pathology. The range of presentations is similar to those cited in a recent UK obstetric triage study (Kenyon, Hewison et al. 2017).

7.4.5 Clinician diagnoses

Final clinician diagnosis was derived from review of the notes. Diagnoses were missing from medical records for 8/50 pain cases (16%) and 6/58 (10.3%) acute controls.

Diagnoses were coded thematically, and cases counted. In some cases multiple diagnoses were given; these have been coded separately. Some diagnoses were expressed as a possibility (such as "?UTI") and no distinction is given here between possible, probable and certain diagnoses.

Table 7-11 and Figure 7-3 compare the final diagnoses for pain cases and acute controls. Among pain cases, the commonest diagnoses were UTI (18/42, 42.9%), musculoskeletal or ligamentous (15/42, 35.7%) and 'nil abnormality / reassure' (15/42, 35.7%). Other diagnoses included heartburn (2/42, 4.8%), and labour, bleeding (no cause identified), Braxton Hicks, ruptured membranes and show, all in 1/42 cases (2.4%).

Among acute controls, the commonest diagnoses were 'nil abnormality / reassure' in 32/52 (61.5%) and bleeding (cause identified) in 5/52 (9.6%). Other diagnoses included obstetric cholestasis and ruptured membranes in 3/52 (5.8%) cases each; UTI, bleeding (no cause identified) and thrush in 2/52 (3.8%) cases each; and migraine, dehydration, labour, anaemia and Bell's palsy in 1/52 (1.9%) cases each.

There were significant differences between the two groups in terms of musculoskeletal / ligamentous ($p < 0.001$) and UTI ($p < 0.001$). All other diagnostic labels were not statistically different between the groups once a Bonferroni correction was applied ($p < 0.0029$).

Table 7-11: Summary of final clinician diagnoses for each acute group

	Pain cases (n=42)	Acute controls (n=52)	Fisher's Exact*
UTI	18/42 (42.9%)	2/52 (3.8%)	p<0.001
Nil abnormality, reassure	15/42 (35.7%)	32/52 (61.5%)	p=0.022
Musculoskeletal / ligamentous	15/42 (35.7%)	0/52 (0%)	p<0.001
Heartburn	2/42 (4.8%)	0/52 (0%)	p=0.197
Ruptured membranes	1/42 (2.4%)	3/52 (5.8%)	p=0.626
Bleeding (no cause identified)	1/42 (2.4%)	2/52 (3.8%)	p=1.000
In labour	1/42 (2.4%)	1/52 (1.9%)	p=1.000
Braxton Hicks	1/42 (2.4%)	0/52 (0%)	p=0.447
Fibroid pain	1/42 (2.4%)	0/52 (0%)	p=0.447
Show	1/42 (2.4%)	0/52 (0%)	p=0.447
Bleeding (cause identified)	0/42 (0%)	5/52 (9.6%)	p=0.063
Obstetric cholestasis	0/42 (0%)	3/52 (5.8%)	p=0.251
Thrush	0/42 (0%)	2/52 (3.8%)	p=0.500
Migraine	0/42 (0%)	1/52 (1.9%)	p=1.000
Dehydration	0/42 (0%)	1/52 (1.9%)	p=1.000
Anaemia	0/42 (0%)	1/52 (1.9%)	p=1.000
Bell's palsy	0/42 (0%)	1/52 (1.9%)	p=1.000

**Bonferroni correction – 17 comparisons therefore significant at p<0.0029*

Figure 7-3: Bar chart comparing final clinician diagnoses for pain cases and acute controls

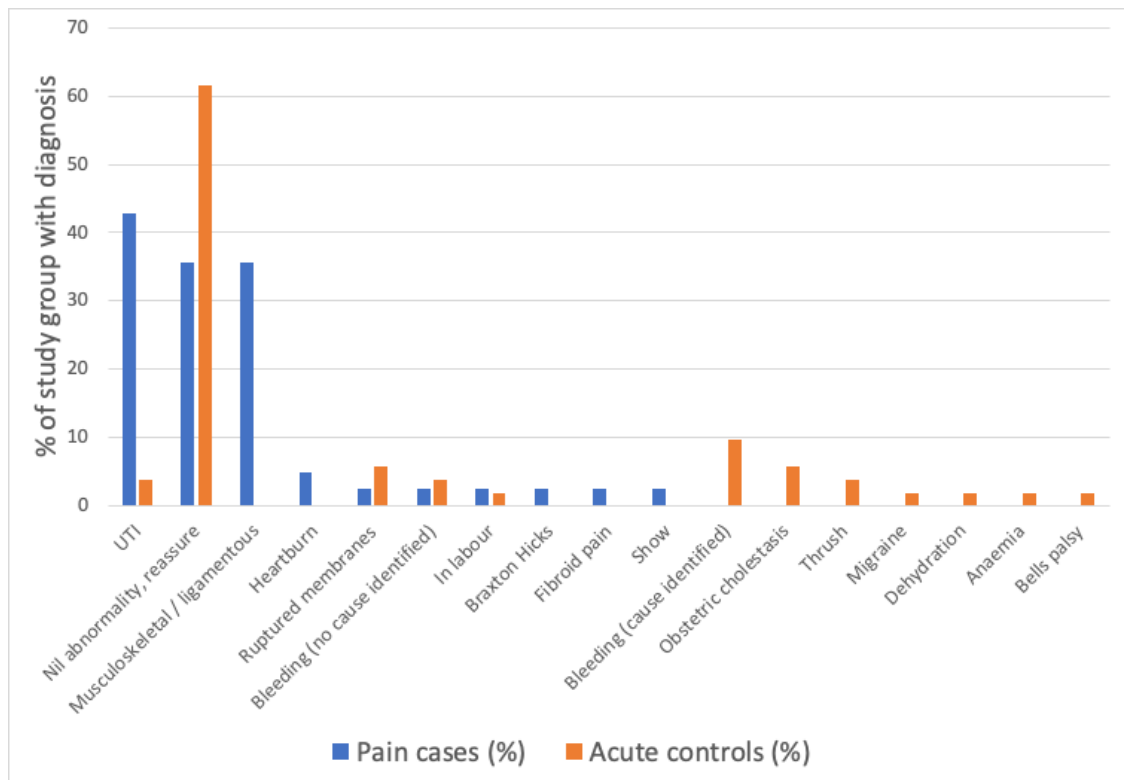


Table 7-12 compares prescription of antibiotics for Pain cases and Acute controls. Of those for whom data was available, 14/43 (32.6%) pain cases were prescribed oral antibiotics, versus 1/52 (1.9%) acute controls. A further 4/95 (4.2%) were advised for antibiotics if MSU culture was positive. 5/95 (5.2%) had other plans for antimicrobials, which included PPRM management with oral erythromycin, two with antifungal treatment, antibiotics for SROM with GBS, and one who was already on antibiotics for suspected UTI but had been advised to increase the dose. The prescription of antimicrobials at time of review did differ between groups ($p < 0.001$).

Table 7-12: Prescribed antimicrobials at time of presentation

	Pain cases (n=43)	Acute controls (n=52)	Totals (n=95)	Fisher's Exact
Prescribed oral antibiotics at time of review	14/43 (32.6%)	1/52 (1.9%)	15/95 (15.8%)	p<0.001
Plan for antibiotics if culture positive	2/43 (4.7%)	2/52 (3.8%)	4/95 (4.2%)	
No antibiotics prescribed	26/43 (60.5%)	45/52 (86.5%)	71/95 (74.7%)	
Other	1/43 (2.3%)	4/52 (7.7%)	5/95 (5.2%)	
Totals	43	52	95	

Similar to presenting symptoms, there was heterogeneity of diagnoses, with a proportion in both groups found not to have pathology.

There were between-group differences, with musculoskeletal / ligamentous accounting for 15/42 (35.7%) and UTI accounting for 18/42 (42.9%) diagnoses in the pain group compared with 0/52 (0%) and 2/52 (3.8%) respectively in the acute controls. These cannot be added as diagnostic categories are not mutually exclusive.

This would be consistent with the original hypothesis that women with abdominal pain in pregnancy may have UTI and that a group of women with abdominal pain would be a way to recruit women with UTI or subclinical UTI.

It is not clear what diagnostic criteria were applied in the diagnosis of musculoskeletal / ligamentous causes of pain. The rate of non-diagnosed pain was similar to a recent Finnish study (Fagerstrom et al 2017). In this,

diagnoses for abdominal pain were compared over a 26-year period (1986, 2003, 2012). It was found that 31-37% of presentations had a final diagnosis of non-specific abdominal pain, a rate which was fairly stable over that time.

In a later subsection (0) I describe the documentation of urinary symptoms in study patients.

There was a different pattern of prescribing antimicrobials between the two groups. In particular, 14/43 (32.6%) pain cases were prescribed antimicrobials at time of presentation, compared with 1/52 (1.9%) acute controls.

The differential use of antibiotics adds complexity to the use of end-of-pregnancy outcomes to compare the groups, as differences or lack of differences may be due to infectious pathology or this may be mitigated by use of antibiotics.

It was not possible to assign diagnoses to all patients. This was due to missing records. Data about diagnosis were missing for 8/50 (16%) pain cases and 6/58 (10.3%) acute controls; data about antimicrobial prescribing were missing for 7/50 (14%) and 6/58 (10.3%) respectively.

7.4.6 Patient diagnosis of UTI

Prior to clinician review, participants were asked if they thought they had a UTI (Table 7-13). Of those for whom data are available, 12/36 (33.3%) pain cases thought they might have, or did have, a UTI, compared with 2/50 (4%) acute controls ($p < 0.001$).

Table 7-14 shows that of the 4 patients who thought they had a UTI, 3/4 (75%) were diagnosed with UTI by the clinician; of the 67 who did not think they had a UTI, 63/67 (94.0%) were not diagnosed with UTI.

Table 7-13: Does the participant think they have a UTI?

Patient belief about UTI	Pain cases (n=36)	Acute controls (n=50)	Total (n=86)	Fisher's Exact
Unlikely UTI	24/36 (66.7%)	48/50 (96%)	72/86 (83.7%)	p<0.001
Possible UTI	9/36 (25%)	1/50 (2%)	10/86 (11.6%)	
Likely UTI	3/36 (8.3%)	1/50 (2%)	4/86 (4.7%)	

Table 7-14: Comparison of clinician diagnosis of UTI versus patient diagnosis of UTI

		Patient diagnosis of UTI			Totals
		UTI	Possible UTI	No UTI	
Clinician diagnosis of UTI	UTI	3	7	4	14
	No UTI	1	2	63	66
Totals		4	9	67	80

Fisher's Exact p<0.001

It could be assumed that clinician and patient assessment are liable to vary widely. However, it is worth questioning this assumption. Respect for patient experience of UTI symptoms, despite negative cultures, has led to research advances in chronic UTI (Swamy, Barcella et al. 2018). Clinician gestalt, or holistic clinical judgement based on knowledge and experience, is valuable, and where a diagnostic test disagrees, it is worth critically examining not just the clinical diagnosis, but also the application of the diagnostic test (Cervellin, Borghi et al. 2014). That is to say, both patient and clinician impressions may be valuable in a diagnostic pathway, regardless of the diagnostic test result.

In this study there was relatively good agreement between patients and clinicians.

A weakness of this study was that these clinician and patient assessments were not made for normal controls, which would have acted as a baseline. In addition, the proportion of participants with missing data means that the reliability of this data can be questioned. This is a problem particularly associated with a paper-based notes system where notes were missing from the files and prescribing data was not systematically filed.

In conclusion, women presenting with pain were more likely to be diagnosed with UTI, and given antibiotics, than women presenting with other acute problems. They were more likely to believe they had a UTI themselves. These clinician and patient beliefs will be examined again later (7.6) once other findings have been presented.

Next, it is necessary to compare prevalence of urinary pathology in the three groups using standard and alternative tests.

7.5 Comparison of the prevalence of urinary pathology using standard and alternative methods

7.5.1 Introduction

In this section I present data comparing the prevalence of urinary pathology in pregnant women with (1) abdominal pain, (2) another acute problem or (3) no current problem, using standard and alternative methods.

7.5.2 Structured assessment of lower urinary tract symptoms (LUTS)

7.5.2.1 Methods

Participants were asked to complete the Artemis LUTS inventory. It was not part of their routine antenatal clinical assessment. All participants filled in the questionnaire although not all completed all questions.

7.5.2.2 Results

Table 7-15 describes LUTS scores for each study group. In summary there were small between-group differences in 24-hour frequency, and differences in pain score appropriate to a group complaining of pain.

151/159 (95.0%) participants reported at least one urinary symptom with no between-group differences ($p=0.282$). Figure 7-4 shows the distribution of total LUTS scores, which was wide. There was no significant difference between groups ($p=0.074$).

Median daytime frequency was 7.5 (95% CI 6.5-7.5, range 2.5-19.5), with a small significant difference between groups (Kruskal Wallis, $p=0.024$).

Median nocturia was 1.5 episodes (IQR 2.0, range 0.5-6.5), with a significant difference between groups (Kruskal Wallis, $p=0.015$). For pain cases median nocturia was higher (2.5, 95% CI 2.5-3.5) than acute controls (1.5, 95% CI 1.5-2.5) and normal controls (1.5, 95% CI 1.5-2.5).

Daytime incontinence was reported in 58/154 (37.7%) participants, with no difference between groups ($p=0.998$). Night incontinence was noted in 21/154 (13.6%) participants, with no difference between groups ($p=0.328$).

LUTS scores were sub-divided into four groups of symptoms. 68/159 (42.8%) reported any stress incontinence symptoms; the median score overall was 0/7 (95% CI 0-1, range 0-5). 110/159 (69.2%) reported any overactive bladder symptoms; the median score overall was 2/12 (95% CI 1-2, range 0-8). 126/159 (79.2%) reported voiding symptoms; the median score overall was 2/8 (95% CI 2-2, range 0-8). There was no difference between groups for these.

35/50 (70%) of pain cases reported any pain symptoms versus 28/58 (48.3%) acute controls and 22/51 (43.1%) normal controls ($p=0.016$). The median score for pain symptoms was significantly different between groups ($p=0.003$). Median pain score for pain cases was 3/13 (95% CI 1-4, range 0-8) which was significantly different to acute controls (median 0, 95% CI 0-2, range 0-9) and normal controls (median 0, 95% CI 0-2, range 0-9).

Table 7-15: Structured lower urinary tract symptoms (LUTS) assessment using Artemis LUTS inventory

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Total LUTS (out of 40)	8	5	5		6
Median	(6-11)	(4-7)	(4-6)	p=0.074	(5-7)
(95% CI)	(0-21)	(0-22)	(0-24)		(0-24)
(range)					
Any LUTS (%)	47/50 (94%)	57/58 (98.3%)	47/51 (92.2%)	p=0.282	151/159 (95.0%)
(95% CI)	(82.5-98.4)	(90.0-99.9)	(80.3-97.5)		(90.0-97.6)
Frequency	7.5	6.5	7.5	p=0.024	7.5
Median	(6.5-10.5)	(5.5-7.5)	(6.5-9.5)		(6.5-7.5)
(95% CI)	(4.5-19.5)	(2.5-16.5)	(3.5-15.5)		(2.5-19.5)
(range)					
Nocturia	(n=48)	1.5	1.5		(n=157)
Median	2.5	(1.5-2.5)	(1.5-2.5)	p=0.015	1.5
(95% CI)	(2.5-3.5)	(0.5-6.5)	(0.5-5.5)		(1.5-2.5)
(range)	(0.5-6.5)				(0.5-6.5)
Any daytime incontinence	(n=48)	(n=56)	(n=50)		(n=154)
(%)	18/48 (37.5%)	21/56 (37.5%)	19/50 (38%)	p=0.998	58/154 (37.7%)
(95% CI)	(24.3-52.7)	(25.2-51.5)	(25-52.8)		(30.1-45.9)
Any night incontinence	(n=48)	(n=56)	(n=50)		(n=154)
(%)	7/48 (14.6%)	10/56 (17.9%)	4/50 (8%)	p=0.328	21/154 (13.6%)
(95% CI)	(6.5-28.4)	(9.3-30.9)	(2.6-20.1)		(8.8-20.3)
Stress incontinence. score (out of 7)	0	0	0	p=0.615	0
Median	(0-1)	(0-1)	(0-1)		(0-1)
(95% CI)	(0-5)	(0-4)	(0-3)		(0-5)
(range)					
Any stress incontinence (%)	18/50 (36%)	27/58 (46.6%)	23/51 (45.1%)	p=0.499	68/159 (42.8%)
(95% CI)	(23.3-50.9)	(33.5-60.0)	(31.4-59.6)		(35.0-50.9)
Overactive bladder score (out of 12)	2	2	1	p=0.823	2
Median	(1-3)	(1-3)	(1-3)		(1-2)
(95% CI)	(0-7)	(0-8)	(0-7)		(0-8)
(range)					

Continued on next page

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Any overactive bladder symptoms (%) (95% CI)	34/50 (68%) (53.2-80.1)	43/58 (74.1%) (60.7-84.4)	33/51 (64.7%) (50.0-77.2)	p=0.560	110/159 (69.2%) (61.3-76.1)
Voiding score (out of 8) Median (95% CI) (range)	2.5 (2-3) (0-7)	2 (1-2) (0-5)	2 (1-2) (0-8)	p=0.084	2 (2-2) (0-8)
Any voiding symptoms (%) (95% CI)	41/50 (82%) (68.1-91.0)	46/58 (79.3%) (66.3-88.4)	39/51 (76.5%) (62.2-86.8)	p=0.805	126/159 (79.2%) (72.0-85.1)
Pain score (out of 13) Median (95% CI) (range)	3 (1-4) (0-8)	0 (0-2) (0-9)	0 (0-2) (0-9)	p=0.003*	1 (0-2) (0-9)
Any pain symptoms (%) (95% CI)	35/50 (70%) (55.2-81.7)	28/58 (48.3%) (35.1-61.7)	22/51 (43.1%) (29.6-57.7)	p=0.016	85/159 (53.5%) (45.4-61.3)

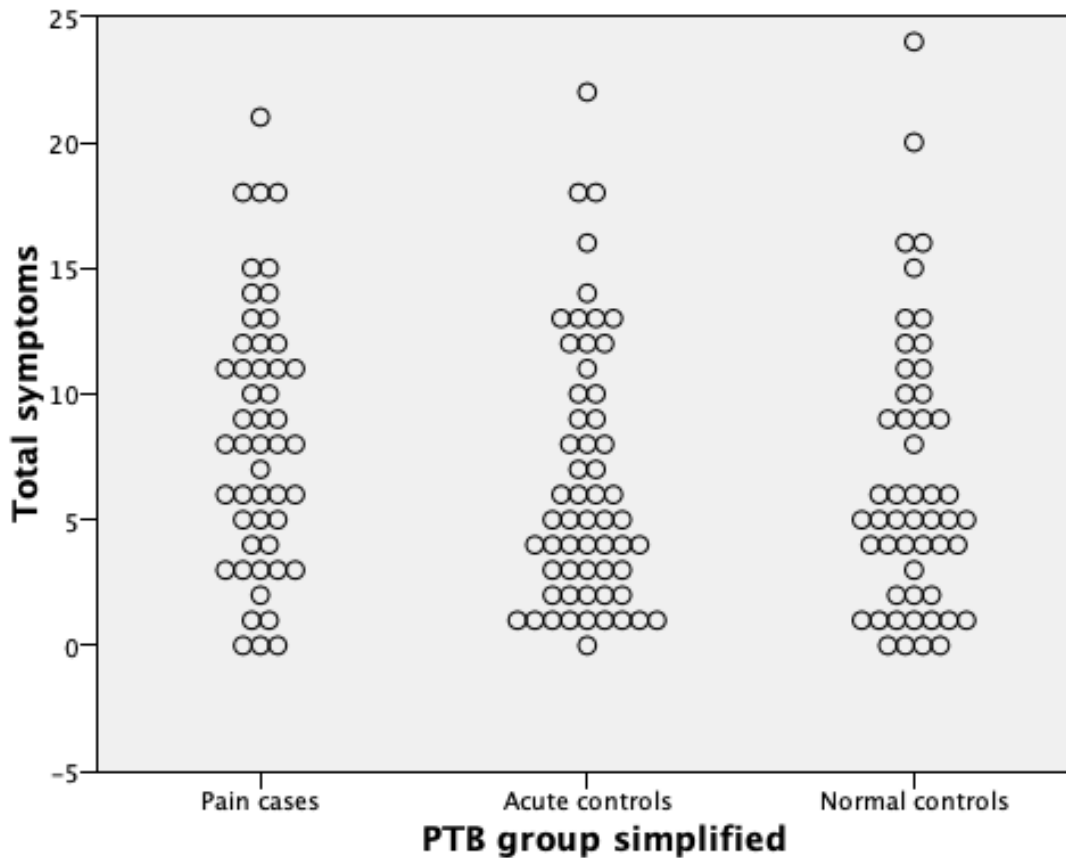
Figure 7-4: Dot plot showing total LUTS scores according to study group

Figure 7-5 compares symptoms reported by participants in each group. The commonest symptoms reported, across all groups, were urinary urgency (75/158, 47.5%), incomplete emptying (72/155, 46.5%), bladder pain on filling (70/157, 44.6%), bladder pain relieved by voiding (61/144, 42.4%), double voiding (65/156, 41.7%), waking rising urgency (61/156, 39.1%), and cough-sneeze incontinence (60/159, 37.7%).

The only differences between groups were expected symptoms of bladder pain on filling ($p=0.046$), bladder or suprapubic pain ($p=0.039$), left or right iliac fossa pain ($p=0.005$), and pain radiating to legs ($p=0.037$), all of which were more frequent in pain cases. Using a Bonferroni correction, none of these remain significant ($p<0.0013$).

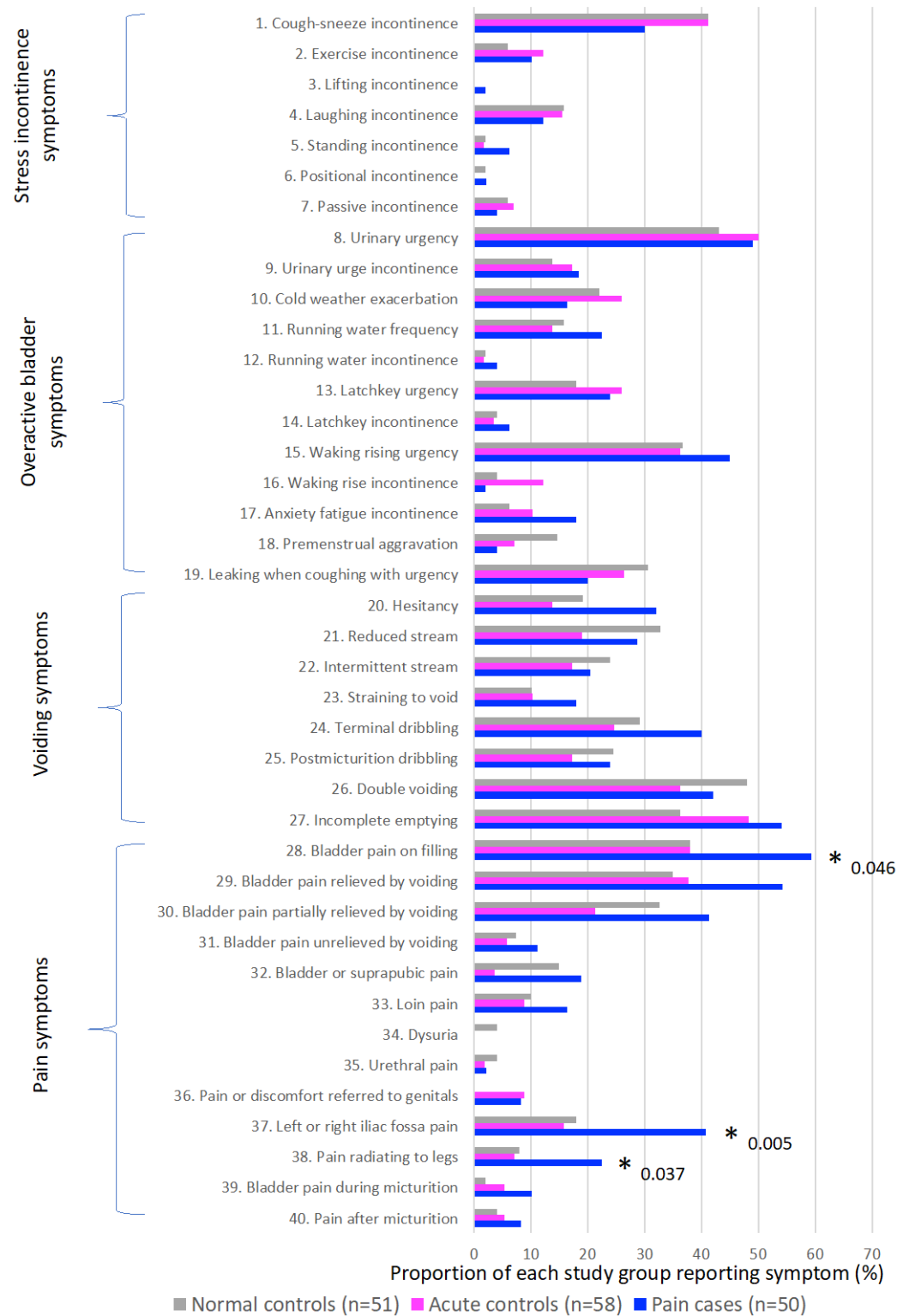
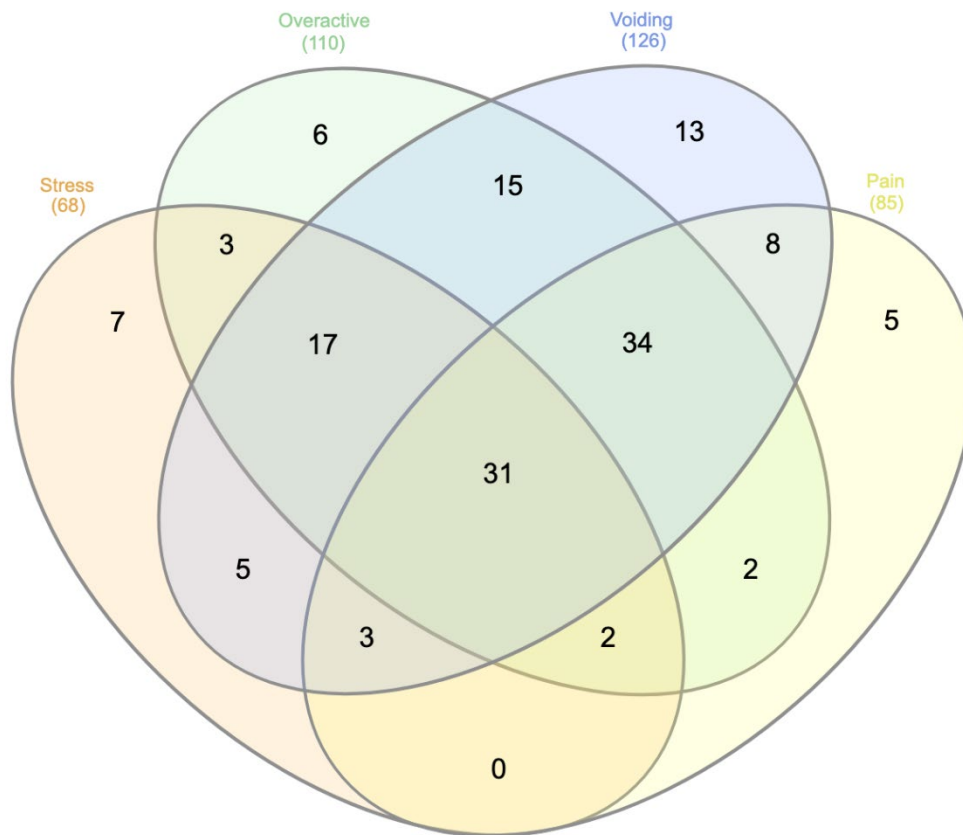
Figure 7-5: Detailed LUTS compared across study groups

Figure 7-6 is a Venn diagram of combinations of the four main LUTS categories. 151/159 (95.0%) participants experienced any LUTS. The commonest combination was overactive, voiding and pain (34/159, 21.4%), followed by all four symptom groups (31/159, 19.5%). Most participants had a combination of symptom groups (120/159, 75.5%) rather than single symptom groups (31/159, 19.5%), demonstrating considerable overlap between the types of symptoms. This contrasts with the chronic LUTS study (Khasriya, Barcella et al. 2018) using the same questionnaire, in which symptom groups occurred in combination, but only 3% reported all four symptom groups and 15.4% reported three of the four symptom groups.

Figure 7-6: Venn diagram showing the relationships between groups of symptoms



(Venn diagram adapted from (Heberle, Meirelles et al. 2015))

7.5.2.3 Discussion

Overall, the prevalence of urinary pathology using the LUTS questionnaire was not different in the three groups. 151/159 (95.0%) reported any LUTS and the median LUTS score was 6/40. Around a third of patients reported any daytime incontinence, and more than one in ten reported night incontinence. This was not an asymptomatic cohort.

The dot plot in Figure 7-4 showed marked heterogeneity in responses in participants in all three groups. This is one of the most striking findings. There is a proportion of women, whether low risk and apparently asymptomatic, or presenting with acute symptoms, who reported a substantial number of LUTS on direct questioning.

Why are high rates of LUTS reported in a group of women who are apparently asymptomatic? LUTS being prevalent is consistent with research described above (Chaliha, Kalia et al. 1999) which reported a peak in LUTS during pregnancy compared with before and after birth.

Most women did not report any stress incontinence symptoms. This contrasts with a previous study reporting both stress and urge incontinence in pregnancy, increasing towards late gestation (Viktrup 1993).

The lack of statistical difference between the study groups is noteworthy. The normal controls, and the acute controls, of whom only 2/54 (3.7%) presented with urinary symptoms, reported similar total LUTS scores as women presenting with pain, of whom 4/48 (8.3%) also presented with urinary symptoms. Women with abdominal pain were not more likely to report urinary symptoms than acute or normal controls.

The only differences between groups were for daytime frequency and nocturia, probably not clinically significant differences, and total pain symptoms, which is consistent with recruitment criteria.

More than 40% of controls reported some pain symptoms. This needs to be explored alongside microbiological and host response data to be understood. In particular, it may have implications for studies of asymptomatic bacteriuria, if apparently normal controls report LUTS.

Only 2/159 (1.3%) patients reported dysuria. This is similar to a 1994 study (MacDermott 1994) which found dysuria in 3/148 women presenting acutely in pregnancy and who had investigations for UTI. This contrasts with a 2020 study of women attending the Emergency Department in the first trimester, which reported dysuria in 26/198 (13.1%) participants (Gendel and Nolan 2020). This cohort is unusual as 46/198 (23.2%) had positive cultures by standard criteria, which is higher than would be expected for asymptomatic bacteriuria and reflects that this was women presenting with acute and varying complaints. Given the prominent role that dysuria has in traditional culture-based diagnosis of acute UTI outside pregnancy (Bent, Nallamotheu et al. 2002), it is interesting that dysuria in this study was one of the least common symptoms, on the background of symptoms in all groups.

My data were compared with historical data collected whilst validating this questionnaire in patients with chronic LUTS, using pyuria as a marker of disease severity (Khasriya, Barcella et al. 2017). In the cohort of 2050 patients with a mean age of 52, the mean number of symptoms was 10 (SD=5.9, median = 9), in controls it was zero.

In another study using the questionnaire (Gill, Horsley et al. 2015), comparing 75 healthy controls with 314 patients with chronic LUTS, the median score was 0 for the controls and 3.5 (IQR 1-6, mean=5) for the patients. In this study controls were excluded if they reported any LUTS.

The median score for the pregnant patients in this study, including controls, was between these two studies (median 6 (95% CI 5-7, range 0 – 24). Note that both these other studies refer to mixed genders and ages.

The LUTS questionnaire is self-completed by the participant; most patients took approximately 5-10 minutes to complete it. It does not include any questions about how bothersome the symptoms are, a deliberate exclusion when the questionnaire was designed, to make it more objective (Khasriya, Barcella et al. 2017). However, this means interpreting one-off scores is harder, for example it is not clear whether a patient reporting 20 symptoms is more bothered or concerned than a patient reporting one symptom. It may be that as a snapshot analysis it is less useful, and has more value as a

longitudinal appraisal of symptoms. A questionnaire developed for patients with chronic LUTS may be more meaningful to them than to pregnant patients with other presenting problems, who may not have previously thought about their urinary symptoms.

I conjecture that, owing to the dogma that positive cultures in obstetrics must be treated, the likelihood of asking in detail about LUTS is less. If specific LUTS are shown to have predictive value in UTI, or relevant obstetric outcomes, this may alter. Urinary symptoms should be appraised in the context of other data, including microbiological factors and outcome data.

In conclusion, the groups did not differ in relation to their symptoms. The symptoms experience was widely dispersed in all groups. Without further work we know not whether this reflects normal physiology or a high prevalence of urinary pathology in pregnancy. It calls into question the credibility of asymptomatic bacteriuria.

7.5.3 Quality of life assessment

7.5.3.1 Methods

Participants completed the EQ-5D-5L questionnaire. Patients self-rated on five index scales, which were then used to calculate an index value. They also used a visual analogue scale (VAS) to rate "Your health today" between 0-100%. These were completed by 152/159 (95.6%) participants.

7.5.3.2 Results

People presenting with pain reported lower quality of life. Table 7-16 summarises the results. Median EQ-5D-5L index value differed between groups ($p=0.032$). Median index value for pain cases was 0.74 (95% CI 0.71-0.84) compared with 0.84 (95% CI 0.84-1.00) for normal controls, with acute controls in between. Median VAS score also differed between groups ($p<0.001$). Median pain cases score was 70% (95% CI 60-80) compared with 88.5% (95% CI 80-90) for normal controls, with acute controls in between.

Table 7-16: Quality of life assessment (EQ-5D-5L) for Abdominal pain study

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall
Index value (n)	(n=49)	(n=55)	(n=47)	$p=0.032^*$ Kruskal- Wallis	(n=151)
Median	0.74	0.84	0.84		0.84
(95% CI)	(0.71-0.84)	(0.84-1.00)	(0.84-1.00)		(0.80-0.84)
(range)	(0.14 – 1.0)	(-0.003 – 1.0)	(-0.126 – 1.0)		(-0.126-1.0)
"Your health today" (VAS) (n)	(n=47)	(n=57)	(n=48)	$p<0.001^*$ Kruskal- Wallis	(n=152)
Median	70.0	80.0	88.5		80.0
(95% CI)	(60-80)	(70-85)	(80-90)		(75-80)
(range)	(20 – 99)	(30 – 100)	(20 – 100)		(20-100)

7.5.3.3 Discussion

Pain cases had lower scores for both measures than normal controls.

The minimally important difference in health-related quality of life, using EQ-5D-5L, has been estimated in England as 0.037 ± 0.008 (McClure, Sayah et al. 2017). Differences of 0.1 between pain cases and acute or normal controls are likely to be clinically meaningful.

EQ-5D-5L has been studied in postnatal women in a low-resource setting and found to be simple to administer and useful for assessing quality of life (Kohler, Sidney Annerstedt et al. 2018). Another large study found it to be useful in comparing quality of life according to mode of delivery, one year post-partum (Petrou, Kim et al. 2017).

The lower quality of life score for pain is clinically relevant. Pain without a serious obstetric cause such as abruption or threatened preterm labour may be dismissed as unimportant. However, if the pain is associated with reduced quality of life then this is a concern. Abdominal pain is a common problem in pregnancy. In a large audit used to develop a maternity triage system in a UK tertiary hospital, 11% of presentations to maternity triage were for abdominal pain (Kenyon, Hewison et al. 2017). The quality of life burden of pain in pregnancy is therefore likely to be high.

The EQ-5D-5L tool was filled in by fewer participants than the LUTS questionnaire (152 versus 159). Some participants may have felt it was not relevant or did not understand the questions.

In conclusion, pain cases reported lower quality of life than normal controls.

7.5.4 Documentation about urinary symptoms

To aid in understanding clinical reasoning regarding LUTS, clinical notes were reviewed, and documentation of UTI symptoms was thematically analysed (Table 7-17).

UTI symptoms were documented in 20/34 (58.8%) of pain cases compared with 11/49 (22.4%) of acute controls for whom data were available ($p=0.001$, χ^2). Where symptoms were documented, LUTS were present in 7/20 (35%) pain cases, and 3/11 (27.3%) acute controls (no difference between groups, $p=0.66$). Documentation was mostly brief, particularly for negative findings (for example, "no urinary symptoms").

UTI symptoms were documented for 11/16 (68.8%) of those who were diagnosed with UTI compared with 18/67 (26.9%) who were not diagnosed with UTI ($p=0.003$, χ^2 , Table 7-18).

Table 7-17: Documentation about urinary symptoms in history by clinician

Pain cases	Acute controls	Significance
(n=34) 20/34 (58.5%)	(n=49) 11/49 (22.4%)	$p=0.001$
Symptoms present (7) <i>All refer to frequency and/or dysuria Single mention of urgency & foul-smelling urine</i>	Symptoms present (3) <i>All refer to frequency and/or dysuria Single mention of abdominal pain</i>	
No symptoms (13) <i>Most 'no other symptoms' or 'no urinary symptoms' Single mention of no dysuria / no frequency</i>	No symptoms (8) <i>Most 'no other symptoms' or 'no urinary symptoms'</i>	
Not documented (14)	Not documented (38)	

Table 7-18: Documentation about urinary symptoms in those diagnosed with UTI

	Diagnosed as UTI or possible UTI	Not diagnosed as UTI	Totals	Significance
Any documentation about urinary symptoms? (%)	11/16 (68.8%)	18/67 (26.9%)	29/83 (34.9%)	$\chi^2=9.967$ p=0.003

Documentation of LUTS was more common in those presenting with pain, and in those in whom UTI was diagnosed. Overall fewer than half of the documented accounts included a mention of LUTS.

Documentation may reflect diagnostic reasoning, in that where the clinician includes UTI as a differential, they may be more likely to document LUTS. If the clinician does not suspect UTI, they may not document LUTS. However, documentation may not reflect the clinical conversation.

This data could suggest that urinary symptoms in pregnant women are under-valued in diagnostic reasoning.

Clinicians may have been aware of the patient's recruitment to a UTI study, with an effect on their documentation.

This is consistent with data from non-pregnant hospital patients, showing a low correlation between self-reported urinary symptoms and medical record documentation in patients with a positive culture UTI, with a trend towards fewer documented symptoms compared with patient self-report (Echaiz, Cass et al. 2015). A similar study showed that the medical record incompletely captures urinary symptoms in patients with positive culture (Mithoowani, Celetti et al. 2015).

Next, I compare the groups according to standard microbiological data throughout the pregnancy.

7.5.5 Standard urine cultures throughout the index pregnancy

7.5.5.1 Results

First, urine cultures at booking were compared (Table 7-19). There were 76/156 (48.7%) screening culture negative, 32/156 (20.5%) non-significant growth, 25/156 (16.0%) mixed growth, and 20/156 (12.8%) positive, with no difference between groups in their results ($p=0.517$).

Table 7-19: Comparison of standard culture at booking against study group

MSU result	Pain cases (n=50) (%)	Acute controls (n=58) (%)	Normal controls (n=48) (%)	Totals (% of total)	Significance
Screening culture negative	20 (40%)	28 (48.3%)	28 (58.3%)	76 (48.7%)	$\chi^2 = 5.277$ $p=0.517$
Non-significant growth	12 (24%)	15 (25.9%)	8 (16.7%)	32 (20.5%)	
Mixed growth	11 (22%)	9 (15.5%)	5 (10.4%)	25 (16.0%)	
Positive	7 (14%)	6 (10.3%)	7 (14.6%)	20 (12.8%)	
Totals	50 (100%)	58 (100%)	48 (100%)	156 (100.0%)	

Next, all cultures throughout the pregnancies were compared (Table 7-20). 554 standard cultures were reported throughout the 159 pregnancies, including booking cultures. Of these, 271/554 (48.9%) were screening culture negative, 130/554 (23.5%) showed non-significant growth, 110/554 (19.9%) showed mixed growth of uncertain significance, and 43/554 (7.8%) were positive. This was not different between groups ($p=0.261$).

Table 7-20: Comparison of all standard MSU results in index pregnancy against study group

MSU result	Pain cases (n=50) (%)	Acute controls (n=58) (%)	Normal controls (n=51) (%)	Totals (% of total)	Significance
Screening culture negative	89 (43.8%)	97 (48.5%)	85 (56.3%)	271 (48.9%)	$\chi^2 = 7.694$ $p=0.261$
Non-significant growth	54 (26.6%)	49 (24.5%)	27 (17.9%)	130 (23.5%)	
Mixed growth	42 (20.7%)	42 (21%)	26 (17.2%)	110 (19.9%)	
Positive	18 (8.9%)	12 (6%)	13 (8.6%)	43 (7.8%)	
Totals	203 (100%)	200 (100%)	151 (100%)	554 (100.0%)	

Table 7-21 shows all standard urine cultures at a participant level. The median number of standard cultures recorded per patient was 4 (95% CI 3-4) for pain cases, 3 (95% CI 2-3) for acute controls, and 3 (95% CI 2-3) for normal controls (between-group difference, $p=0.009$). 33/159 (20.8%) participants had a positive MSU at some point during the pregnancy, which did not differ between groups ($p=0.402$).

Table 7-21: Details of all other standard urine culture tests reported during the index pregnancy

Microbiological parameter	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance	Overall (n=159)
Number of cultures reported					
Median	4	3	3	Kruskal-	3
(95% CI)	(3–4)	(2–3)	(2–3)	Wallis	(3–3)
(range)	(2–12)	(1–10)	(1–7)	p=0.009	(1–12)
Number of screening culture negative					
Median	2	1	2	Kruskal-	2
(95% CI)	(1–2)	(1–2)	(1–2)	Wallis	(1–2)
(range)	(0–5)	(0–6)	(0–5)	p=0.814	(0–6)
Number of non-significant growth					
Median	1	1	0	Kruskal-	1
(95% CI)	(0–1)	(0–1)	(0–1)	Wallis	(0–1)
(range)	(0–4)	(0–5)	(0–3)	p=0.036	(0–5)
Number of mixed growth					
Median	0	0	0	Kruskal-	0
(95% CI)	(0–1)	(0–1)	(0–1)	Wallis	(0–1)
(range)	(0–6)	(0–3)	(0–2)	p=0.493	(0–6)
Number of positive cultures					
Median	0	0	0	Kruskal-	0
(95% CI)	(0–0)	(0–0)	(0–0)	Wallis	(0–0)
(range)	(0–2)	(0–2)	(0–2)	p=0.390	(0–2)
Proportion with any positive MSUs this pregnancy (%)	13/50 (26%)	9/58 (15.5%)	11/51 (21.6%)	$\chi^2 = 1.8243$ p=0.402	33/159 (20.8%)

7.5.5.2 Discussion

There were no differences in booking culture between study groups ($p=0.517$), with a rate of 12.8% bacteriuria at booking. There were high rates of non-negative results, with 36.5% having a non-significant or mixed growth culture at booking. These rates are similar to those throughout pregnancy (see Table 7-20), with 43/554 (7.8%) of all MSUs positive, and 240/554 (43.4%) reported as non-significant or mixed growth. Approximately 1/5 participants had a positive culture at some point during the pregnancy, with no difference between study groups. If a positive MSU is taken as a proxy for antibiotic treatment for UTI, this suggests more than ten percent would have been offered antibiotics by the end of the first trimester, and twenty percent by the end of the pregnancy.

It is of interest that the rate of bacteriuria at booking is higher than has been reported previously (2-10% (Smaill and Vazquez 2015)), and that there was no difference with the normal controls, in whom it was 7/48 (14.6%). This implies a higher-than-normal burden of urinary pathology in the study participants, and in a group of apparently normal controls, and this should be borne in mind when interpreting the findings. However with a small group such as this no real conclusions can be drawn, as shown by the wide 95% confidence intervals of these proportions.

Patients presenting with pain had a higher median number of cultures, however even normal controls had a median of 3 cultures sent, which would have included one at booking, one for the current study, and one additional one during the pregnancy. It is imperative that the performance in pregnancy of a test that is used so commonly is well understood, to avoid waste and potential harm.

7.5.6 Other microbiology investigations throughout the index pregnancy

7.5.6.1 Results

Table 7-22 demonstrates the other microbiological investigations reported for participants during the index pregnancy.

15/159 (9.4%) had blood cultures sent, with no difference between groups ($p=0.890$). Of these, eleven were intrapartum, and four postpartum. None were positive.

86/159 (54.1%) patients had at least one vaginal swab. The distribution was different between groups, with 20/51 (39.2%) normal controls having a swab sent versus 34/58 (58.6%) of acute controls and 32/50 (64%) pain cases ($p=0.031$). In total 150 swabs were sent.

14/86 (16.3%) had at least one swab reported as GBS positive (no differences, $p=0.869$). 5/86 (5.8%) had a swab reported with something other than *Candida spp* or GBS ($p=1.00$). This included *Gardnerella vaginalis* & mixed anaerobes, and *Streptococcus pyogenes* (Group A Strep) (pain cases), *Streptococcus milleri* and *Escherichia coli* (acute controls), and mixed anaerobes (normal controls).

Other microbiological tests included Methicillin-resistant *Staphylococcus aureus* (MRSA, all negative), faeces cultures (all negative), perineal swabs (one negative, one GBS), a respiratory swab (negative) and a placental swab (mixed anaerobes). 11 patients had placental histology, of which 4/11 (36.4%) reported chorioamnionitis and/or funisitis. No significance testing was carried out because of low numbers.

Table 7-22: Other microbiological results in pregnancy

Test	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance
Any blood cultures sent during index pregnancy* (%)	(n=50) 5/50 (10%)	(n=58) 6/58 (10.3%)	(n=51) 4/51 (7.8%)	Fisher's Exact=0.292 p=0.890
Any vaginal swabs sent in index pregnancy (%)	(n=50) 32/50 (64%)	(n=58) 34/58 (58.6%)	(n=51) 20/51 (39.2%)	$\chi^2= 7.001$ p=0.031
Any GBS positive swabs (%)	(n=32) 5/32 (15.6%)	(n=34) 5/34 (14.7%)	(n=20) 4/20 (20%)	Fisher's Exact=0.410 p=0.869
Any positive swabs reported as non-GBS, non- <i>Candida spp</i> (%)	(n=32) 2/32 (6.3%)	(n=34) 2/34 (5.9%)	(n=20) 1/20 (5%)	Fisher's Exact=0.268 p=1.000
Faeces culture	2/50 (negative)	2/58 (negative)	0/51	n/a
Perineal wound swab	2/50 (GBS, negative)	0/58	0/51	n/a
Respiratory swab	0/50	1/58 (negative)	0/51	n/a
Placental swab	0/50	0/58	1/51 (mixed anaerobes)	n/a
MRSA (all negative)	13/50	15/58	8/51	n/a
Chorioamnionitis / funisitis on placental histology (%)	(n=2) 1/2 (50%)	(n=4) 1/4 (25%)	(n=5) 2/5 (40%)	n/a

**All blood cultures were reported as 'no growth after 5 days'; only one set sent per person*

7.5.6.2 Discussion

No antenatal blood cultures were sent for any of the participants, consistent with no cases of antenatal sepsis or pyelonephritis reported in clinical records.

There was a high rate of vaginal swabs, even in normal controls (39.2%). 16.3% of those who had a swab sent were found to be GBS positive. This is comparable with the estimated UK carriage rate of 22% (Bevan, White et al. 2019).

Only one participant had a placental swab sent, although 4/11 placentas analysed histologically had evidence of chorioamnionitis or funisitis.

Other than number of vaginal swabs sent, groups were comparable for other microbiological results.

So far, we have compared the study groups in terms of demographics, clinical risk factors, obstetric and neonatal outcomes, diagnoses, urine culture results and standard microbiological results through the pregnancy, and considered documentation of LUTS in pain cases and acute controls. Groups were heterogeneous but largely comparable.

7.5.7 Standard microscopy and culture

7.5.7.1 Methods

For methods see 6.15.2.

7.5.7.2 Results

Standard microscopy and culture results are described in Table 7-23.

Microscopy was performed for 97/159 (61.0%) participants, which differed between groups ($p < 0.001$), with 36/50 (72%) pain cases, 18/58 (31.0%) acute controls and 43/51 (84.3%) normal controls.

White blood cells were reported in 68/97 (61.0%) participants with no difference between groups ($p = 0.078$). Red blood cells were reported in 16/97 (16.5%), which ranged from 1/43 (2.3%) normal controls to 9/36 (25%) pain cases and 6/18 (33.3%) acute controls ($p = 0.003$). Epithelial cells were reported in 94/97 (96.9%) participants, which did not differ between groups ($p = 0.794$).

Standard culture was performed in 157/159 (98.7%) participants. It was positive (with $>10^5$ CFU/ml) in 10/157 (6.4%) participants, with no difference between groups ($p = 0.455$).

Table 7-23: Standard microscopy and culture result

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance	Overall
Proportion with microscopy performed (%)	(n=50) 36/50 (72%)	(n=58) 18/58 (31.0%)	(n=51) 43/51 (84.3%)	$\chi^2=36.089$ $p<0.001$	97/159 (61.0%)
White cells (any seen) (%)	(n=36) 23/36 (63.9%)	(n=18) 10/18 (55.6%)	(n=43) 35/43 (81.4%)	$\chi^2=5.097$ $p=0.078$	(n=97) 68/97 (70.1%)
Red blood cells (any seen) (%)	(n=36) 9/36 (25.0%)	(n=18) 6/18 (33.3%)	(n=43) 1/43 (2.3%)	$\chi^2=11.8635$ $p=0.003$	(n=97) 16/97 (16.5%)
Epithelial cells (any seen) (%)	(n=36) 35/36 (97.2%)	(n=18) 17/18 (94.4%)	(n=43) 42/43 (97.7%)	$\chi^2=0.4606$ $p=0.794$	(n=97) 94/97 (96.9%)
Culture positive >10 ⁵ CFU/ml (%)	(n=50) 2/50 (4%)	(n=56) 3/56 (5.4%)	(n=51) 5/51 (9.8%)	$\chi^2=1.5757$ $p=0.4548$	(n=157) 10/157 (6.4%)

Detailed culture results are described in Table 7-24, for illustrative purposes only given the small numbers. Of the 10 positive results, 5/157 (3.2%) were *Escherichia coli*, 2/157 (1.3%) were *Enterococcus spp.*, 2/157 (1.3%) were *Streptococcus agalactiae*, and 1/157 (0.6%) were *Citrobacter koseri*. Of the non-positive results, 98/157 (62.4%) were reported as screening culture negative, 26/157 (16.6%) as no significant growth, and 12/157 (14.6%) as mixed growth of uncertain significance. Rates of these categories did not differ between groups ($p=0.191$, Fisher's Exact test).

Table 7-24: Standard culture result in detail

	Pain cases (n=50) (% of total)	Acute controls (n=56)* (% of total)	Normal controls (n=51) (% of total)	Overall (% of total)
>10 ⁵ CFU/ml <i>Enterococcus</i>	0 (0%)	1 (1.8%)	1 (2.0%)	2 (1.3%)
>10 ⁵ CFU/ml <i>Escherichia coli</i>	1 (2%)	1 (1.8%)	3 (5.9%)	5 (3.2%)
>10 ⁵ CFU/ml <i>Streptococcus agalactiae</i>	1 (2%)	1 (1.8%)	0 (0%)	2 (1.3%)
>10 ⁵ CFU/ml <i>Citrobacter koseri</i>	0 (0%)	0 (0%)	1 (2.0%)	1 (0.6%)
A mixed growth of uncertain significance	4 (8%)	7 (4.5%)	12 (23.5%)	23 (14.6%)
No significant growth	12 (24%)	8 (14.3%)	9 (17.6%)	26 (16.6%)
Screening culture negative	32 (64%)	38 (67.9%)	28 (54.9%)	98 (62.4%)
Totals	50	56	51	157

**2 missing culture results, one with no sample on system and one with sample not processed by lab*

Standard culture result was compared with antibiotic prescribing at the time of the visit (Table 7-25). 3/4 (75%) of those with positive cultures were not prescribed antibiotics whereas 7/60 (11.7%) of those with negative cultures were prescribed antibiotics.

Table 7-25: comparison of standard culture versus prescription of antibiotics

	Standard culture result			Totals	Significance
	Positive	Mixed	Negative		
No antibiotics prescribed	3	21	45	69	Fisher's Exact=0.100
Antibiotics prescribed	0	8	7	15	
Other plan involving antibiotics	0	0	5	5	
Antibiotics planned if culture positive	1	0	3	4	
Totals	4	29	60	93	

7.5.7.3 Discussion

Microscopy was not carried out for all requested samples. This is likely due to laboratory policy not to perform microscopy where screening for asymptomatic bacteriuria may be required, which is consistent with Public Health England guidance (Standards Unit 2017). This makes it difficult to compare the groups. It is noteworthy that epithelial cells were seen in 94/97 (96.9%), which could be interpreted as contamination, as previously discussed.

Culture was positive in 10/157 (6.4%) cases. There was no difference between study groups ($p=0.455$), despite pain cases being more likely than acute controls and normal controls to report pain urinary symptoms, to score lower for quality of life, and more likely than acute controls to self-diagnose UTI, to receive a clinician diagnosis of UTI, and to receive antimicrobials.

Regarding 'mixed growth of uncertain significance', and 'no significant growth', there was no difference between study groups ($p=0.191$). Mixed growth or no significant growth accounted for nearly a quarter of samples; these may be labelled as contaminated but true clinical relevance is not known.

There was a mismatch between antibiotic prescription and standard culture results. If standard culture remains gold standard, this suggests simultaneous under-treatment of patients with UTI, and over-treatment of patients without UTI, suggesting that clinical reasoning tools available do not correlate well with standard culture results. Apparent over-diagnosis of UTI may have been a consequence of study involvement, in terms of clinician and patient awareness of UTI and UTI symptoms.

7.5.8 Urinary dipstick

7.5.8.1 Methods

For methods see 6.15.1.

7.5.8.2 Results

Urinary dipstick results are described in Table 7-26. In line with typical antenatal practice, dipstick test was recorded positive given any reaction to leucocytes, protein or nitrite. Thus 109/159 (68.6%) participants showed positive with no difference between groups ($p=0.110$); 1/159 (0.6%) was positive for nitrite.

Table 7-27 compares overall dipstick result with clinician diagnosis of UTI.

Table 7-26: Standard dipstick for Abdominal pain study

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance	Overall (n=159)
Leucocyte esterase (any with trace or more) (%)	28/50 (56%)	29/58 (50.0%)	30/51 (58.8%)	$\chi^2=0.901$ $p=0.637$	87/159 (54.7%)
Blood (any with trace or more) (%)	18/50 (36%)	17/58 (29.3%)	12/51 (23.5%)	$\chi^2=1.888$ $p=0.389$	47/159 (29.6%)
Protein (any with trace or more) (%)	23/50 (46%)	18/58 (31.0%)	22/51 (43.1%)	$\chi^2=2.902$ $p=0.234$	63/159 (39.6%)
Nitrite (any positive) (%)	0/50 (0%)	1/58 (1.7%)	0/51 (0%)	Fisher's Exact=1.656 $p=1.00$	1/159 (0.6%)
Glucose (any with trace or more) (%)	5/50 (10%)	2/58 (3.4%)	2/51 (3.9%)	$\chi^2=2.584$ $p=0.275$	9/159 (5.7%)
Overall dipstick positive (%)	36/50 (72%)	34/58 (58.6%)	39/51 (76.5%)	$\chi^2=4.413$ $p=0.110$	109/159 (68.6%)

Table 7-27: Comparison of dipstick versus clinician diagnosis of UTI

	Clinician diagnosis of UTI		Total	Significance
	UTI	No UTI		
Positive (any of protein / nitrite / leucocyte)	19	42	61	$\chi^2=10.108$ p=0.001
Negative	1	32	33	
Total	20	74	94	

7.5.8.3 Discussion

There were no statistically significant differences between groups with respect to leucocyte esterase, blood, nitrite, protein or glucose or a combination using dipstick positive for protein / nitrite / leucocyte esterase.

There was an association between dipstick and clinician diagnosis of UTI. This would be commensurate with clinicians using dipstick to diagnose UTI in women with abdominal pain.

7.5.9 Fresh unspun urine microscopy

7.5.9.1 Methods

For methods see 6.16.3

7.5.9.2 Results

Using fresh unspun microscopy (see Table 7-28), white cells were noted in 139/159 (87.4%) samples, with median white cell count of $14\mu\text{l}^{-1}$ (95% CI 10-24) and no difference between groups ($p=0.054$, $p=0.102$ respectively). Cell counts ranged from 0-1190 μl^{-1} .

Red cells were noted in 77/159 (48.4%), with median red cell count of $0\mu\text{l}^{-1}$ (95% CI 0-2), and no difference between groups ($p=0.754$, $p=0.634$ respectively). Cell counts ranged from 0-7520 μl^{-1} .

Epithelial cells were noted in 140/159 (88.1%), with median epithelial cell count of $14\mu\text{l}^{-1}$ (95% CI 8-18), with no difference between groups ($p=0.863$, $p=0.233$ respectively). Cell counts ranged from 0-184 μl^{-1} .

Table 7-28: Fresh unspun urine microscopy for Abdominal pain study

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Significance	Overall
White cell count (median WBC μl^{-1}) (95% CI) (Range)	13 (6-24) (0 – 326)	10 (6-28) (0 – 1190)	20 (14-32) (0 – 640)	p=0.102 Kruskal- Wallis	14 (10-24) (0 - 1190)
Any white cells (%) (95% CI)	45/50 (90%) (77.4-96.3)	46/58 (79.3%) (66.3-88.4)	48/51 (94.1%) (82.8-98.5)	$\chi^2=5.852$ p=0.054	139/159 (87.4%) (81.0-92.0)
Red cell count (median RBC μl^{-1}) (95% CI) (Range)	1.5 (0-4) (0 – 2560)	0 (0-2) (0 – 930)	0 (0-6) (0 – 7520)	p=0.634 Kruskal- Wallis	0 (0-2) (0 - 7520)
Any red cells (%) (95% CI)	26/50 (52%) (37.6-66.1)	26/58 (44.8%) (32.0-58.4)	25/51 (49.0%) (35.0-63.2)	$\chi^2=0.564$ p=0.754	77/159 (48.4%) (40.5-56.5)
Epithelial cell count (median EPC μl^{-1}) (95% CI) (Range)	14 (8-26) (0 – 142)	14 (6-30) (0 – 184)	8 (4-16) (0 – 160)	p=0.233 Kruskal- Wallis	14 (8-18) (0 – 184)
Any epithelial cells (%) (95% CI)	44/50 (88%) (75.0-95.0)	52/58 (89.7%) (78.2-95.7)	44/51 (86.3%) (73.1-93.9)	$\chi^2=0.295$ p=0.863	140/159 (88.1%) (81.7-92.5)

7.5.9.3 Discussion

There was no difference between study groups in terms of any of the fresh unspun microscopy measures.

Although pain cases reported more frequency, nocturia and pain symptoms than controls, they were not more likely to have pyuria. Importantly, the study was powered to detect a difference in pyuria.

Most participants had some white cells and epithelial cells present, and nearly half had some red blood cells present. There was a wide range in cell counts in all three groups. Further understanding of the relationship between fresh microscopy, particularly white cells and epithelial cells, and relevant clinical

outcomes, is needed to be able to interpret these findings. These will be examined later in the synthesis of pooled data from the three related studies.

Next, I will describe analysis of urinary ATP.

7.5.10 Urinary ATP

7.5.10.1 Methods

For methods see 6.16.4

7.5.10.2 Results

Urinary ATP was measured for all participants (see Table 7-29). Median ATP was 5947 RLU (95% CI 4653-8064) for pain cases, 8203 RLU (95% CI 6967-9149) for acute controls, and 5157 RLU (95% CI 3649-7186) for normal controls. There was a distribution difference between groups ($p=0.005$). The range of ATP values was wide across all three groups.

Table 7-29: ATP measurement for Abdominal pain study

Measure (units)	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
ATP (relative light units, RLU)					
Median	5947	8203	5157	p=0.005 Kruskal-Wallis	6717
(95% CI)	(4653-8064)	(6967-9149)	(3649-7186)		(5513-7900)
(Range)	(718–48800)	(1095–21889)	(679–16347)		(679–48800)

7.5.10.3 Discussion

Although there was a distribution difference between groups, confidence intervals for median ATP values were wide and overlapping so clinical application would be inappropriate. Acute controls included a wide variety of presenting symptoms, such that sub-analysis would be inappropriate.

The reproducibility of ATP readings was not analysed in this study.

7.5.11 Enhanced sediment culture with counts and identification to species level of cultured organisms

7.5.11.1 Methods

For methods see 6.16.5

7.5.11.2 Results

Sediment culture was carried out for all participants (Table 7-30). Median total colony counts were 1220 CFU/ml (95% CI 720-1945) with no difference between groups ($p=0.239$). The overall range was 0–1390319 CFU/ml. The median colony counts for the dominant species were 976 CFU/ml (95% CI 603-692, range 0–1280000) with no difference between groups ($p=0.246$). The number of isolates identified was a median of 3.0 (95% CI 3-3), with no difference between groups ($p=0.875$). The proportion with any bacteriuria was 157/159 (98.7%) with no difference between groups ($p=0.534$).

Table 7-30: Sediment culture with counts and identification to species level of cultured organisms

Measure (units)	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Total colony counts (CFU/ml)					
Median	1515	1246	884	Kruskal-	1220
(95% CI)	(663-13470)	(399-2902)	(280-1945)	Wallis	(720-1945)
(Range)	(0-472005)	(3-1280000)	(0-1390319)	p=0.239	(0-1390319)
Colony count for dominant species (CFU/ml)					
Median	1160	950	972	Kruskal-	976
(95% CI)	(550-11883)	(237-2258)	(167-2125)	Wallis	(603-1692)
(Range)	(0-472000)	(2-1280000)	(0-1280000)	p=0.246	(0-1280000)
Number of isolates					
Median	3	3	3	Kruskal-	3
(95% CI)	(3-4)	(3-4)	(3-3)	Wallis	(3-3)
(Range)	(0-6)	(1-7)	(0-6)	p=0.875	(0-7)
Proportion with any bacteriuria (colony count > 0) (%)					
(95% CI)	49/50 (98%) (88.0-99.9)	58/58 (100%) (92.3-100)	50/51 (98.0%) (88.2-99.9)	Fisher's Exact p=0.534	157/159 (98.7%) (95.1-99.8)

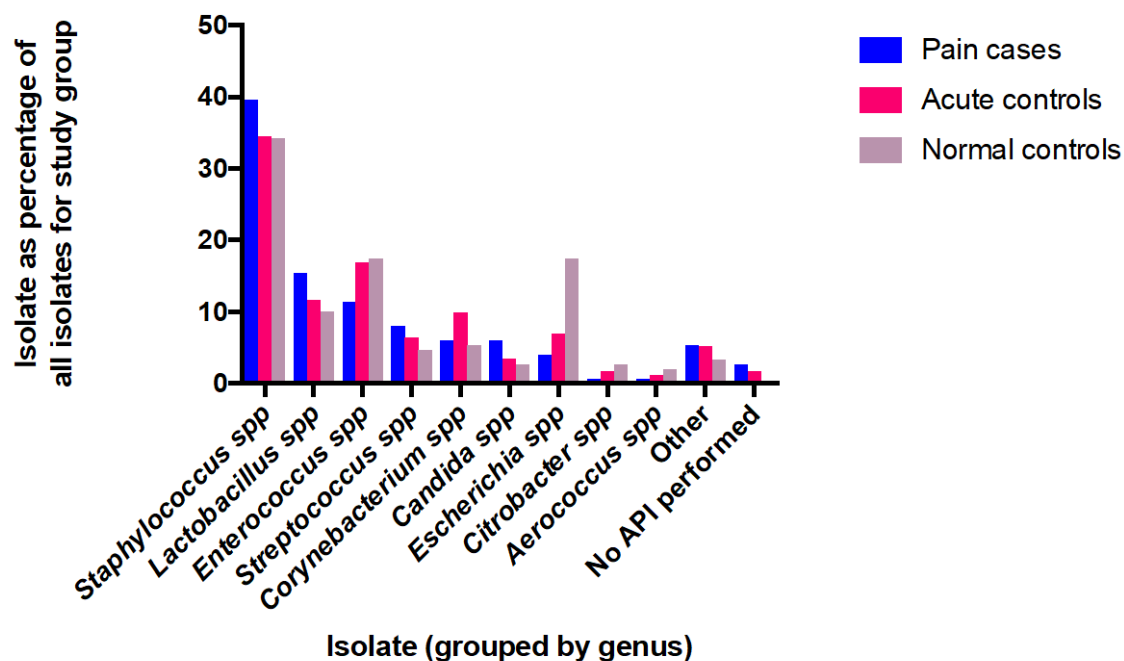
Isolates identified by sediment culture, categorised by genus, are shown in Table 7-31 and Figure 7-7. No API was performed in 7/471 cases (1.5%). The commonest three genera were *Staphylococcus spp* (present in 169/471 (35.9%) samples), *Enterococcus spp* (74/471, 15.7%), and *Lactobacillus spp* (58/471, 12.3%). Other genera identified were *Escherichia spp* (44/471, 9.3%), *Corynebacterium spp* (34/471, 7.2%), *Streptococcus spp* (30/471, 6.4%), *Candida spp* (19/471, 4.0%), *Citrobacter spp* (8/471, 8%), and *Aerococcus spp* (6/471, 1.3%). Other genera were identified in 22/471 (4.7%) cases. These distributions are significantly different (p=0.023, Chi square).

Table 7-31: Most common isolates by genus, compared by study group

Isolate genus (number of samples in which isolate was identified)	Pain cases (% of all pain case isolates)	Acute controls (% of all acute control isolates)	Normal controls (% of all normal control isolates)	Totals (% of all isolates)
<i>Staphylococcus spp</i>	59 (39.6)	59 (34.5)	51 (34.2)	169 (35.9)
<i>Enterococcus spp</i>	17 (11.4)	29 (17.0)	26 (17.5)	74 (15.7)
<i>Lactobacillus spp</i>	23 (15.4)	20 (11.7)	15 (10.1)	58 (12.3)
<i>Escherichia spp</i>	6 (4.0)	12 (6.9)	26 (17.5)	44 (9.3)
<i>Corynebacterium spp</i>	9 (6.0)	17 (9.9)	8 (5.4)	34 (7.2)
<i>Streptococcus spp</i>	12 (8.1)	11 (6.4)	7 (4.7)	30 (6.4)
<i>Candida spp</i>	9 (6.0)	6 (3.5)	4 (2.7)	19 (4.0)
<i>Citrobacter spp</i>	1 (0.7)	3 (1.8)	4 (2.7)	8 (1.7)
<i>Aerococcus spp</i>	1 (0.7)	2 (1.2)	3 (2.0)	6 (1.3)
Other	8 (5.4)	9 (5.3)	5 (3.4)	22 (4.7)
Other (specified)	<i>Brevibacterium spp</i> (2) <i>Bulkholderia spp</i> <i>Cellulomonas spp</i> / <i>Microbacterium spp</i> <i>Klebsiella spp</i> <i>Pseudomonas spp</i> (2) <i>Rhodococcus spp</i>	<i>Arthrobacter spp</i> <i>Cellulomonas spp</i> / <i>Microbacterium spp</i> <i>Enterobacter spp</i> (2) <i>Klebsiella spp</i> (2) <i>Pasteurella spp</i> <i>Rothia spp</i> <i>Serratia spp</i>	<i>Brevibacterium spp</i> <i>Pseudomonas spp</i> <i>Micrococcus spp</i> <i>Morganella spp</i> <i>Pantoea spp</i>	
No API performed	4 (2.7)	3 (1.8)	0 (0.0)	7 (1.5)
Totals	149 (100)	173 (100)	149 (100)	471 (100)

 $\chi^2=34.46$ $df=20$ $p=0.0232$

Figure 7-7: Ten most common isolates obtained using sediment culture, categorised by genus, arranged in descending order of frequency by pain cases



Isolates identified by sediment culture, categorised by species, are shown in Table 7-32 and Figure 7-8. No API was performed in 7/471 cases (1.5%).

The commonest three isolates overall, categorised to species level (except *Lactobacillus spp*), were *Staphylococcus haemolyticus* (72/471 isolates, 15.3%), *Enterococcus faecalis* (69/471, 14.7%) and *Lactobacillus spp* (58/471, 12.3%). Other common species included *Staphylococcus epidermidis* (53/471, 11.3%), *Escherichia Coli 1* (44/471 (9.3%), *Streptococcus agalactiae* (26/471, 5.5%), *Candida albicans* (15/471, 3.2%), *Corynebacterium striatum* / *amycolatum* (15/471, 3.2%), *Corynebacterium Group G* (15/471, 3.2%) and *Staphylococcus aureus* (14/471, 3.0%).

These distributions are significantly different ($p=0.0075$, $\chi^2=38.61$). The ten commonest species did occur in all three groups. The most striking difference between the groups is that *E. coli* occurred in 26/149 (17.5%, 95% CI 11.9-24.7) isolates in normal controls compared with 6/149 (4.0%, 95% CI 1.7-9.0) pain cases and 12/173 (6.9%, 95% CI 3.8-12.1) acute controls.

All species identified according to study group are illustrated in a Venn diagram in Figure 7-9. This shows that 15 species were identified in all study groups, while many others were only identified in one study group.

Table 7-32: Ten most common enhanced culture isolate species, according to study group

Isolate (number of samples in which isolate was identified)	Pain cases (% of all pain case isolates)	Acute controls (% of all acute control isolates)	Normal controls (% of all normal control isolates)	Overall totals (% of all isolates)
<i>Staphylococcus haemolyticus</i>	24 (16.1)	22 (12.7)	26 (17.5)	72 (15.3)
<i>Enterococcus faecalis</i>	16 (10.7)	28 (16.2)	25 (16.8)	69 (14.7)
<i>Lactobacillus spp</i>	23 (15.4)	20 (11.6)	15 (10.1)	58 (12.3)
<i>Staphylococcus epidermidis</i>	20 (13.4)	24 (13.9)	9 (6.0)	53 (11.3)
<i>Escherichia Coli 1</i>	6 (4.0)	12 (6.9)	26 (17.5)	44 (9.3)
<i>Streptococcus agalactiae</i>	10 (6.7))	9 (5.2)	7 (4.7)	26 (5.5)
<i>Candida albicans</i>	7 (4.7)	6 (3.5)	2 (1.3)	15 (3.2)
<i>Corynebacterium striatum / amycolatum</i>	5 (3.4)	8 (4.6)	2 (1.3)	15 (3.2)
<i>Corynebacterium Group G</i>	4 (2.7)	6 (3.5)	5 (3.4)	15 (3.2)
<i>Staphylococcus aureus</i>	2 (1.3)	9 (5.2)	3 (2.0)	14 (3.0)
Other isolates	28 (18.8)	26 (15.0)	29 (19.5)	90 (19.1)
Totals	149 (100)	173 (100)	149 (100)	471 (100)

$$\chi^2=38.61 \text{ df}=20 \text{ p}=0.0075$$

Figure 7-8: Ten most common isolates obtained using sediment culture, categorised by species, arranged in descending order of frequency by pain cases

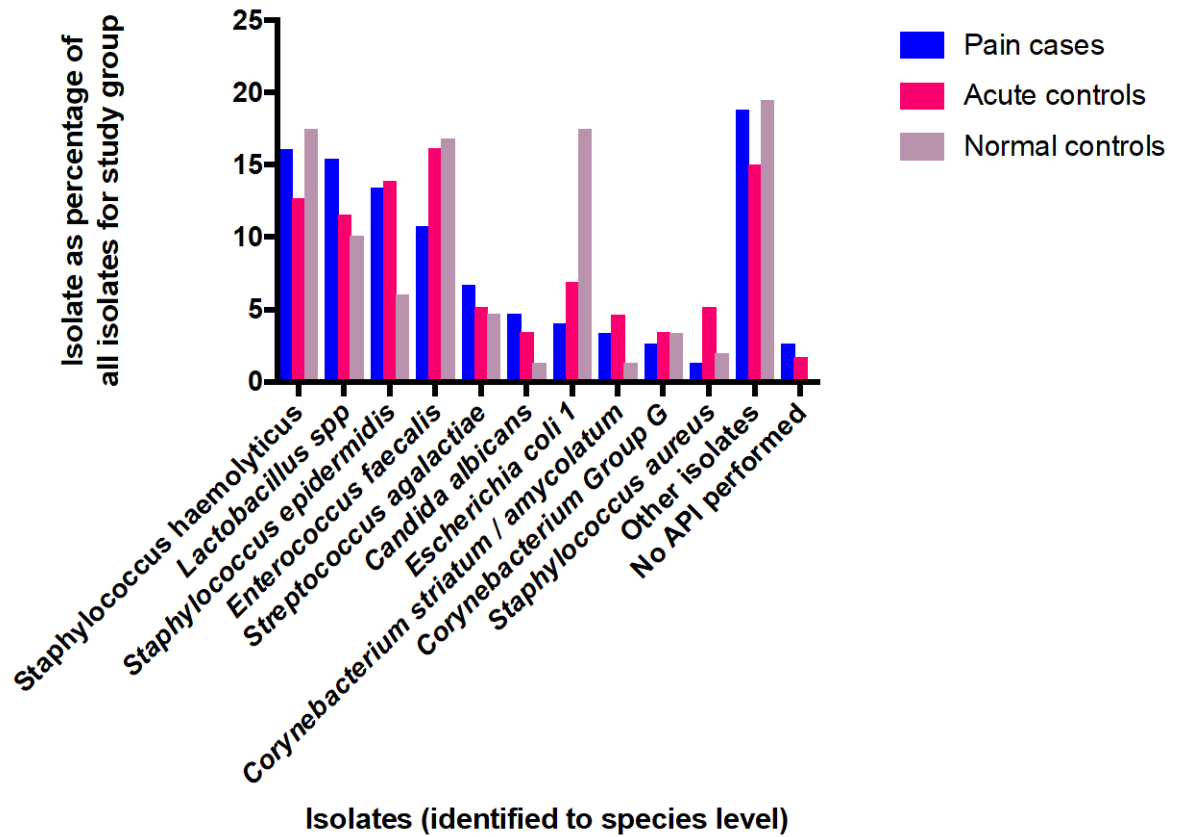


Figure 7-9: Venn diagram of species identified by enhanced culture

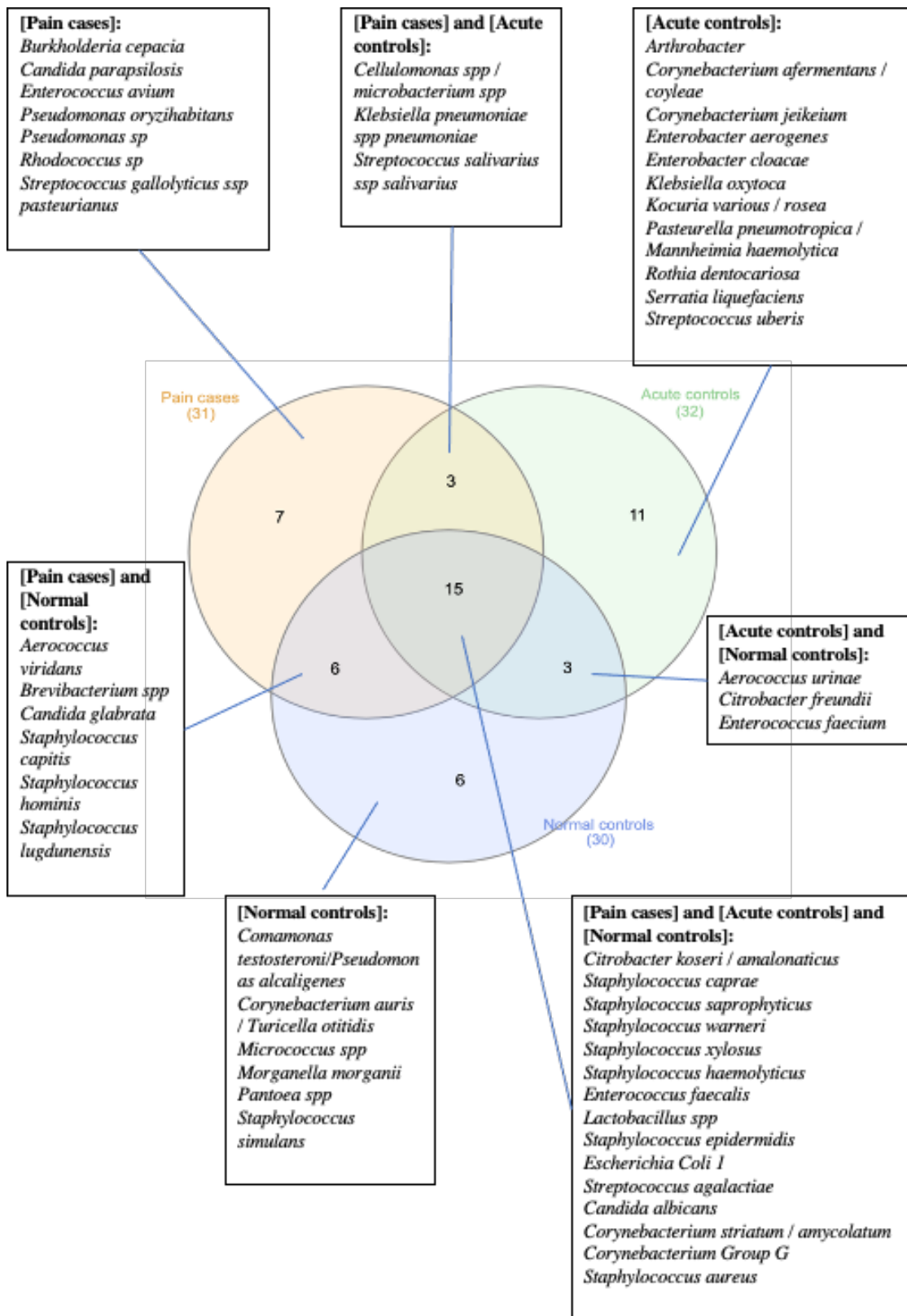


Diagram constructed using <http://www.interactivenn.net/index2.html>

7.5.11.3 Discussion

Enhanced culture identified bacteria in nearly all samples (98.7%), compared with standard culture where over half were culture negative (62.4%). This supports evidence that urine is not sterile.

The median colony count was 10^3 CFU/ml, which would be reported as non-significant growth in a standard culture. However, the basis on which this is called non-significant has been discussed previously; we need to better understand the clinical implications of lower colony counts.

The median number of bacterial isolates per sample was three. This polymicrobial model is consistent with a microbiome understanding of the bladder. Most polymicrobial samples would be reported as mixed growth without further information for clinical interpretation.

There were no differences between groups for any of these measures. This may reflect the heterogeneity of the study groups, and lack of substantial difference between the groups in terms of symptoms. A secondary analysis according to clinical outcomes will be reported in chapter 10.

When comparing isolate genera, the distributions were different. Whilst the top two genera for all groups were *Staphylococcus spp* and *Enterococcus spp*, the third commonest for normal controls was *Escherichia spp*, which at 17.5 % of isolates for normal controls appears overrepresented compared with pain cases (4.0%) and acute controls (6.9%). The list of genera common to all groups contains both traditional uropathogens and apparent commensals or contaminants. This is further evidence that this division may need to be re-evaluated.

The traditional understanding of detection of non-uropathogens in urine is that these must be contaminants. Some of the organisms detected are known to represent flora of surrounding anatomical structures, for example gastrointestinal, genital and skin flora. An explanation for the lack of difference between study groups could be that all groups were equally likely to be contaminated by these. It is important to remember that enhanced cultures were performed using the same samples as standard culture which either did not detect (negative cultures) or report (mixed growth and non-significant

growth) these organisms. By not detecting or reporting them we will be unable to resolve the issue of whether they are associated with negative outcomes, or determine whether they may also represent bladder microbiome or bladder flora. Other methods are needed to establish the origin of these organisms, such as uroplakin-staining of the shed epithelial cells in the urinary sediment which is what is cultured in this enhanced culture. We also need to question the superiority of a single positive culture, which arguably may also represent nearby flora, but may have been isolated in culture owing to its ability to be grown.

In a study of enhanced cultures using the same sediment culture technique (Sathiananthamoorthy, Malone-Lee et al. 2019), comparing patients with chronic LUTS with asymptomatic controls, a similar pattern was found. In that study, seven genera were found in both patients and controls:

Corynebacterium, *Enterococcus*, *Escherichia*, *Klebsiella*, *Proteus*, *Staphylococcus* and *Streptococcus*, with the commonest being *Staphylococcus*, *Streptococcus* and *Enterococcus*. The only difference in prevalence was that *Enterococcus* was more common in new patients than controls. *Candida*, *Lactobacillus* and *Citrobacter* were found only in patients, which is different to this study where they were in all three groups.

The isolate species also differed in their distribution between study groups, again with *E. coli* being overrepresented amongst normal controls. Species included a mixture of contaminants or commensals (such as *S. haemolyticus* or *S. epidermidis*) and traditional uropathogens (such as *E. coli* and *E. faecalis*). The overrepresentation of *E. coli* in normal controls may be clinically important, or may just represent a chance finding given the relatively small numbers in the study, and multiple comparisons being made. This would need to be explored in future studies of enhanced cultures in pregnancy.

An important difference between the chronic LUTS study and the current study is the definition of study groups. In that study, patients with chronic LUTS had a defined phenotype of symptomatic suffering, compared with asymptomatic controls. In the current study, a broad recruitment criteria was chosen to maximise the likelihood of identifying urinary pathology, however it made it less

likely that any between-group differences or lack of differences could be explained.

S. agalactiae (Group B Streptococcus or GBS) was isolated in 26 samples using enhanced culture, representing 26/159 (16.3%) of patients, however using standard culture there were only two reports (1.3% of patients). This has implications for pregnant women as any degree of GBS bacteriuria has been shown to have an impact on GBS pathology as described previously. An analysis of GBS bacteriuria against outcomes will be performed in the synthesis chapter. While GBS is known to be part of vaginal flora, it is also known that detection of it in the urine is associated with negative neonatal outcomes as described previously (Hughes RG 2017). If enhanced sediment culture detects it more than standard culture then this has grave implications. This logic may apply to other microorganisms, or combination of microorganisms.

It is frustrating that lactobacillus could not be classified at species level, given associations with healthy and symptomatic vaginal and urinary microbiomes of different lactobacillus species.

The enhanced culture approach used here is not without flaws. The use of chromogenic agar and aerobic conditions favours traditional uropathogens, which will influence the species isolated compared with a sequencing approach.

The use of API testing for species identification is a potential weakness. At the time of designing the study, this phenotypic approach was being used by the research laboratory as it is rapid and cost-effective. However, there are flaws with the approach as described previously (see 4.6.2). In addition, genetic approaches would also be able to identify non-culturable organisms.

Next, I compare urinary cytokines across the three groups.

7.5.12 Urinary cytokines – IL-6, IL-8, lactoferrin

7.5.12.1 Methods

See methods in 6.16.6.

7.5.12.2 Results

Urinary cytokine results are demonstrated in Table 7-33 and Figure 7-10 to Figure 7-12.

Median urinary IL-6 for pain cases was 1.71pg/ml (95% CI 1.0-3.2), for acute controls was 1.90pg/ml (95% CI 1.3-5.0) and for normal controls was 1.22pg/ml (95% CI 0.5-2.3). There was a difference between groups ($p=0.046$). The mean proportion with IL-6 below the LLOD was 24/159 (15.1%), with no difference between groups ($p=0.121$). The range was 0.14pg/ml to 281pg/ml (Figure 7-10).

Median urinary IL-8 for pain cases was 60.6pg/ml (95% CI 32.9-117.1), for acute controls was 38.7pg/ml (95% CI 17.1-80.2) and for normal controls was 21.9pg/ml (10.4-52.2). There was no difference between groups ($p=0.063$). The mean proportion with IL-8 below the LLOD was 10/159 (6.3%) with no difference between groups ($p=0.848$). The range was 0.71pg/ml to 2782pg/ml (Figure 7-11).

Median urinary lactoferrin for pain cases was 6.0ng/ml (95% CI 3.5-19.2), for acute controls was 5.2ng/ml (95% CI 2.2-8.3) and for normal controls was 2.2ng/ml (2.2-3.3). Note the LLOD was 2.2ng/ml. There was a difference between groups ($p=0.006$) with median for pain cases higher than normal controls. The proportion with lactoferrin below the LLOD was 71/159 (44.7%) which was different between groups ($p=0.016$). The range was 2.2ng/ml to 895.5ng/ml (Figure 7-12).

Median protein concentration for pain cases was 86.5 μ g/ml (95% CI 57.6-103.5), for acute controls was 89.2 μ g/ml (95% CI 76.4-105.5), and for normal controls was 60.1 μ g/ml (95% CI 44.9-77.7). There was a different distribution between groups ($p=0.008$).

When normalised for protein, there was no difference between groups for any of urinary IL-6, IL-8 or lactoferrin (respectively $p=0.134$, $p=0.213$, $p=0.251$).

Table 7-33: Urinary cytokines – IL-6, IL-8, lactoferrin by study group

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Total protein concentration (µg/ml) Median (95% CI) (Range)	86.5 (57.6-103.5) (22.5-316.0)	89.2 (76.4-105.5) (19.7-348.5)	60.1 (44.9-77.7) (15.6-209.5)	p=0.008 Kruskal- Wallis	77.7 (67.4-92.7) (15.6-348.5)
Proportion with protein below lower limit of detection (%) (95% CI)	0/50 (0%) (0-9)	0/58 (0%) (0-8)	0/51 (0%) (0-9)	n/a	0/159 (0%) (0-3)
IL-6 concentration (pg/ml) Median (95% CI) (Range)	1.71 (1.0-3.2) (0.14-281.4)	1.90 (1.3-5.0) (0.14-213.5)	1.22 (0.5-2.3) (0.14-47.5)	p=0.046 Kruskal- Wallis	1.59 (1.2-2.3) (0.14-281.4)
Proportion with IL-6 below lower limit of detection (%) (95% CI)	6/50 (12%) (5-25)	6/58 (10.3%) (4-22)	12/51 (23.5%) (13-38)	$\chi^2=4.226$ p=0.121	24/159 (15.1%) (10-22)
IL-6 normalised for total protein (ng/µg) Median (95% CI) (Range)	19 (13-34) (2.3-1377)	25 (16-43) (1.9-649)	16 (9-2.7) (3.3-248)	p=0.134 Kruskal- Wallis	20 (15-27) (1.9-1377)
IL-8 concentration (pg/ml) Median (95% CI) (Range)	60.6 (32.9-117.1) (0.71-1120)	38.7 (17.1-80.2) (0.71-2782)	21.9 (10.4-52.2) (0.71-928)	p=0.063 Kruskal- Wallis	46.4 (22.0-61.6) (0.71-2782)

Continued next page

Measure	Pain cases (n=50)	Acute controls (n=58)	Normal controls (n=51)	Sig.	Overall (n=159)
Proportion with IL-8 below lower limit of detection (%)	4/50 (8%) (3-20)	3/58 (5.2%) (1-15)	3/51 (5.9%) (2-17)	Fishers exact = 0.487 p=0.848	10/159 (6.3%) (3-12)
IL-8 normalised for total protein (pg/ μ g) Median (95% CI) (Range)	0.83 (0.30-1.31) (0.0068– 17.38)	0.40 (0.21-0.53) (0.0118– 28.19)	0.36 (0.25-0.79) (0.0128– 8.284)	p=0.213 Kruskal- Wallis	0.45 (0.30-0.76) (0.0068– 28.19)
Lactoferrin concentration (ng/ml) Median (95% CI) (Range)	6.0 (3.5-19.2) (2.21–895.5)	5.2 (2.2-8.3) (2.21–224.9)	2.2 (2.2-3.3) (2.21–82.43)	p=0.006 Kruskal- Wallis	4.2 (2.2-5.8) (2.21–895.5)
Proportion with lactoferrin below lower limit of detection (%) (95% CI)	17/50 (34%) (22-49)	23/58 (39.7%) (27-53)	31/51 (60.8%) (46-74)	$\chi^2=8.252$ p=0.016	71/159 (44.7%) (37-53)
Lactoferrin normalised for total protein (pg/ μ g) Median (95% CI) (Range)	92 (47-178) (12–20880)	68 (44-116) (11–1659)	68 (46-85) (11–1395)	p=0.251 Kruskal- Wallis	71 (52-89) (11–20880)

Figure 7-10: Dot plot of IL-6 (logarithmic scale) versus study group

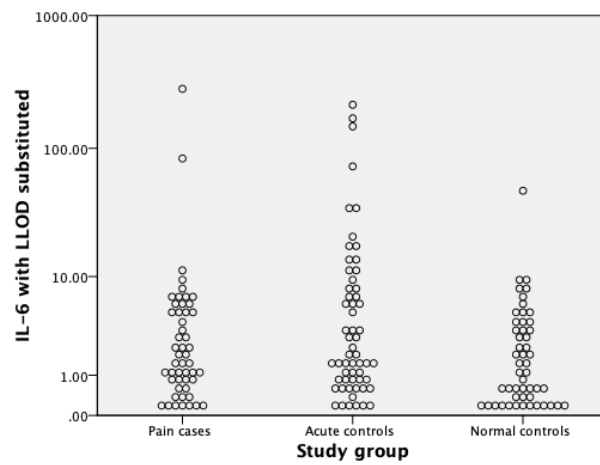


Figure 7-11: Dot plot of IL-8 (logarithmic scale) versus study group

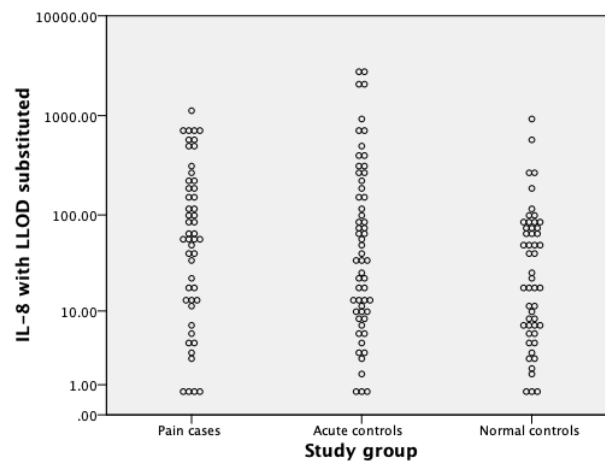
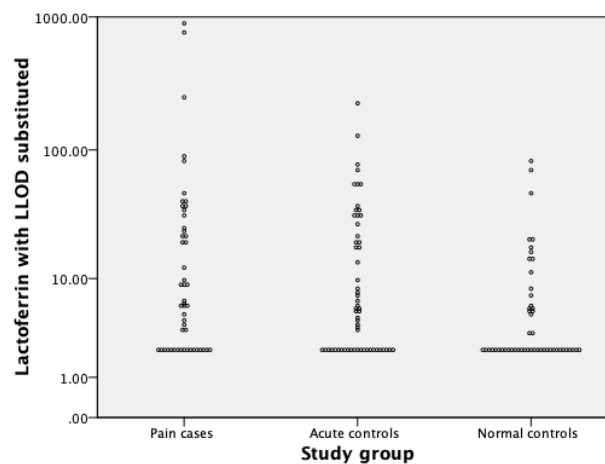


Figure 7-12: Dot plot of lactoferrin (logarithmic scale) versus study group



7.5.12.3 Discussion

In this study, median IL-6 was 1.59pg/ml (95% CI 1.2-2.3, range 0.14-281.4). To compare this with other studies, in pregnant women taking cranberry supplements, median IL-6 was 3.16pg/ml (range 0.01-7.34), and those taking placebo it was 9.32pg/ml (range 0.53-29.61) (Wing, Rumney et al. 2010). None of these patients had UTI. In patients undergoing amniocentesis to detect intra-amniotic infection, urinary IL-6 ranged from 0.1pg/ml to <1000pg/ml, which included patients with and without infection or preterm birth and no control for UTI (Dulay, Buhimschi et al. 2015).

In this study, median IL-8 was 46.4pg/ml (95% CI 22.0-61.6, range 0.71–2782). In pregnant women with asymptomatic bacteriuria, median IL-8 concentrations were 356pg/ml (range 22-5620), compared with 125pg/ml (6-5365) in women without asymptomatic bacteriuria ($p<0.01$) (Shelton, Boggess et al. 2001). In women with asymptomatic urinary infection it was 1200.72pg/ml $\pm$ 375, compared with 40.2pg/ml $\pm$ 17 in healthy pregnant women ((Basso, Gimenez et al. 2005))

Lactoferrin concentrations ranged from 2.2 to 895ng/ml, with a median of 4.2 (95% CI 2.2-5.8). Mean lactoferrin was 26.4ng/ml (95% CI 11.2-41.6). This is comparable to the values reported in (Arao, Matsuura et al. 1999) in non-pregnant patients, where mean urinary lactoferrin was 30.4 $\pm$ 2.7ng/ml (average $\pm$ standard error of the mean) in healthy controls and 60.3 $\pm$ 14.9ng/ml in patients without UTI. In patients with UTI levels were 3300 $\pm$ 646.3ng/ml. They cited a cut-off of 200ng/ml lactoferrin concentration for diagnosis of UTI. This is the first report of urinary lactoferrin values in pregnancy, to the best of our knowledge.

Without normalisation, there was a trend to higher urinary cytokine levels in the pain cases compared with normal controls, although this was only statistically significant for lactoferrin. There was no difference between groups in normalised cytokine values. The decision about whether and how to normalise urinary cytokines is complex as discussed previously. Some published studies have normalised and some have not. Using protein to normalise showed no overall difference between study groups for any of IL-6, IL-8 or lactoferrin.

Whilst a theoretical approach is one way to settle the dilemma (what is most likely to be clinically meaningful?), another is to compare results against clinical outcomes (what is best at predicting outcomes?). This will be explored later using pooled data.

Normalising for protein allows a direct comparison with two other studies from related authors, using lactoferrin normalised to total protein using a Bradford Assay (Nienhouse, Gao et al. 2014), (Plichta, Holmes et al. 2017). Higher lactoferrin was found in urogynaecology patients who were culture-positive for UTI on day of surgery (POS) compared with those who were culture negative on day of surgery (NEG) or those who were culture negative on day of surgery but subsequently culture positive (Nienhouse, Gao et al. 2014). Median values (adapted from graph in publication) were around $\sim 1.5\text{ng}/\mu\text{g}$ for POS patients and $\sim 0.1\text{ng}/\mu\text{g}$ for negative patients. There were no differences in urinary lactoferrin between controls and patients who had suffered cutaneous burns (Plichta, Holmes et al. 2017). Values were around $0.0010\text{--}0.0020\text{ng}/\text{mg}$ for controls (adapted from graph in publication, not clear if median or mean displayed). In this study median lactoferrin/protein was $71\text{pg}/\mu\text{g}=0.071\text{ng}/\mu\text{g}$ which falls in between. These other studies were in other settings with non-pregnant patients. It is not clear why the values are markedly different to each other. Comparison with other non-pregnant cohorts is particularly important for lactoferrin as this is the first report of urinary lactoferrin levels in pregnancy, as far as we can determine.

There was a high rate of lactoferrin below LLOD using this assay (71/159, 44.7%). This suggests either that lactoferrin was often low, or that the assay was not sensitive enough. Comparing proportions below LLOD did show a significant difference ($p=0.016$) with normal controls having the highest proportion, which may suggest a difference not detected using this test.

All three assays showed wide variation from below LLOD to several orders of magnitude higher, necessitating logarithmic conversion to display them graphically.

Quality control of ELISA measurements was careful as described in methods. Although assays were performed in duplicate, and normalised by protein, other

factors such as time of day, time since last void, and other normalisation methods were not taken into account.

7.5.13 Bacterial-associated epithelial cell proportions

7.5.13.1 Methods

For methods see 6.16.8. Slides were prepared for every sample, however one slide (acute control) was unreadable and was excluded from the results.

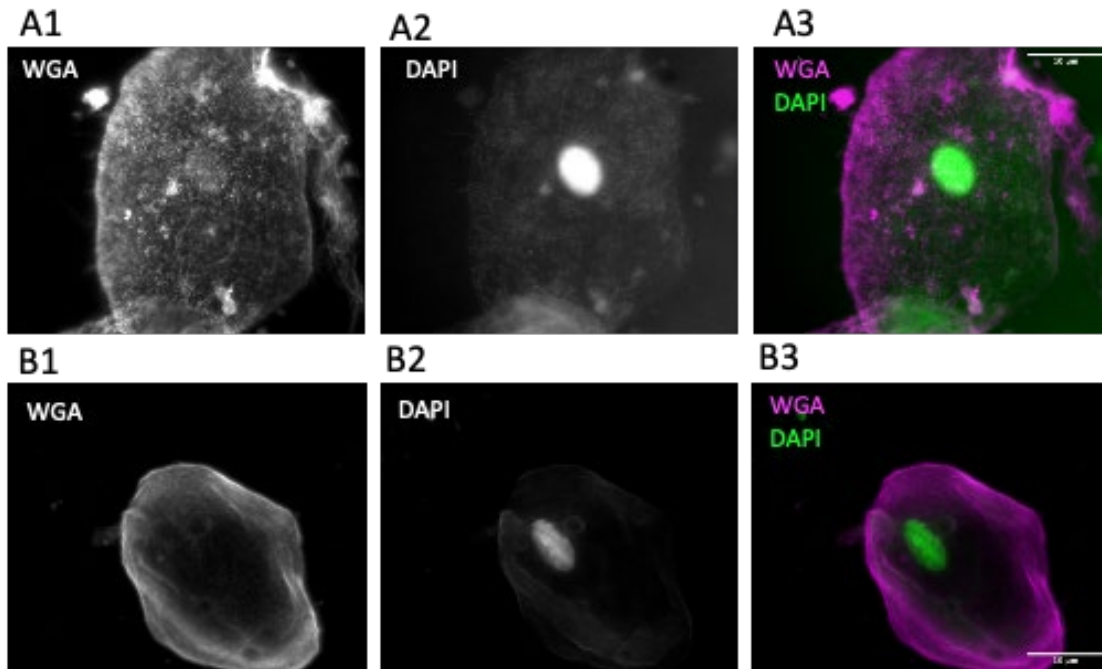
7.5.13.2 Results

Figure 7-13 shows examples of epithelial cells with and without associated bacteria (clue cells). The top line (A) shows a clue cell from a pain case, the bottom line (B) shows a non-clue cell from an acute control.

Table 7-34 shows that the median proportion of epithelial cells that were clue cells was 21.1% (95% CI 14.0-27.8) with no difference between groups ($p=0.464$). This distribution is shown in Figure 7-14.

The proportion of slides with any clue cells was 111/157 (70.7%, 95% CI 62.8-77.6) with no difference between groups ($p=0.482$).

Figure 7-13: Epithelial cells with (A) and without (B) associated bacteria (clue cells)

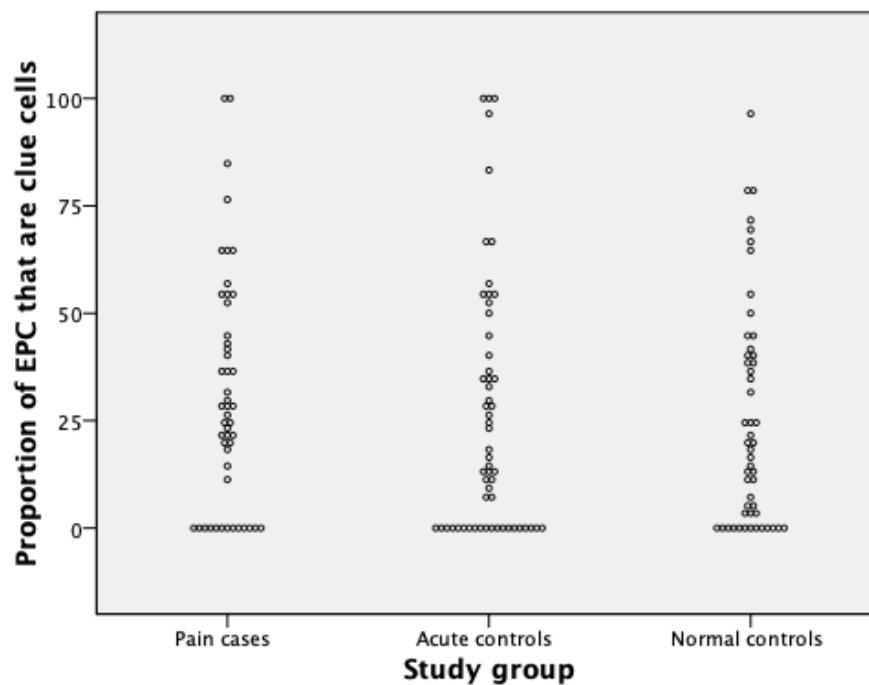


Immunofluorescence under epi-fluorescent microscopy. DAPI and WGA stain for host / pathogen DNA and cell membrane respectively. Each channel for WGA (1) and DAPI (2) shown in monochrome. Composite (3) shows DAPI in green and WGA in magenta. Each image viewed at 100x magnification. Scale bar shows 10 μ m. (A) Epithelial cell with associated bacteria, from a pain case. (B) Epithelial cell without associated bacteria, from an acute control.

Table 7-34: Epifluorescent microscopy for bacterial-associated epithelial cell proportions

Measure	Pain cases (n=49)*	Acute controls (n=57)	Normal controls (n=51)	Sig.	Overall (n=157)
Proportion (%) of epithelial cells that are clue cells					
Median	26.7	14.3	19.0	p=0.464	21.1
(95% CI)	(19.4-36.2)	(6.7-29.2)	(7.3-32.2)	Kruskal- Wallis	(14.0-27.8)
(Range)	(0 – 100)	(0 – 100)	(0 – 96)		(0 – 100)
Proportion with any clue cells (clue cell proportion > 0)					
(%)	36/49	37/57	38/51	$\chi^2=1.461$	111/157
(95% CI)	(73.5%) (58.7-84.6)	(64.9%) (51.1-76.8)	(74.5%) (60.1-85.2)	p=0.482	(70.7%) (62.8-77.6)

**One proportion was 0/0 so is excluded from analysis*

Figure 7-14: Dot plot to show proportion of epithelial cells that have associated bacteria

7.5.13.3 Discussion

There was no difference between groups in terms of the proportion of epithelial cells that had associated bacteria (clue cells). Overall, 111/157 (70.7%) of samples had at least one clue cell, again with no between-group differences. Previous studies using this technique found that 75% of patients with chronic LUTS had clue cells compared with 17% of healthy controls (Horsley, Malone-Lee et al. 2013), and that 56% of renal transplant recipient patients had clue cells (Kelley, Courtneidge et al. 2014). The discrepancy in the proportion of clue cells between those healthy controls and the normal controls in this study requires further understanding. It is possible that shedding of colonised urothelial cells is higher in pregnancy, or it may represent an increase in colonisation, or a difference in technique.

The appearance of clue cells does not confirm that bacteria are intracellular, or a biofilm formation. However, it is support for the hypothesis that epithelial cell shedding may be one means of clearing microbes from the bladder.

As far as we know, this series of studies is the first report of bacterial-associated urothelial cells in pregnancy and serves as a baseline for comparison for future research.

The staining in this method does not confirm a urothelial origin for the epithelial cells. That requires uroplakin staining, which was performed as a subset of the entire cohort.

7.5.14 Uroplakin-positive clue cells

7.5.14.1 Methods

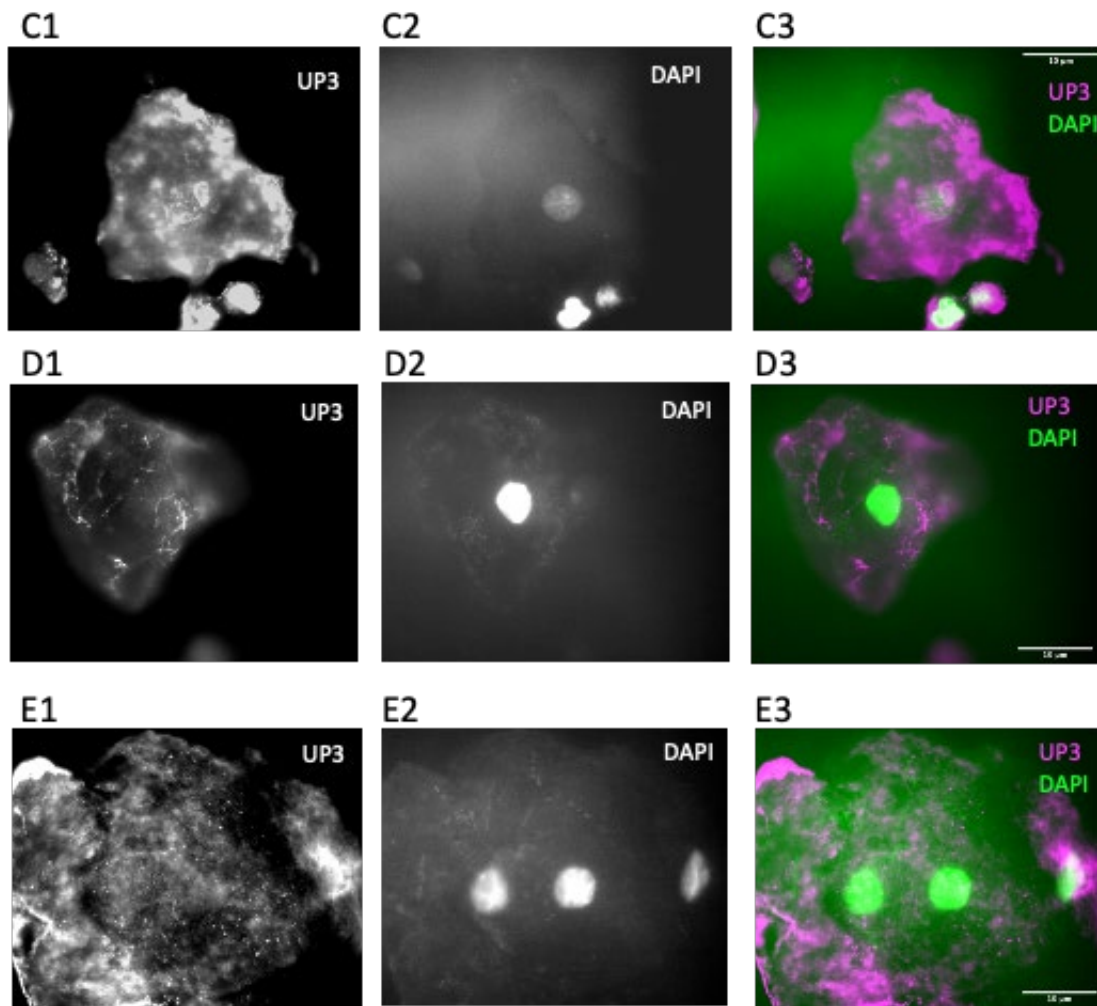
For methods see 6.16.8. 20 slides underwent uroplakin-staining, however two were unreadable (one from Pain cases, one from Acute controls).

7.5.14.2 Results

Figure 7-15 shows uroplakin-3 stained epithelial cells (urothelial cells). These are without associated bacteria (C) and with associated bacteria (D and E).

Table 7-35 shows results of uroplakin-stained epifluorescent analysis of epithelial cells. The median proportion of epithelial cells that were uroplakin-3-positive was 100% (95% CI 94-100), with no difference between groups ($p=0.864$). The median proportion of uroplakin-3-positive epithelial cells that were clue cells (had associated bacteria) was 1.3% (95% CI 0-31). 9/18 (50%) slides had any uroplakin-3-positive clue cells.

The proportion of urothelial cells with associated bacteria was 1.3% (95% CI 0-31), compared with 21.1% (95% CI 14-28) for simple clue cells. The proportion of samples with any urothelial cells with associated bacteria was 9/18 (50%, 95% CI 27-73), compared with 15/18 (83.3%, 95% CI 58-96) for simple clue cells for the same samples. There was no difference in distribution, however (Fisher's Exact, $p=0.206$).

Figure 7-15: Uroplakin-3 stained epithelial cells (urothelial cells)

Immunofluorescence under epi-fluorescent microscopy. DAPI and UP3 (uroplakin-3) stain for host / pathogen DNA and urothelial surface membrane glycoprotein respectively. Each channel for UP3 (1) and DAPI (2) shown in monochrome. Composite (3) shows DAPI in green and UP3 in magenta. Each image viewed at 100x magnification. Scale bar shows 10 μ m. (C) Urothelial cell without associated bacteria, from an acute control. (D) Urothelial cell with associated bacteria, from a normal control. (E) Two overlapping urothelial cells with associated bacteria, from a normal control.

Table 7-35: Uroplakin-positive epithelial cell proportions

Measure	Pain cases (n=2)	Acute controls (n=9)	Normal controls (n=7)	Sig.	Overall (n=18)
Proportion of uroplakin-3 positive epithelial cells (median %) (95% CI) (Range)	93.6 (87-100) (87.1–100)	100 (92-100) (90–100)	100 (43-100) 42.9–100)	p=0.864 Kruskal- Wallis	100 (94-100) (42.9–100)
Proportion of uroplakin-3 positive epithelial cells that are clue cells (median %) (95% CI) (Range)	10.0 (0-20) (0–20)	2.7 (0-55) (0–100)	0.0 (0-67) (0–66.7)	p=0.914 Kruskal- Wallis	1.3 (0-31) (0–100)
Proportion of uroplakin-3 positive epithelial cells with any associated bacteria (clue cell proportion > 0) (%) (95% CI)	1/2 (50%) (3-97)	5/9 (55.6%) (23-85)	3/7 (42.9%) (12-80)	Fisher's Exact p=1.000	9/18 (50%) (27-73)

7.5.14.3 Discussion

There was a high proportion of uroplakin-3 positive epithelial cells, demonstrating urothelial origin of the shed epithelial cells in this subset of samples.

The proportion of slides with any uroplakin-3-positive clue cells was comparable with simple clue cell counting. It should be noted that this was based on a small sample size with a high risk of type II error.

7.6 Sub-group analysis comparing alternative tests according to clinical diagnosis of UTI

I performed a secondary analysis of pain cases and acute controls, comparing women who were diagnosed with UTI on the day of recruitment against those who were not diagnosed with UTI (Table 7-36). This showed that IL-8, lactoferrin and fresh microscopy white cell counts were higher in those diagnosed with UTI. This applied for cytokines normalised for protein as well. Applying a Bonferroni correction ($p < 0.004$) the difference in lactoferrin remained significant.

Table 7-36: Comparison of alternative tests according to clinical diagnosis of UTI

Median (95% CI of median)	Diagnosed with UTI (n=20)	Not diagnosed with UTI (n=74)	Significance
Total LUTS	8 (6-11)	5.5 (4-8)	MWU=887 p=0.173
24-hour frequency	11 (8-14)	9 (8-11)	MWU=859 p=0.110
Quality of life %VAS	75 (60-84)	75 (70-80)	MWU=674 p=0.922
Quality of life index value	0.77 (0.71-0.84)	0.84 (0.80-0.91)	MWU=575 p=0.217
White cell count	28 (14-78)	8 (6-16)	MWU=1038 p=0.006
Epithelial cell count	24 (10-50)	14 (7-20)	MWU=885 p=0.180
ATP	6600 (4340-16980)	8030 (6920-8850)	MWU p=0.868
Total colony count	23330 (815-126970)	1250 (620-2900)	MWU=939 p=0.066
Number of isolates	3.5 (3-4)	3 (3-3)	MWU=891 p=0.150
IL-6	1.2 (0.5-5.0)	1.9 (1.3-3.2)	MWU=592 p=0.171
IL-8	191 (53-471)	34 (17-70)	MWU=999 p=0.017
Lactoferrin	21.7 (8.7-36.7)	4.1 (2.2-6.1)	MWU=1098 p=0.001

* $p < 0.05$ Bonferroni correction significance = $0.05/12 = 0.004$

We should remember that there was no difference in the proportion that were positive by standard culture. This may reflect that diagnosis was likely to be influenced by dipstick leucocyte esterase, or it may reflect clinical gestalt (Cervellin, Borghi et al. 2014).

7.7 Conclusions

I argued at the beginning of this chapter that abdominal pain could provide a paradigm for exploring UTI in pregnancy, without making assumptions about what defines UTI. Importantly, such a group would be expected to include some women with infective urinary pathology.

The primary hypothesis of the Abdominal pain study was that pregnant women with abdominal pain may have urinary pathology not detected by standard tests. I set out to answer this by comparing standard and alternative urinary tests in women with abdominal pain with acute controls who had other presenting symptoms, and normal controls attending routine antenatal care. The answer to this is that all groups had findings suggestive of urinary pathology not detected by standard tests.

Symptoms were prevalent in all groups, with scores equivalent to those seen in patients with chronic LUTS. Pain cases reported more pain symptoms and nocturia, but there were no other differences between groups. Despite this, pain cases were more likely to be diagnosed with UTI and prescribed antibiotics than acute controls, despite equivalent rates of positive standard culture. Interestingly, pain cases diagnosed with UTI had higher urinary IL-8, lactoferrin and fresh microscopy white cell counts, which may validate the clinical diagnosis of UTI.

There were a few other differences between the three groups using the alternative tests, particularly a different distribution of genera and species using enhanced culture, and urinary cytokines IL-8 and lactoferrin.

The other noticeable finding from these data are the ranges of results seen. There was a marked range in symptom scores and inflammatory markers such as cell counts, ATP and urinary cytokines. This demands further exploration to understand the pathophysiology and clinical relevance of this. Polymicrobial enhanced cultures were almost universal, supporting the concept of the (non-sterile) bladder microbiome in pregnancy.

As a cross-sectional study, strength of conclusions is likely to be limited, and although comparison was made with clinical outcomes, in a study of this size it is not possible to reach firm conclusions about to what extent this prevalence

of urinary inflammatory and microbiological signals represents pathological processes. However this study also provides important baseline work about the role in pregnancy of alternative tests.

I hypothesised that epithelial cells in the urine may represent urothelial cells and in the subset of cases that underwent uroplakin-3 testing, a urothelial origin was confirmed. This questions the assumption that epithelial cells must represent contamination.

This is the first study to report values for ATP, urinary lactoferrin and bacterial-associated epithelial cells (clue cells) in pregnancy. It is also unusual in taking a multifaceted and open-minded approach to UTI in pregnancy, incorporating clinical outcome data, symptoms and patient experience, as well as microbiological and immunological tests.

I approached this research with a critical appreciation of the current standard tests for UTI in pregnancy. The use of alternative tests, well validated in a chronic UTI research programme, has not helped to clarify the situation. The data raise questions about current testing practice and the assumptions that drive them.

8 Urinary pathology in women at risk of preterm birth, using standard and alternative tests; a prospective case-control study ('PTB study')

8.1 Introduction

UTI has been associated with preterm birth since Kass' work. Recent meta-analyses have described only a weak association between asymptomatic bacteriuria and preterm birth; symptomatic UTI has been implicated in some cases of preterm birth. If a subset of women at risk of preterm birth have urinary pathology, this would open a seam of new research. We chose to study standard and alternative tests for UTI in women at risk of preterm birth to (1) understand if there is urinary pathology not detected by standard tests in such women and (2) understand the relationship between standard and alternative tests (this will be addressed in chapter 10).

8.2 Aims

To compare the prevalence of urinary pathology in (1) women at risk of preterm birth owing to previous preterm birth (HR-PTB) (2) women at risk of preterm birth owing to other risk factors (HR-non-PTB) (3) women at low risk of preterm birth owing to previous term births (LR).

Hypothesis:

Women at increased risk for preterm birth may have urinary pathology detected using alternative tests.

Research questions:

- (1) Compare the prevalence of urinary pathology using standard and alternative methods:
 - a. Structured lower urinary tract symptoms assessment using Artemis
 - b. Standard dipstick, microscopy and culture
 - c. Fresh unspun urine microscopy
 - d. Urinary ATP measurement

- e. Sediment culture with counts and identification to species level of cultured organisms
- f. Urinary cytokines – IL-6, IL-8, lactoferrin
- g. Bacterial-associated clue cell proportions

8.3 Methods

Methods for the PTB study were the same as for the Abdominal pain study except for the elements described below.

8.3.1 Tests

It is important to note the chronology of the studies. Recruitment to the PTB study started first. Initially the protocol did not include quality of life assessment or species-level identification from enhanced culture (just genus-level identification). When development of the Abdominal pain study started, it was clear that it would be beneficial to follow this enhanced protocol, to maximise learning from the study and also to facilitate pooling of results between studies.

8.3.1.1 Quality of life

Quality of life assessment (using EQ-5D-5L) was collected only from 12/80 PTB study patients, owing to the late introduction of this test. I have not therefore included these data in this analysis although will include them in the pooled data in chapter 10.

8.3.1.2 Enhanced culture

At the start of the PTB study I did not freeze isolates for subsequent species identification by API, and based isolate genus analysis on bench testing using chromogenic agar, catalase and indole assays. Part-way through I started freezing isolates for subsequent API testing, as would occur for the Abdominal pain study. Therefore, I have analysed separately bench level identification (largely genus) and API analysis (species level).

8.3.1.3 Bacterial-associated epithelial cell proportions

Clue cell slides were counted in a batch at the end of the study, all using the 'count-to-20' method described in 6.16.8 to avoid any change in methodology affecting results.

8.3.2 PTB study – participants

8.3.2.1 Eligibility criteria

Eligibility included pregnant women (≥ 18 years of age) presenting to the UCLH preterm birth clinic with risk factors for preterm birth, or multiparous women without risk factors attending a routine ANC/US appointment, all at 13-17 weeks of gestation.

Following the earlier pilot study, a recruitment checklist (see Appendix 13.1) was created (by Dr Penny James), as it was found that some patients did not meet eligibility criteria. This included gestation, parity, details of preterm birth risk factors and some potential confounders for preterm birth including postcode, occupation, ethnicity, BMI and smoking status.

8.3.2.2 Exclusion criteria

Cases: Unlike the Abdominal pain study, women not booking at UCLH were not excluded. The preterm birth clinic is a tertiary referral service so were from disparate health authorities. All controls were booked at UCLH.

Potential normal controls were excluded if meeting acceptance criteria for the preterm birth clinic, or if nulliparous.

8.3.2.3 Deviation from planned protocol

Originally it was planned to recruit 34 participants into six sub-groups, representing particular risk factors for preterm birth, and I intended to include participants from the pilot study. It transpired that there were insufficient stored samples and inconsistent recruitment for the pilot study. Because of time imperatives, recruitment to the Abdominal pain study was prioritised. In the end recruitment was just not achievable for this ambition so I modified my goals: High risk of PTB, for reasons other than previous preterm birth were

combined into one group, to compare against high risk because of previous preterm birth.

8.3.2.4 Methods of case ascertainment

Study groups were allocated on the basis of risk factors for preterm birth.

8.3.2.5 Case recruitment

To reduce recruitment bias, consecutive recruitment of patients meeting recruitment criteria, by appointment time, was used, on days when a researcher was present for immediate sample processing.

8.3.2.6 Control selection

Recruitment was from midwife-led antenatal clinics. These routine appointments clustered at specific gestations according to the maternity care schedule. Women between 13-17 weeks were most likely to be attending a 16-week appointment, at a fixed time which was problematic for recruitment. Recruitment at this gestation was therefore challenging and 1-1 matched recruitment was not possible.

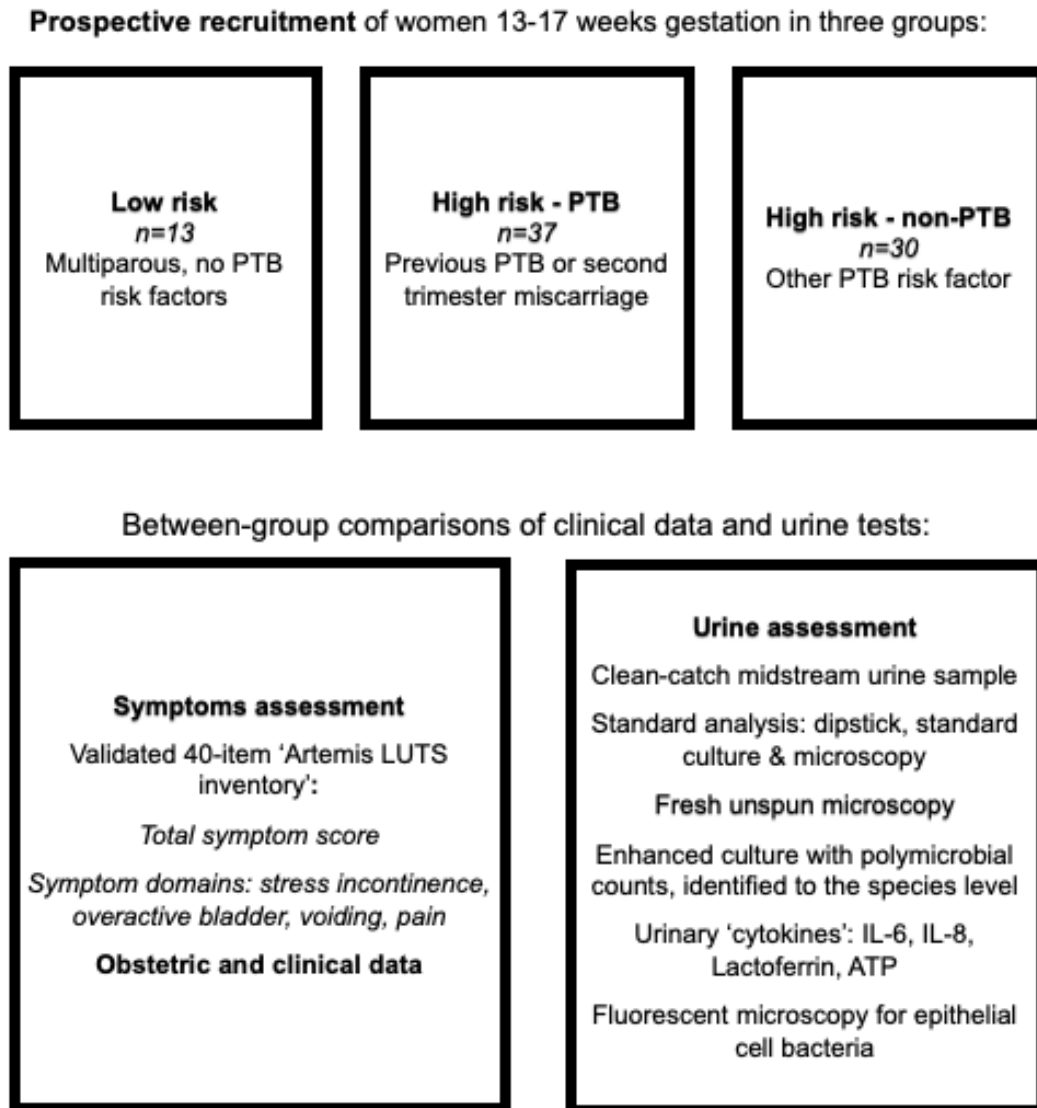
8.3.2.7 Confounding issues

Parity may be a confounding factor. LR and HR-PTB were either multiparous or had second trimester deliveries; HR-non-PTB could be nulliparous.

8.3.2.8 Study design

The study design is demonstrated in Figure 8-1.

Figure 8-1: Diagram to summarise study design and methods for the PTB study



8.4 Demographics, medical and obstetric risk factors, clinical outcomes and group characteristics

8.4.1 Demographics

Initially, cases were recruited into groups depending on their risk factors for preterm birth: low risk (n=13), previous preterm birth (n=34), previous cone biopsy (n=6), previous LLETZ (n=17), uterine anomaly (n=3), and other (including combinations of risk factors, n=7). As described above previously, these were collapsed into: low risk (LR, n=13); high risk with previous preterm birth (HR-PTB, n=37); high risk without previous preterm birth (HR-non-PTB, n=30).

Demographic features in the three groups are compared in Table 8-1. Median gestational age was 114 days (95% CI 111-117, range 92-125), equivalent to 16⁺² weeks (range 13⁺¹ to 17⁺⁶). There was no difference between groups (p=0.074).

Median maternal age at recruitment was 36.2 years (95% CI 35-37.3, range 27.0–49.6 years), with no difference between groups (p=0.721).

55/80 (68.8%) participants lived in the 50% most deprived postcodes, with no difference between groups.

7/13 (53.8%) of LR group were from a non-white ethnic group, compared with 15/37 (40.5%) HR-PTB group, and 3/30 (10%) HR-non-PTB group. This was significantly different (p=0.002).

Table 8-1: Demographic features for PTB study

Variable	Low risk (n=13)	High risk – PTB (n=37)	High risk – non-PTB (n=30)	Sig.	Overall (n=80)
Gestation at recruitment (days) (95%CI)	117.0 (110-121)	110 (107-116)	115.5 (114-117)	p=0.074 Kruskal- Wallis	114 (111-117)
Age (years) (95% CI)	37.4 (32.7-38.3)	35.5 (34.1-38.1)	36.5 (33.0-37.3)	p=0.721 Kruskal- Wallis	36.2 (35-37.3)
Socio-economic status (proportion in most deprived 50% of deciles) (%) (95% CI)	7/13 (53.8%) (26.1-79.6)	28/37 (75.7%) (58.5-87.6)	20/30 (66.7%) (47.1-82.1)	Fisher's Exact p=0.304	55/80 (68.8%) (57.3-78.4)
Ethnicity (proportion of non-white ethnic groups) (%) (95% CI)	7/13 (53.9%) (26.1-79.6)	15/37 (40.5%) (25.2-57.8)	3/30 (10%) (2.6-27.7)	Fisher's Exact p=0.002	25/80 (31.3%) (21.6-42.7)

8.4.2 Medical and obstetric risk factors

Table 8-2 shows clinical features of participants.

Parity differed between the three groups ($p < 0.001$). HR-PTB included 8/37 (21.6%) technically nulliparous women, who had miscarried between 16 and 23 weeks. 18/30 (60%) women in the HR-non-PTB group were nulliparous.

Median BMI in LR group was 24.1kg/m², compared with 24.8kg/m² in the HR-PTB group and 22.0kg/m² in HR-non-PTB group. This was significantly different ($p = 0.041$) although confidence intervals were overlapping.

UTI risk factors differed between the groups ($p=0.010$). They were present in none of the LR group, 11/35 (31.4%) HR-PTB group, and 2/25 (8%) HR-non-PTB group, although confidence intervals were wide and overlapping. In HR-PTB the risk factors were recurrent UTI pre-pregnancy ($n=9$) and UTI in this pregnancy ($n=2$). In HR-non-PTB the risk factors were recurrent UTI pre-pregnancy ($n=1$) and UTI this pregnancy (1).

7/80 (8.8%) of participants were non-smokers at recruitment, with no difference between groups. All pregnancies were singleton.

Table 8-2: Clinical features of participants (medical and obstetric history and risk factors)

Variable	Low risk ($n=13$)	High risk – PTB ($n=37$)	High risk – non-PTB ($n=30$)	Sig.	Overall ($n=80$)
Nulliparous (%) (95% CI)	0/13 (0%) (0-28.3)	8/37 (21.6%) (20.4-38.7)	18/30 (60%) (40.1-76.8)	Fisher's Exact $p<0.001^*$	26/80 (32.5%) (22.7-44.0)
Median body mass index (kg/m^2) (95% CI)	24.1 (21.2-28.0)	24.8 (22.9-26.1)	22.0 (21.0-24.3)	$p=0.041^*$ Kruskal- Wallis	23.8 (22.2-25.4)
UTI risk factors present (%) (95% CI)	0/13 (0%) (0-28.3)	11/35 (31.4%) (17.4-49.4)	2/25 (8%) (1.4-27.5)	Fisher's Exact $p=0.010^*$	13/73 (17.8%) (10.2-28.9)
Current smokers (%) (95% CI)	1/13 (7.7%) (0.4-37.9)	4/37 (10.8%) (3.5-26.4)	2/30 (6.7%) (1.2-23.5)	Fisher's Exact $p=0.871$	7/80 (8.8%) (3.9-17.8)

Table 8-3 shows clinical information originating from the preterm birth clinic (therefore HR-PTB and HR-non-PTB groups only).

18/80 (22.5%) of participants had cervical cerclage in the index pregnancy, all of whom were in the HR-PTB group (18/37, 48.6%). Two cerclages were inserted abdominally pre-pregnancy, the rest between 12 and 19 weeks. 14/18 (77.8%) had been inserted prior to recruitment.

18/79 participants took progesterone, 15/36 (41.7%) in HR-PTB and 3/30 (10%) in HR-non-PTB. In 6 cases this was from first trimester and the other 12 from second trimester.

Median number of cervical length scans was 5 (95% CI 4-6) in the HR-PTB group and 2.5 (95% CI 2-3) in the HR-non-PTB group. This was different between groups ($p < 0.001$).

Shortest cervical length was less than 15mm for 1/37 (2.7%) HR-PTB and 0/30 (0%) HR-non-PTB ($p = 1.000$). Shortest cervical length was at least 15mm but less than 25mm for 6/37 (16.2%) HR-PTB group and 3/30 (10%) HR-non-PTB group ($p = 0.500$). The median shortest ever measured cervical length was 29mm (95% CI 28-30, range 0-44, no between-group difference, $p = 0.263$).

Fetal fibronectin was performed in 33/37 (89.2%) HR-PTB and 22/30 (73.3%) HR-non-PTB. 8 participants had 2 fetal fibronectin tests and 3 had 3. This result was qualitatively positive in 3/33 (9.1%), all from the HR-PTB group. Quantitative results did not differ between groups ($p = 0.077$), with median 7.0 (95% CI 3-14, range 0-144) for HR-PTB and 3.0 (95% CI 3-5, range 2-7) for HR-non-PTB.

Table 8-3: Preterm birth clinic-specific clinical information and outcomes

Variable	High risk – PTB (n=37)	High risk – non-PTB (n=30)
Cervical cerclage this pregnancy (%) (95% CI)	18/37 (48.6%) (32.2-65.3)	0/30 (0%) (0-14.1)
Progesterone treatment this pregnancy (%) (95% CI)	15/36 (41.7%) (26.0-59.1)	3/30 (10%) (2.6-27.7)
Scans in PTB clinic (95% CI) (Range)	5 (4-6) (1-9)	2.5 (2-3) (1-8)
Cervix less than 15mm (%) (95% CI)	1/37 (2.7%) (0.1-15.8)	0/30 (0%) (0-14.1)
Cervix 15 – 25mm (%) (95% CI)	6/37 (16.2%) (6.8-32.7)	3/30 (10%) (2.6-27.7)
Shortest ever measured cervix (mm) (95% CI) (Range)	29.0 (27-30) (15-44)	29.0 (28-32) (19-42)
Fetal fibronectin (fFN) test done (%) (95% CI)	33/37 (89.2%) (73.6-96.5)	22/30 (73.3%) (53.8-87.0)
fFN result positive (%) (95% CI)	3/33 (9.1%) (2.4-25.5)	0/22 (0%) (0-18.5)
fFN value (95% CI) (Range)	(n=32) 7.0 (3-14) (0-144)	(n=19) 3.0 (3-5) (2-7)

8.4.3 Obstetric and neonatal outcomes

Table 8-4 shows obstetric and neonatal outcomes. Delivery information was incomplete for four external patients who were uncontactable for follow-up.

3/76 participants experienced preterm prelabour rupture of membranes (PPROM), all in HR-PTB group.

Median gestation at delivery differed between groups ($p=0.013$). Median gestation in LR group was 274 days (95% CI 265-278), compared with 269 days (95% CI 263-275) in HR-PTB and 279 days (95% CI 273-282) in HR-non-PTB (see Figure 8-2).

The overall rate of preterm birth was 11/77 (14.3%), with 2/12 (16.7%) in LR group, 9/36 (25%) in HR-PTB, and 0/29 (0%) in HR-non-PTB; this was different between groups ($p=0.005$).

The two preterm births in the LR group were spontaneous. One occurred in a woman whose first delivery was preterm at 36+6 weeks, which the patient did not mention at booking, was not known at recruitment to this study, and which was only discovered incidentally. This would not have met criteria for preterm birth clinic referral as it would not have been considered high risk for preterm birth. The patient was therefore kept in the planned study analysis.

Of the nine preterm births in the HR-PTB group, four were due to PPRM or spontaneous preterm labour; the rest were medically indicated.

Median time from recruitment to delivery overall was 161 days (range 8 to 185), with no significant difference between groups ($p=0.074$).

There were no between-group differences in induction of labour or mode of delivery. There were no differences in other outcomes including suspected intrapartum, postpartum and neonatal sepsis, admission to NNU, birthweight or Apgars.

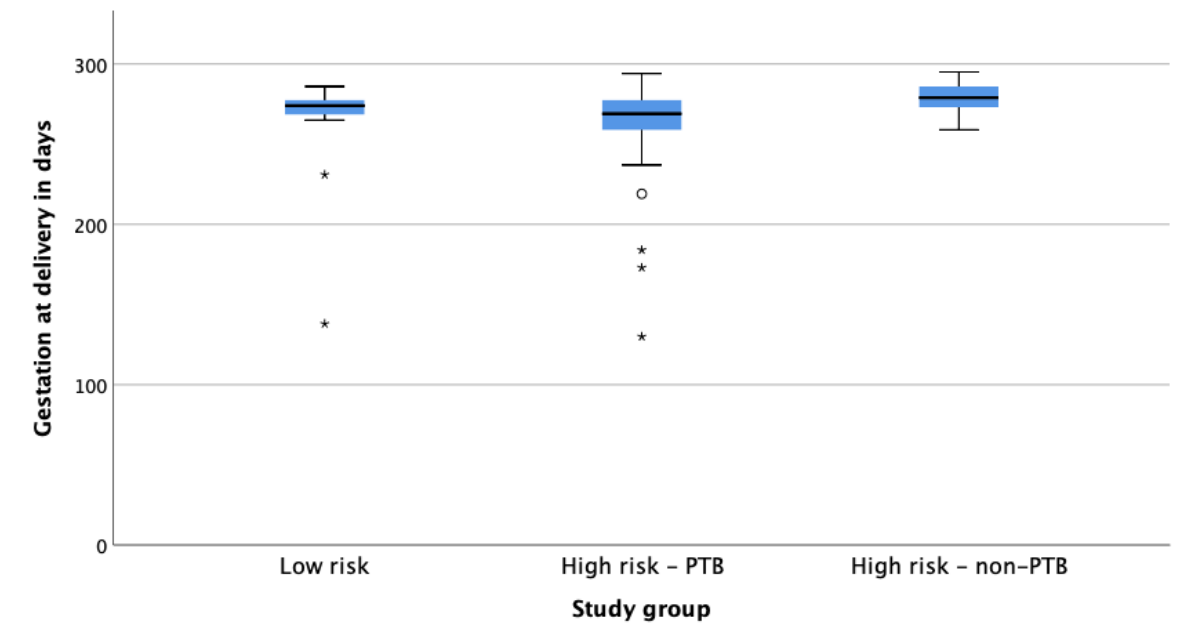
Table 8-4: Obstetric and neonatal outcomes for PTB study

	Low risk (n=13)	High risk – PTB (n=37)	High risk – non-PTB (n=30)	Significance
PPROM this pregnancy (%) (95% CI)	(n=12) 0/12 (0%) (0-30.1)	(n=34) 3/34 (8.8) (2.3-24.8)	(n=30) 0/30 (0%) (0-14.1)	Fisher's Exact p=0.280
Gestation at delivery (days) Median (95% CI) (Range)	(n=12) 274 (265-278) (138–286)	(n=36) 269 (263-275) (130–294)	(n=29) 279 (273-282) (259–295)	p=0.013 Kruskal-Wallis
PTB or second trimester miscarriage this pregnancy (%) (95% CI)	(n=12) 2/12 (16.7%) (2.9-49.1)	(n=37) 9/37 (24.3%) (12.4-41.6)	(n=29) 0/29 (0%) (0-14.6)	Fisher's Exact p=0.005
Spontaneous PTB this pregnancy (%) (95% CI)	(n=12) 2/12 (16.7%) (2.9-49.1)	(n=37) 4/37 (10.8%) (3.5-26.4)	(n=29) 0/29 (0%) (0-14.6)	Fisher's Exact p=0.054
Days from recruitment to delivery (days) Median (95% CI) (Range)	(n=12) 158.0 (148-165) (16–166)	(n=36) 158.5 (146-168) (8–184)	(n=29) 162.0 (159-170) (135–185)	p=0.074 Kruskal-Wallis
Induction of labour (proportion induced) (%) (95% CI)	(n=10) 0/10 (0%) (0-34.5)	(n=36) 9/36 (25%) (12.7-42.5)	(n=28) 6/28 (21.4%) (9-41.5)	Fisher's Exact p=0.475
Mode of delivery	(n=11) SVD 5/11 Inst 1/11 CS 5/11	(n=36) SVD 20/36 Inst 0/36 CS 16/36	(n=29) SVD 17/29 Inst 1/29 CS 11/29	Fisher's Exact p=0.482

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	Low risk (n=13)	High risk – PTB (n=37)	High risk – non-PTB (n=30)	Significance
Suspected intrapartum sepsis (%) (95% CI)	2/10 (20%) (3.5-55.8)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.114
Suspected postnatal sepsis (%) (95% CI)	1/10 (10%) (0.5-45.9)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.407
Admission to NNU (%) (95% CI)	2/10 (20%) (3.5-55.8)	6/36 (16.7%) (7.0-33.5)	1/29 (3.5%) (0.2-19.6)	Fisher's Exact p=0.119
Apgar at 1 minute (95% CI)	(n=9) 9 (9-9)	(n=31) 9 (9-9)	(n=26) 9 (9-9)	Kruskal-Wallis p=0.691
Apgar at 5 minutes (95% CI)	(n=9) 10 (9-10)	(n=31) 10 (9-10)	(n=26) 10 (10-10)	Kruskal-Wallis p=0.464
Suspected neonatal infection (%) (95% CI)	1/10 (10%) (0.5-45.9)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.119
Birth weight median /g 95% CI	(n=11) 3360 (2100-3770)	(n=35) 3316 (2970-3610)	(n=29) 3302 (2980-3600)	Kruskal-Wallis p=0.986
Fetal or neonatal loss	1 (miscarriage)	1 (neonatal death)	0	n/a

Figure 8-2: Box-and-whisker plot comparing gestation at delivery for PTB study



8.4.4 Discussion

The intended recruitment target of 13 to 17 weeks was achieved. Women in HR-non-PTB had lower BMI, were more likely to be nulliparous, and more likely to be white ethnicity; these potential confounders would need to be assessed if any difference were to be found between groups. For example, in England and Wales, all minority ethnic groups had higher odds of preterm birth compared with White British except for 'Other white' (Li, Quigley et al. 2019).

There was no difference in smoking or socio-economic status. preterm birth rates have been found to increase with deprivation, although this effect appeared to be mediated via maternal weight and smoking status (Taylor-Robinson, Agarwal et al. 2011).

Every patient in the HR-PTB and HR-non-PTB groups had at least one cervical length scan, as per clinic protocol. Most had a fetal fibronectin. Nearly half of the HR-PTB group had a cervical cerclage, with over three quarters having it prior to recruitment. These investigations and interventions may have affected the natural history and thus gestational age at delivery.

The differences in gestation at delivery and proportions of preterm birth suggests that HR-non-PTB have a lower risk of preterm birth compared with HR-PTB. This is consistent with literature which says that the strongest risk factor for spontaneous preterm birth is previous preterm birth, with an odds ratio of 4.6-6.0 (Ferrero, Larson et al. 2016).

The rate of preterm birth in LR women was 2/13 (16.7%, 95% CI 2.9-49.1); this is such a small group with a wide confidence interval that the rate cannot be interpreted. The rate should be low: a Dutch longitudinal linked national cohort study found a preterm birth rate of 2.46% after previous term vaginal delivery and 2.79% after previous term Caesarean (Visser, Slaager et al. 2019). At the time of starting this study, the increased risk of preterm birth following previous caesarean section, particularly at advanced gestation, had not been widely reported (Glazewska-Hallin, Story et al. 2019). This data was therefore not collected and may be a confounder.

There were no cases of pyelonephritis, and no differences between groups in terms of maternal or neonatal sepsis, with only 4 possible cases of intrapartum sepsis, 3 postpartum sepsis, and 3 neonatal. Sepsis in this context includes investigation for sepsis using blood cultures owing to maternal pyrexia, and treatment with intravenous antibiotics.

Between-group comparisons with LR women are limited by the small group size; this comparison is underpowered, and lack of a difference may not be meaningful. However, the comparison between HR-PTB and HR-non-PTB is adequately powered.

Having compared demographics, risk factors and clinical outcomes, next I compared microbiological results throughout the index pregnancies.

8.5 Full microbiological results in index pregnancy

8.5.1 Standard urine cultures throughout pregnancy

Comparing booking cultures (Table 8-5), there were 33/80 (41.3%) screening culture negative, 21/80 (26.3%) non-significant growth, 18/80 (22.5%) mixed growth, and 8/80 (10%) positive, with no difference between groups ($p=0.459$).

Table 8-5: Table comparing standard culture at booking against study group

MSU result (% of study group)	LR (n=13)	HR-PTB (n=37)	HR-non- PTB (n=30)	Totals (n=80)	Significance
Screening culture negative	3 (23.1%)	18 (48.7%)	12 (40%)	33 (41.3%)	Fisher's Exact $p=0.459$
Non-significant growth	4 (30.8%)	9 (24.3%)	8 (26.7%)	21 (26.3%)	
Mixed growth	5 (38.5%)	5 (13.5%)	8 (26.7%)	18 (22.5%)	
Positive	1 (7.7%)	5 (13.5%)	2 (6.7%)	8 (10%)	
Totals	13 (100%)	37 (100%)	30 (100%)	80 (100%)	

Comparing all urine cultures throughout pregnancy (Table 8-6), 371 standard cultures were reported throughout the 80 pregnancies. Of these, 180/371 (48.5%) were screening culture negative, 92/371 (24.8%) were non-significant growth, 63/371 (17.0%) showed mixed growth of uncertain significance, and 36/371 (9.7%) were positive. This was not different between groups ($p=0.159$). This was comparable with the booking cultures.

Table 8-6: All standard urine culture results in index pregnancy compared by study group

MSU result (% of study group)	LR (n=13)	HR-PTB (n=37)	HR-non- PTB (n=30)	Totals (n=80)	Significance
Screening culture negative	14 (33.3%)	113 (48.1%)	53 (56.4%)	180 (48.5%)	$\chi^2=9.277$ p=0.159
Non-significant growth	11 (26.2%)	61 (26.0%)	20 (21.3%)	92 (24.8%)	
Mixed growth	9 (21.4%)	39 (16.6%)	15 (16.0%)	63 (17.0%)	
Positive	8 (19.1%)	22 (9.4%)	6 (6.4%)	36 (9.7%)	
Totals	42 (100%)	235 (100%)	94 (100%)	371 (100%)	

The median number of MSU samples sent per patient (Table 8-7) was different between study groups ($p<0.001$) with HR-PTB having 6 samples per patient (95% CI 4-7, range 2-13) compared with 3 per patient (95% CI 2-5, range 2–5) for LR group and 3 per patient (95% CI 2-4, range 2–7) for HR-non-PTB group. The rates of screening culture negative and NSG were higher for the HR-PTB group with no difference in rates of mixed growth and positive samples.

22/80 (27.5%) patients in the study had at least one positive MSU; this did not differ between groups.

It should be noted that there was a difference in distribution of total number of samples in the pregnancy, total number screening culture negative, and total number non-significant growth. However, for the latter, the median is 1 for each. This is unusual, and reflects properties of the median when there is a small range of responses.

Table 8-7: Full standard culture results for index pregnancy for PTB study (per patient)

Microbiological parameter	Low risk (n=13)	High risk – PTB (n=37)	High risk – non- PTB (n=30)	Sig.
Median number of samples this pregnancy (n=373) (95% CI) (Range)	3 (2-5) (2-5)	6 (4-7) (2-13)	3 (2-4) (2-7)	p<0.001 Kruskal- Wallis
Median number screening culture negative (n=180) (95% CI) (Range)	1 (1-2) (0-2)	3 (2-4) (0-7)	2 (1-2) (0-4)	p<0.001 Kruskal- Wallis
Median number of non- significant growth (n=92) (95% CI) (Range)	1 (0-1) (0-2)	1 (1-2) (0-6)	1 (0-1) (0-2)	p=0.013 Kruskal- Wallis
Median number of mixed growth (n=63) (95% CI) (Range)	0 (0-2) (0-2)	0 (0-1) (0-4)	0 (0-1) (0-3)	p=0.453 Kruskal- Wallis
Median number of positive cultures (n=36) (95% CI) (Range)	0 (0-1) (0-4)	0 (0-0) (0-5)	0 (0-0) (0-2)	p=0.191 Kruskal- Wallis
Proportion with any positive MSUs this pregnancy (%) (95% CI)	5/13 (38.5%) (15.1-67.7)	12/37 (32.4%) (18.6-49.9)	5/30 (16.7%) (6.3-35.5)	p=0.209 Fisher's Exact

8.5.2 Other microbiology results throughout pregnancy

Table 8-8 shows all other microbiology results. 3/80 (3.8%) patients had blood cultures, 1 in each group (no difference, $p=0.709$), with only 1 positive result (*Escherichia coli* in HR-non-PTB group).

144 cervico-vaginal swabs were sent for 68/80 (85%) patients during the index pregnancy which was different between groups (4/13 (30.8%) low risk, 35/37 (94.6%) HR-PTB, 29/30 (93.3%) HR-non-PTB, $p<0.001$). Patients at high risk for preterm birth (median HR-non-PTB 1.5 (95% CI 1-2), median HR-PTB 2.0 (95% CI 1-3)) had more swabs sent than low risk patients (median 0.0 (95% CI 0-1)), $p<0.001$). Of those that had swabs sent, there was no difference in the rate of positive swab results, with 12/68 (17.6%) having a swab showing *Streptococcus agalactiae* ($p=0.360$), and 4/68 (5.9%) having a swab showing anaerobes ($p=1.0$). There were no other positive swab findings.

Other microbiology sent included MRSA, faeces, perineal wound, skin wound, ear swab and placental culture, all of which were negative except a perineal swab showing mixed growth of uncertain significance. Three cervical sutures were sent for microbiology after removal, one with no significant growth, one with vaginal flora, and one with heavy growth of *Staphylococcus aureus*.

Placentas were sent for histopathology for 2/13 low risk, 8/37 HR-PTB, and 0/30 HR-non-PTB patients. 1/2 (50%) and 4/8 (50%) respectively were reported as showing chorioamnionitis or funisitis.

Table 8-8: Other microbiological results for index pregnancy for PTB study

Test	Low risk (n=13)	High risk – PTB (n=37)	High risk – non-PTB (n=30)	Sig.
Blood cultures sent during index pregnancy (NB all postpartum)	1/13 (7.7%) (0.4-37.9)	1/37 (2.7%) (0.1-15.8)	1/30 (3.3%) (0.2-19.1)	$\chi^2=0.687$ p=0.709
Number of vaginal / cervical swabs sent in index pregnancy (total = 144 swabs) Median (95% CI) (Range)	0.0 (0-1) (0-2)	2.0 (1-3) (0–9)	1.5 (1-2) (0 –5)	Kruskal- Wallis p<0.001
Any swabs sent in index pregnancy	4/13 (30.8%)	35/37 (94.6%)	29/30 (96.7%) (81.0-99.8)	Fisher's Exact p<0.001
Any GBS positive swabs	1/4 (25%)	8/35 (22.9%)	3/29 (10.3%)	Fisher's Exact p=0.360
Any positive swabs reported as non-GBS, non- <i>Candida</i> <i>spp</i>	(n=4) 0/4 (0%)	(n=35) 2/35 (5.7%)	(n=29) 2/30 (6.7%)	Fisher's Exact p=1.000
Faeces culture	0/13 (0%)	1/37 (neg) (2.7%)	0/30 (0%)	
Perineal or abdominal wound swab	2/13 (15.4%) <i>Mixed growth, no growth</i>	1/37 (2.7%) <i>No growth</i>	0/30 (0%)	
Placental swab	2/37 (neg)			
Cervical suture	3/37			
MRSA (all negative)	3/13	22/37	8/30	
Other micro	Ear swab 1/37 (neg)			
Chorioamnionitis / funisitis on placental histology (%)	(n=2) 1/2 (50%)	(n=8) 4/8 (50%)	(n=0) n/a	

8.5.3 Discussion

Given routine practice of treating asymptomatic bacteriuria in pregnancy, it is likely that women with positive cultures were prescribed antibiotics. Nearly 10% of samples were positive by standard criteria and 27.5% of patients had at least one positive result, suggesting over a quarter were prescribed antibiotics. Unfortunately, prescribing data was not collected, so this cannot be confirmed.

The median number of standard cultures for HR-PTB patients was 6. Although more MSU samples were sent, they were no more likely to have a positive result than HR-non-PTB or LR patients.

There were substantial numbers of mixed growth and non-significant growth reported, accounting for 155/373 (41.6%) samples. Whether these represent contamination, or pathology, this is concerning, for economic reasons and clinical uncertainty. It is important to understand what mixed growth and non-significant growth represent, particularly in women at increased risk of preterm birth.

High risk patients had more genital swabs sent than LR patients, consistent with clinic protocols. However, they were not more likely to have a positive result, including *Streptococcus agalactiae*, or anaerobes, and there were no other positive findings. The rate of *Streptococcus agalactiae* at 17.6% is similar to the Abdominal pain study.

3/80 (3.8%) patients, one in each group, had blood cultures reported during the index pregnancy; one set of these was positive. Blood cultures could be considered to be a proxy for suspected sepsis since local sepsis protocols during the time of this study required sending blood cultures for suspected sepsis.

There were insufficient placentas sent for histopathology to draw conclusions, however in future studies, combining histological and microbiological findings from delivered placentas would be useful.

A case control study of placentas in spontaneous and iatrogenic preterm birth found that chorioamnionitis was the main finding in spontaneous preterm birth compared with vascular malperfusion lesions in medically indicated preterm

birth (Nijman, van Vliet et al. 2016). The earlier the gestation, the more likely there was to be chorioamnionitis.

8.6 Compare the prevalence of urinary pathology using standard and alternative methods

8.6.1 Introduction

In this section I present data comparing the prevalence of urinary pathology in pregnant women with (1) high risk of preterm birth owing to previous preterm birth (HR-PTB), (2) high risk of preterm birth owing to other risk factors (HR-non-PTB) or (3) low risk for preterm birth with previous term births (LR), using standard and alternative methods.

8.6.2 Methods

Methods for all standard and alternative tests are as described in chapter 6, unless otherwise specified.

8.6.3 Structured assessment of lower urinary tract symptoms

8.6.3.1 Results

Table 8-9 demonstrates LUTS scores for the PTB study. Across all groups, there was a wide range in total LUTS scores, from 0-17 in LR group to 0-32 in HR-PTB group.

There were no between-group differences in frequency, nocturia or incontinence. Median frequency was 6.5 (95% CI 5.5-8.5), median nocturia was 1.5 (95% CI 1.0-1.5). 28/79 (35.4%) reported any daytime incontinence, and 6/78 (7.7%) reported any night incontinence.

75/80 (93.8%) participants reported some LUTS. The commonest group of symptoms were voiding symptoms in 62/80 (77.5%), followed by overactive bladder symptoms in 50/80 (62.5%), pain symptoms in 47/80 (58.8%) and stress incontinence symptoms in 36/80 (45%). There were no between-group differences.

Median stress incontinence score was 0/7 (95% CI 0-1), overactive bladder was 1/12 (95% CI 1-2), voiding was 1.5/8 (95% CI 1-2) and pain was 1/13 (95% CI 0-2). There were no between group differences.

Figure 8-3 demonstrates individual LUTS scores compared across groups. The commonest specific symptoms were incomplete emptying (45%), bladder pain on filling (44%), cough-sneeze incontinence (41%), urinary urgency (35%), waking rising urgency (35%), double voiding (35%) and terminal dribbling (35%). There were no differences between groups.

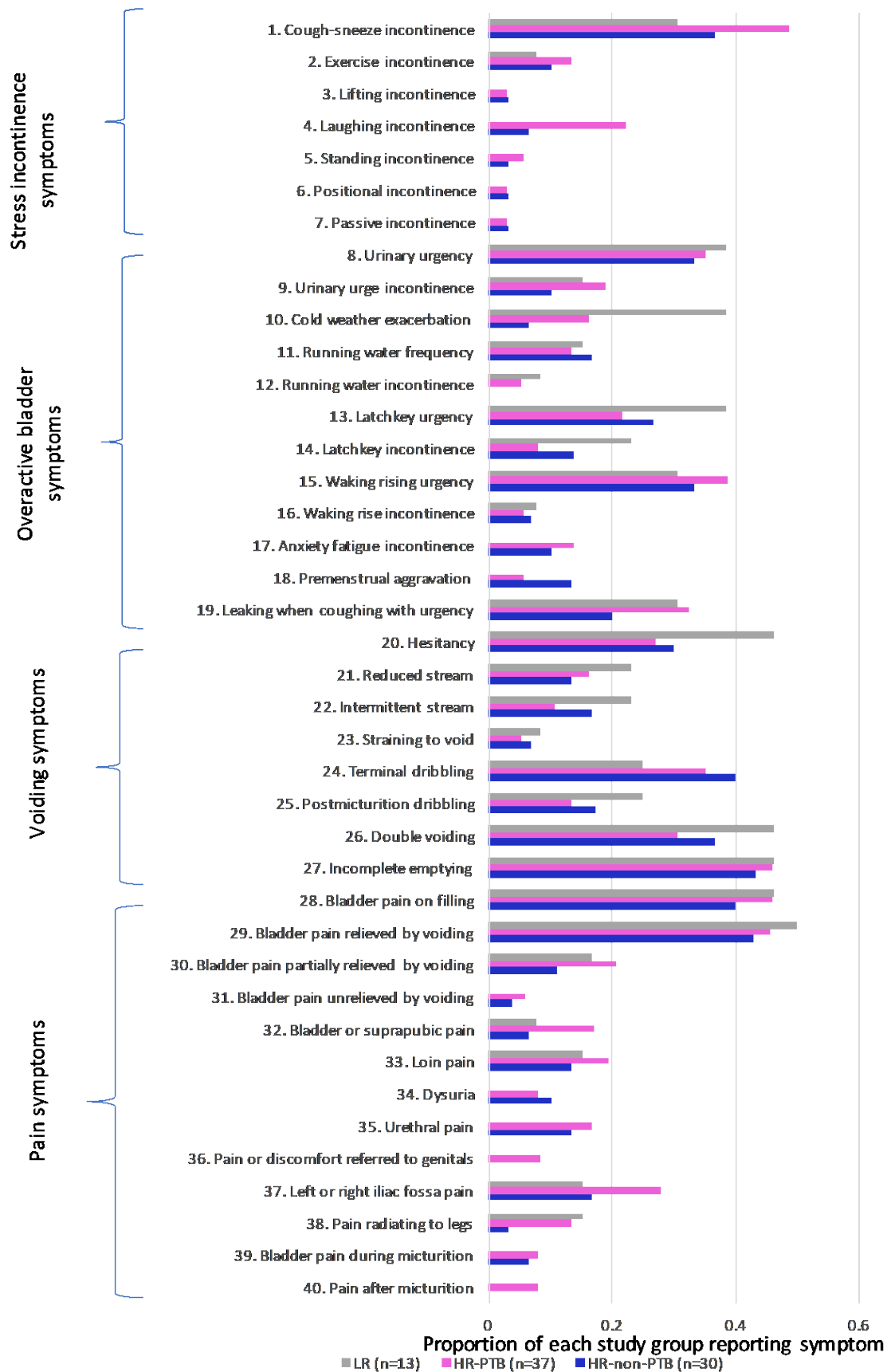
Table 8-9: LUTS for PTB study

	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Significance	Overall (n=80)
Total LUTS (max 40)					
Median	5.0	6.0	5.0	p=0.806 Kruskal- Wallis	5.0
(95% CI)	(3-12)	(4-7)	(3-8)		(4-7)
(Range)	(0–17)	(0–32)	(0–23)		(0–32)
Any LUTS (%)	12/13 (92.3%)	35/37 (94.6%)	28/30 (93.3%)	Fisher's Exact p=1.000	75/80 (93.8%)
(95% CI)	(62.1-99.6)	(80.5-99.1)	(76.5-98.8)		(85.4-97.7)
Frequency (number of episodes)		(n=36)			(n=79)
Median	5.5	7.5	7.0	p=0.417 Kruskal- Wallis	6.5
(95% CI)	(2.5-8.5)	(5.5-8.5)	(5.5-8.5)		(5.5-8.5)
(Range)	(2.5–11.5)	(2.5–17.5)	(3.5–18.5)		(2.5–18.5)
Nocturia (number of episodes)					
Median	1.0	1.5	1.25	p=0.428 Kruskal- Wallis	1.5
(95% CI)	(0.5-2.0)	(1.0-2.5)	(0.5-1.5)		(1.0-1.5)
(Range)	(0–4.5)	(0–6.5)	(0–5.5)		(0.0–6.5)
Any daytime incontinence (%)	(n=12) 3/12 (25%)	(n=37) 17/37 (45.9%)	(n=30) 8/30 (26.7%)	Fisher's Exact p=0.206	(n=79) 28/79 (35.4%)
(95% CI)	(66.9-57.2)	(29.9-62.9)	(13.0-46.2)		(25.2-47.1)
Any night incontinence (%)	(n=13) 0/13 (0%)	(n=35) 5/35 (14.3%)	(n=30) 1/30 (3.3%)	Fisher's Exact p=0.169	(n=78) 6/78 (7.8%)
(95% CI)	(0-28.3)	(5.4-31.1)	(0.2-19.1)		(3.2-16.6)
Stress incontinence (max 7)					
Median	0.0	1.0	0.0	p=0.172 Kruskal- Wallis	0.0
(95% CI)	(0-1)	(0-1)	(0-1)		(0-1)
(Range)	(0-2)	(0–5)	(0–7)		(0–7)
Any stress incontinence (%)	4/13 (30.8%)	20/37 (54.1%)	12/30 (40%)	$\chi^2=2.592$ p=0.308	36/80 (45%)
(95% CI)	(10.4-61.1)	(37.1-70.2)	(23.2-59.3)		(34.0-56.5)

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	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Significance	Overall (n=80)
Overactive bladder symptoms (max 12) Median (95% CI) (Range)	2.0 (0-6) (0–8)	1.0 (0-3) (0–10)	1.0 (0-2) (0–7)	p=0.829 Kruskal- Wallis	1.0 (1-2) (0–10)
Any overactive bladder symptoms (%) (95% CI)	9/13 (69.2%) (38.9-89.6)	22/37 (59.5%) (42.2-74.8)	19/30 (63.3%) (43.9-79.5)	Fisher's Exact p=0.864	50/80 (62.5%) (50.9-72.9)
Voiding symptoms (max 8) Median (95% CI) (Range)	3.0 (0-4) (0–5)	1.0 (1-2) (0–7)	2.0 (1-2) (0–7)	p=0.633 Kruskal- Wallis	1.5 (1-2) (0–7)
Any voiding symptoms (%) (95% CI)	10/13 (76.9%) (46.0-93.8)	30/37 (81.1%) (64.3-91.4)	22/30 (73.3%) (53.8-87.0)	Fisher's Exact p=0.772	62/80 (77.5%) (66.5-85.8)
Pain symptoms (max 13) Median (95% CI) (Range)	1.0 (0-3) (0–6)	1.0 (0-3) (0–13)	1.0 (0-2) (0–9)	p=0.836 Kruskal- Wallis	1.0 (0-2) (0–13)
Any pain symptoms (%) (95% CI)	8/13 (61.5%) (32.3-84.9)	21/37 (56.8%) (39.7-72.5)	18/30 (60%) (40.8-76.8)	$\chi^2=0.12$ p=0.955	47/80 (58.8%) (47.2-69.5)

Figure 8-3: Detailed LUTS compared across study groups



8.6.3.2 Discussion

There were no differences between groups for LUTS, despite the difference in preterm birth rates. This may reflect a lack of urinary pathology contributing to pathophysiology of preterm birth. It may also reflect the small group sizes with wide confidence intervals.

The range of LUTS scores is again very wide. While a few patients reported no symptoms, most reported some, and some reported multiple symptoms. This requires further exploration of associated factors.

Having compared LUTS, I now compare standard urine tests at recruitment.

8.6.4 Dipstick

8.6.4.1 Results

Table 8-10 compares dipstick results. 46/80 (57.5%) samples had a dipstick positive for nitrite or at least trace of leucocyte esterase or protein. 33/80 (41.3%) samples were positive for leucocytes, 27/80 (33.8%) positive for protein, and no samples were nitrite positive. 10/80 (12.5%) samples were positive for blood. There were no between-group differences.

Table 8-10: Dipstick results for PTB study

Measure	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Sig.	Overall (n=80)
Leucocyte esterase (≥ trace) (%) (95% CI)	3/13 (23.1%) (6.2-54.0)	13/37 (35.1%) (20.7-52.6)	17/30 (56.7%) (37.7-74.0)	Fisher's Exact p=0.078	33/80 (41.3%) (30.5-52.8)
Blood (≥ trace) (%) (95% CI)	0/13 (0%) (0-28.3)	7/37 (18.9%) (8.6-35.7)	3/30 (10%) (2.6-27.7)	Fisher's Exact p=0.222	10/80 (12.5%) (6.5-22.2)
Protein (≥ trace) (%) (95% CI)	4/13 (30.8%) (10.4-61.1)	14/37 (37.8%) (22.9-55.2)	9/30 (30%) (15.4-49.6)	Fisher's Exact p=0.814	27/80 (33.8%) (23.8-45.3)
Nitrite (%) (95% CI)	0/13 (0%) (0-28.3)	0/37 (0%) (0-11.7)	0/30 (0%) (0-14.1)	n/a	0/80 (0%) (0-5.7)
Dipstick positive for leu/pro/nit (%) (95% CI)	5/13 (38.5%) (15.1-67.7)	19/37 (51.4%) (34.7-67.8)	21/30 (70%) (50.4-84.6)	$\chi^2=4.337$ p=0.117	45/80 (56.3%) (44.7-67.2)

8.6.4.2 Discussion

There were no between-group differences on any parameters of dipstick testing. Comparison of dipstick against other tests will be part of a synthesis of the three studies.

8.6.5 Standard microscopy and culture

8.6.5.1 Results

Table 8-11 shows standard microscopy and culture results.

Very few samples underwent standard lab microscopy; all 5 were HR-PTB. Epithelial cells were seen in all samples with white cells in 2/5 (40%) and red cells in 3/5 (60%).

Positive cultures were present in LR group for 2/14 (14.3%, 95% CI 2.7-46.3), HR-PTB group for 0/34 (0%, 95% CI 0-12.6) and HR-non-PTB group for 1/28 (3.6%, 95% CI 0.2-19.6). This was different between groups ($p=0.036$) although confidence intervals were wide.

Table 8-12 shows detailed culture results. Of the positive cultures, one was *Enterococcus spp* (HR-non-PTB) and two were *E. coli* (LR). 7/76 (9.2%, 95% CI 4.1-18.6) were mixed growth of uncertain significance, and 17/76 (22.4%, 13.9-33.7) were non-significant growth, with 49/76 (64.5%, 95% CI 52.6-74.9) screening culture negative. The distribution of detailed culture results did not differ between groups ($p=0.215$).

Table 8-11: Standard microscopy and culture for PTB study

Measure	LR (n=13)	HR-PTB (n=37)	HR- non-PTB (n=30)	Significance	Overall (n=80)
Proportion where microscopy performed (%) (95% CI)	0/13 (0%) (0-28.3)	5/37 (13.5%) (5.1-29.6)	0/30 (0%) (0-14.1)	Fisher's Exact p=0.080	5/80 (6.3%) (2.3-14.6)
White cells (any white cells seen) (%) (95% CI)	(n=0)	(n=5) 2/5 (40%) (7.3-83.0)	(n=0)	n/a	(n=5) 2/5 (40%) (7.3-83.0)
Red blood cells (any red cells seen) (%) (95% CI)	(n=0)	(n=5) 3/5 (60%) (17.0-92.7)	(n=0)	n/a	(n=5) 3/5 (60%) (17.0-92.7)
Epithelial cells (any epithelial cells seen) (%) (95% CI)	(n=0)	(n=5) 5/5 (100%) (46.3-100)	(n=0)	n/a	(n=5) 5/5 (100%) (46.3-100)
Culture positive >10 ⁵ CFU/ml (%) (95% CI)	(n=13) 2/13 (15.4%) (2.7-46.3)	(n=34) 0/34 (0%) (0-12.6)	(n=29) 1/29 (3.4%) (0.2-19.6)	Fisher's Exact p=0.036	(n=76) 3/76 (3.9%) (1.0-11.9)

Table 8-12: Detailed standard culture for PTB study

Measure	LR (n=13)	HR-PTB (n=37)	HR- non-PTB (n=30)	Totals
>10 ⁵ CFU/ml <i>Enterococcus</i>	0	0	1	1
>10 ⁵ CFU/ml <i>Escherichia coli</i>	2	0	0	2
A mixed growth of uncertain significance	2	4	1	7
No significant growth	3	8	6	17
Screening culture negative	6	22	21	49
Totals	13	34	29	76

Fisher's Exact p=0.215

8.6.5.2 Discussion

The lack of standard microscopy results was due to the laboratory policy about when to run microscopy in pregnancy, as described previously.

There were only 3/76 (3.9%, 95% CI 1.0-11.9) positive cultures overall, with none in the HR-PTB group. This compares to 10/157 (6.4%, 95% CI 3.3-11.7) in the Abdominal pain study. Although this rate appears lower, confidence intervals for the two studies are wide and overlapping. Again, there is a moderate rate of non-negative non-positive results, with 24/76 (31.6%) samples either mixed growth or non-significant growth.

There is no *Streptococcus agalactiae* reported in these urine samples. This will be compared with enhanced culture results later.

8.6.6 Fresh unspun urine microscopy

8.6.6.1 Results

Table 8-13 shows fresh unspun microscopy results. White cells were detected in 42/80 (52.5%) samples, with a median count of 2.0 WBC μl^{-1} (95% CI 0-4). Red cells were detected in 34/80 (42.5%) samples, with a median count of 0.0 RBC μl^{-1} (95% CI 0-2). Epithelial cells were detected in 68/80 (85%) samples, with a median count of 8.0 EPC μl^{-1} (95% CI 4-12). There were no differences between groups.

Table 8-13: Fresh unspun microscopy for PTB study

Measure	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Sig.	Overall (n=80)
Median white cell count (WBC μl^{-1}) (95% CI) (Range)	0.0 (0-34) (0-54)	2.0 (0-4) (0-122)	3.0 (0-12) (0-190)	Kruskal- Wallis p=0.660	2.0 (0-4) (0-190)
Any white cells (%) (95% CI)	5/13 (38.5%) (15.1-67.7)	19/37 (51.4%) (34.7-67.8)	18/30 (60%) (40.8-76.8)	$\chi^2=1.724$ p=0.456	42/80 (52.5%) (41.1-63.7)
Median red cell count (RBC μl^{-1}) (95% CI) (Range)	0.0 (0-14) (0-206)	0.0 (0-2) (0-660)	0.0 (0-68) (0-740)	Kruskal- Wallis p=0.805	0.0 (0-2) (0-740)
Any red cells (%) (95% CI)	4/13 (30.8%) (10.4-61.1)	16/37 (43.2%) (27.5-60.4)	14/30 (46.7%) (28.8-65.4)	$\chi^2=0.954$ p=0.657	34/80 (42.5%) (31.7-54.1)
Median epithelial cell count (EPC μl^{-1}) (95% CI) (Range)	10.0 (2-32) (0-64)	8.0 (4-14) (0-800)	8.0 (4-12) (0-300)	Kruskal- Wallis p=0.889	8.0 (4-12) (0-800)
Any epithelial cells (%) (95% CI)	11/13 (84.6%) (53.7-97.3)	32/37 (86.5%) (70.4-94.9)	25/30 (83.3%) (64.6-93.7)	Fisher's Exact p=0.918	68/80 (85%) (74.9-91.7)

8.6.6.2 Discussion

There was no difference between groups for any of the cell counts. As noted previously, the LR group was small.

Again, it is interesting that the range of cell counts was so wide, suggesting some participants had high levels of cell loss in the urine. Further analysis of fresh microscopy counts against other measures will be presented in chapter 10.

8.6.7 ATP measurement

8.6.7.1 Results

Table 8-14 compares ATP measurements. ATP (measured in relative light units, RLU) was detected in every sample, ranging from 608 to 24523. Median for LR was 3871 (95% CI 2158-13004), for HR-PTB was 7915 (95% CI 4473-9527), and for HR-non-PTB was 5631 (95% CI 4225-6998). There was no difference between groups ($p=0.380$).

Table 8-14: ATP measurements for PTB study

Measure	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Sig.	Overall (n=80)
Median ATP (RLU)	3871	7915	5631	Kruskal-	5773
(95% CI)	(2158-13004)	(4473-9527)	(4225-6998)	Wallis	(4827-7915)
(Range)	(1483–16502)	(608–24523)	(1050–19985)	$p=0.380$	(608–24523)

8.6.7.2 Discussion

There was no difference between groups; confidence intervals of the median and ranges were very wide. ATP was not able to distinguish study groups.

There is a wide range of readings in each group and again, it will be interesting to compare ATP against other measures to understand its role in urinary pathology.

8.6.8 Sediment culture with counts and identification to species level of cultured organisms

8.6.8.1 Results

Table 8-15 shows enhanced (sediment) culture results. Bacteriuria was detected in 80/80 (100%) samples. Median total colony count was 1920CFU/ml (range 2-1280000) and median colony count for the dominant species was 1540CFU/ml (range 2-1280000). The median number of isolates was 3 (range 1-7). There were no between-group differences.

Table 8-15: Sediment culture results for PTB study

Measure	LR (n=13)	HR-PTB (n=37)	HR-non-PTB (n=30)	Sig.	Overall (n=80)
Total colony counts					
Median CFU/ml	1019	2987	1073	p=0.158	1920
(95% CI)	(52-41430)	(694-11746)	(208-2578)	Kruskal-Wallis	(537-2969)
(Range)	(2-1280000)	(2-405823)	(2-880081)		(2-1280000)
Colony count for dominant isolate					
Median CFU/ml	1280	2400	657	p=0.066	1540
(95% CI)	(46-27536)	(592-7040)	(89-1941)	Kruskal-Wallis	(559-2531)
(Range)	(2-1280000)	(2-403200)	(2-88720)		(2-1280000)
Number of isolates					
Median	3	3	3	p=0.675	3
(95% CI)	(1-4)	(2-3)	(2-3)	Kruskal-Wallis	(2-3)
(Range)	(1-6)	(1-7)	(1-6)		(1-7)
Proportion with any bacteriuria (%)	13/13	37/37	30/30	n/a	80/80
(95% CI)	(100%) (71.7-100)	(100%) (88.3-100)	(100%) (85.9-100)		(100%) (94.3-100)

Table 8-16 and Figure 8-4 show the genera of isolates as identified by bench testing, compared by study group. The most commonly identified genera by

bench testing were *Staphylococcus spp* (84/236, 35.6%), *E. coli* (33/236, 14.0%), *Enterococcus spp* (30/236, 12.7%) and *Lactobacillus spp* (28/236, 11.9%).

I was unable to perform Chi square on the entire table as expected values were too low, therefore I combined the rows for the two least common genera, *Corynebacterium spp* and *Candida spp* ($\chi^2=23.01$, $p=0.0276$). 31/236 (13.1%) of isolates were not identified by bench testing. When I removed the isolates that were not identified by bench testing, there was no difference, ($\chi^2=9.503$, $p=0.485$).

Table 8-16: Comparison of genus of isolates as identified by bench testing with study group

	LR (n=13) (% of all LR isolates)	HR-PTB (n=37) (%) of all HR- PTB isolates)	HR- non- PTB (n=30) (% of all HR-non- PTB isolates)	Totals (n=80) (% of all isolates)	Significance*
<i>Staphylococcus spp</i>	13 (36.6%)	39 (33.9%)	32 (38.1%)	84 (35.6%)	Condensed table: $\chi^2=23.01$ df=12 $p=0.0276$ Excluding non- identified isolates: $\chi^2=9.503$ df=10 $p=0.485$
<i>Escherichia coli spp</i>	6 (16.2%)	18 (15.7%)	9 (10.7%)	33 (14.0%)	
<i>Enterococcus spp</i>	2 (5.4%)	17 (14.8%)	11 (13.1%)	30 (12.7%)	
<i>Lactobacillus spp</i>	1 (2.7%)	15 (13.0%)	12 (14.3%)	28 (11.9%)	
<i>Streptococcus spp</i>	1 (2.4%)	10 (8.7%)	4 (4.8%)	15 (6.4%)	
<i>Corynebacterium spp</i>	2 (5.4%)	5 (4.3%)	5 (6.0%)	12 (5.1%)	
<i>Candida spp</i>	0 (0%)	0 (0%)	3 (3.6%)	3 (1.3%)	
Not identified by bench testing	12 (32.4%)	11 (9.6%)	8 (9.5%)	31 (13.1%)	
Total isolates	37 (100%)	115 (100%)	84 (100%)	236 (100%)	

Figure 8-4: Bar chart comparing sediment culture isolates identified to genus level by bench testing

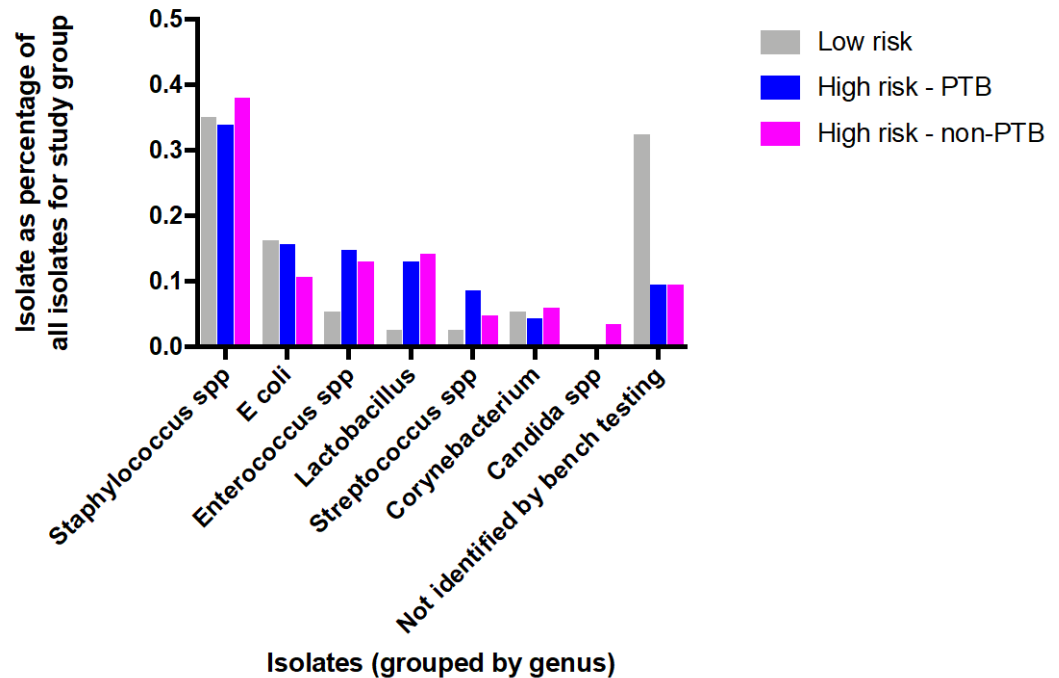


Table 8-17 shows the proportion of isolates identified by bench testing. This differed between groups ($p < 0.001$). LR isolates were less likely to be identified (25/37, 67.6%) compared with HR-PTB (104/115, 90.4%) and HR-non-PTB (76/84, 90.5%).

Table 8-17: Comparison of whether isolates were identified by bench testing against study group

	Low risk (n=13)	High risk PTB (n=37)	High risk non-PTB (n=30)	Significance	Overall
Identified by bench testing (%)	25/37 (67.6%)	104/115 (90.4%)	76/84 (90.5%)	$\chi^2=14.32$ $p < 0.001$	205/236 (86.9%)
(95% CI)	(50.1-81.5)	(83.2-94.9)	(81.6-95.5)		(81.7-90.8)

Frozen isolates underwent API testing to determine the species. The number of isolates that did not undergo API testing (Table 8-18) was different between groups ($p < 0.001$). This ranged from 29/115 (25.2%, 95% CI 17.8-34.3) in HR-non-PTB group to 29/41 (70.7%, 95% CI 61.3-89.6) in LR group.

This discrepancy may mean results obtained from API testing do not represent the three groups equally. It is therefore important to compare the genera identified by bench testing as well as the genera and species identified by API testing.

Table 8-19 shows the proportion of genera correctly identified by initial bench testing. This was 99/115 (86.1%, 95% CI 78.1-91.6). There was no between-group difference ($p=1.000$).

Table 8-18: Comparison of isolates that did not undergo API testing, against study group

	LR (n=13)	HR-PTB (n=37)	HR-non-PTB (n=30)	Significance	Overall (n=80)
No API done (%) (95% CI)	29/37 (78.4%) (61.3-89.6)	29/115 (25.2%) (17.8-34.3)	41/84 (48.8%) (37.8-59.9)	$\chi^2=35.01$ $p<0.001$	97/236 (41.1%) (34.8-47.7)

Table 8-19: Comparison of whether bench genus was correctly identified according to API, against study group

	LR (n=13)	HR-PTB (n=37)	HR-non-PTB (n=30)	Significance	Overall (n=80)
Genus correctly identified (%) (95% CI)	10/11 (90.9%) (57.1-99.5)	61/72 (84.7%) (73.9-91.8)	28/32 (87.5%) (70.1-95.9)	Fisher's Exact $p=1.000$	99/115 (86.1%) (78.1-91.6)

Table 8-20 shows the accuracy of genus identification according to genus. There were 236 isolates from the 80 patients. Of these, 97/236 (41.1%) did not undergo API testing. Of those 139 isolates that underwent API testing, the genus was correctly identified by bench testing for 125/139 (90%). This included 13/20 (65%) *Escherichia coli* spp, 6/9 (67%) *Streptococcus* spp and 6/9 (67%) *Corynebacterium* spp, to 49/50 (98%) *Staphylococcus* spp, 2/2 (100%) *Candida* spp and 21/21 (100%) *Enterococcus* spp. *Lactobacillus* spp is assumed to have been correctly identified to genus level as described previously.

The seven mis-identified *Escherichia coli* spp included five other Gram-negative organisms, one *Corynebacterium* spp, and one other. The two mis-identified *Staphylococcus* spp were *Corynebacterium* spp. The three mis-identified *Streptococcus* spp and three *Corynebacterium* spp were all classified as 'other'.

Table 8-20: Comparison of accuracy of genus identification by bench testing against genus identification by API testing

Genus as identified by bench tests	Correctly identified (% of total by genus that underwent API testing)	Incorrectly identified (% of total by genus that underwent API testing)	Did not undergo API testing	Total
<i>Staphylococcus spp</i>	49/50 (98%)	1/50 (2%)	34	84
<i>Streptococcus spp</i>	6/9 (67%)	3/9 (33%)	6	15
<i>Enterococcus spp</i>	21/21 (100%)	0/21 (0%)	9	30
<i>Escherichia coli spp</i>	13/20 (65%)	7/20 (35%)	13	33
<i>Corynebacterium spp</i>	6/9 (67%)	3/9 (33%)	3	12
<i>Lactobacillus spp</i>	28/28 (100%)	n/a	n/a	28
<i>Candida spp</i>	2/2 (100%)	0/2 (0%)	1	3
Not identified by bench tests	n/a	n/a	31	31
Totals	125/139 (90%)	14/139 (10%)	97	236

Table 8-21 and Figure 8-5 show how genera of isolates as identified by API testing compared with study group. Over 40% had no API test done. The commonest genera were *Staphylococcus spp* (20.8%), *Lactobacillus spp* (11.9%), *Enterococcus spp* (8.9%), and *E. coli* (5.5%) and this order did not appear to differ between the groups.

Table 8-21: Comparison of genus of isolates as identified by API testing

	Low risk (n=13)	High risk PTB (n=37)	High risk non-PTB (n=30)	Totals
No API done	29 (78.4%)	29 (25.2%)	39 (46.4%)	97 (41.1%)
<i>Staphylococcus spp</i>	3 (8.1%)	31 (27.0%)	15 (17.9%)	49 (20.8%)
<i>Lactobacillus spp</i>	1 (2.7%)	15 (13.0%)	12 (14.3%)	28 (11.9%)
<i>Enterococcus spp</i>	1 (2.7%)	13 (11.3%)	7 (8.3%)	21 (8.9%)
<i>E. coli</i>	2 (5.4%)	8 (7.0%)	3 (3.6%)	13 (5.5%)
<i>Corynebacterium spp</i>	0 (0.0%)	6 (5.2%)	2 (2.4%)	8 (3.4%)
Other	1 (2.7%)	2 (1.7%)	4 (4.8%)	7 (3.0%)
Other gram negatives	0 (0.0%)	5 (4.4%)	0 (0.0%)	5 (2.1%)
<i>Candida spp</i>	0 (0.0%)	0 (0.0%)	2 (2.4%)	2 (%)
<i>Streptococcus spp</i>	0 (0.0%)	6 (5.2%)	0 (0.0%)	6 (2.5%)
Total	37 (100%)	115 (100%)	84 (100%)	236 (100%)

Figure 8-5: Bar chart comparing sediment culture isolates identified to genus level by API testing

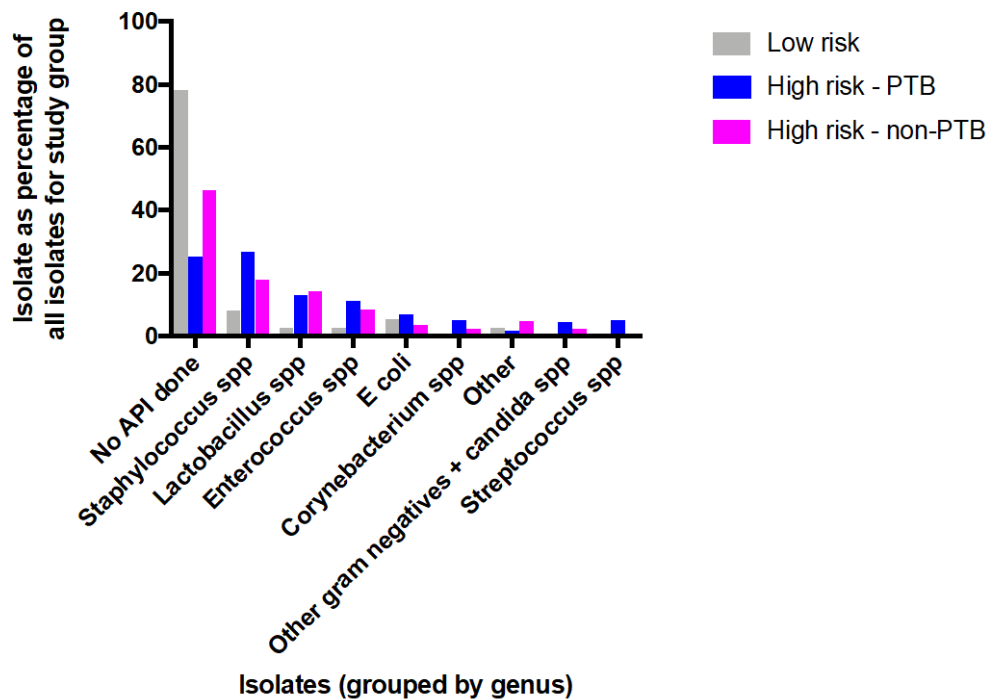


Figure 8-6 and Table 8-22 show the distribution of species identified by API testing according to study group. The largest group is those which did not undergo API testing, followed by *Lactobacillus spp*, which also did not undergo API testing. The next commonest species were *Staphylococcus haemolyticus* (9.8%), *Enterococcus faecalis* (8.9%), *Staphylococcus epidermidis* (6.4%), and *Escherichia Coli 1* (5.5%).

Figure 8-7 shows only those species identified. Of the 139/236 (58.9%) isolates that were identified to species level, the commonest were *Staphylococcus haemolyticus* (23/139, 16.7%), *Enterococcus faecalis* (21/139, 15.2%), *Staphylococcus epidermidis* (15/139, 10.9%) and *E. coli 1* (13/139, 9.4%). 28/139 (19.6%) were *Lactobacillus spp.*, 39/139 (28.3%) were other species. There was no difference between the groups on contingency testing once the unidentified species were excluded ($p=0.737$). Typical uropathogens and non-uropathogens were identified in each group.

Figure 8-6: Graph showing sediment culture isolates identified to species level by API testing, comparing across study groups

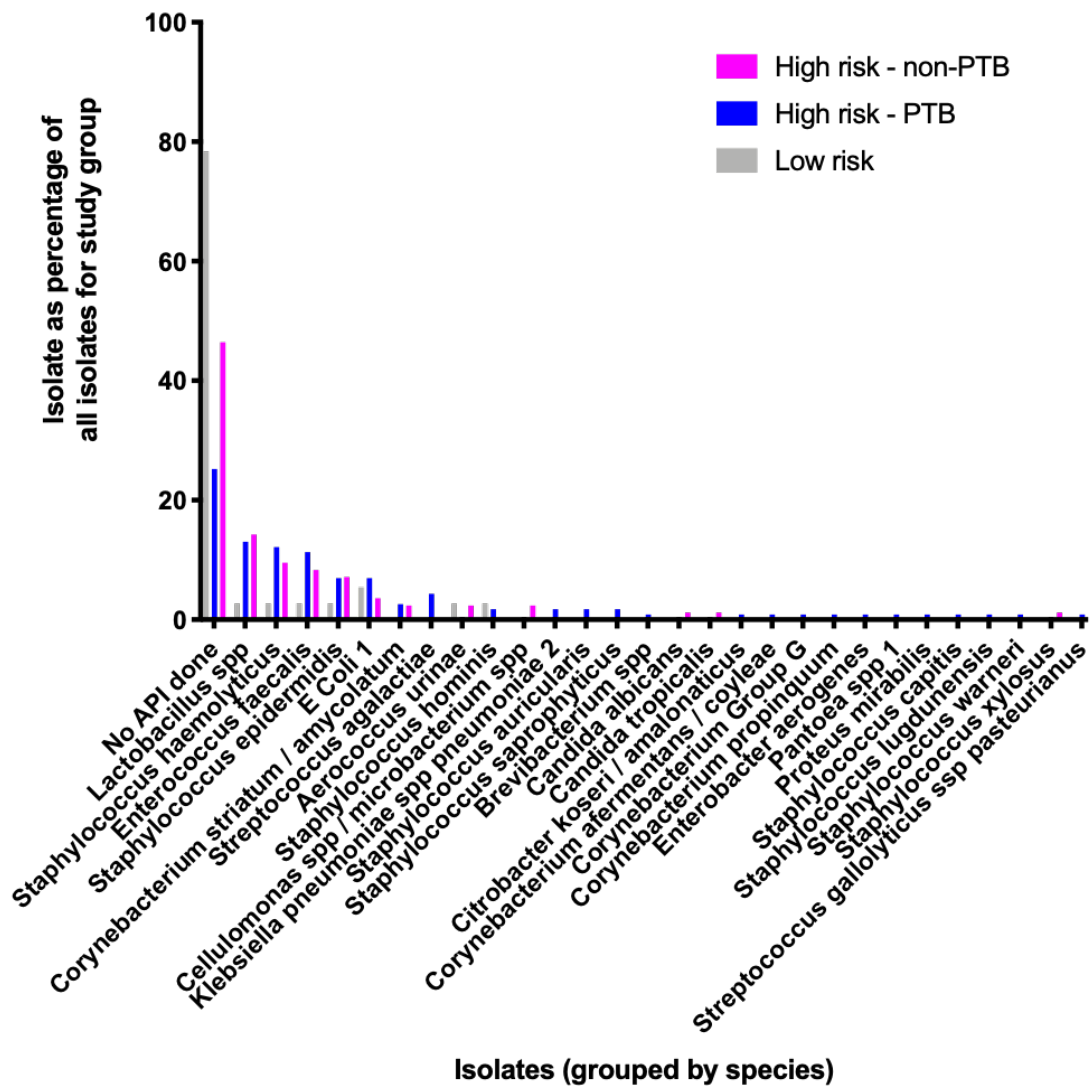


Table 8-22: Overall species by API testing compared with study group

	LR (n=37) (%)	HR-PTB (n=115) (%)	HR-non- PTB (n=84) (%)	Total (n=236) (%)
No API done	29 (78.4%)	29 (25.2%)	39 (46.4%)	97 (41.1%)
<i>Lactobacillus spp</i>	1 (2.7%)	15 (13.0%)	12 (14.3%)	28 (11.9%)
<i>Staphylococcus haemolyticus</i>	1 (2.7%)	14 (12.2%)	8 (9.5%)	23 (9.8%)
<i>Enterococcus faecalis</i>	1 (2.7%)	13 (11.3%)	7 (8.3%)	21 (8.9%)
<i>Staphylococcus epidermidis</i>	1 (2.7%)	8 (7.0%)	6 (7.1%)	15 (6.4%)
<i>E. coli 1</i>	2 (5.4%)	8 (7.0%)	3 (3.6%)	13 (5.5%)
<i>Corynebacterium striatum / amycolatum</i>	0 (0.0%)	3 (2.6%)	2 (2.4%)	5 (2.1%)
<i>Streptococcus agalactiae</i>	0 (0.0%)	5 (4.4%)	0 (0.0%)	5 (2.1%)
<i>Aerococcus urinae</i>	1 (2.7%)	0 (0.0%)	2 (2.4%)	3 (1.3%)
<i>Staphylococcus hominis</i>	1 (2.7%)	2 (1.7%)	0 (0.0%)	3 (1.3%)
<i>Cellulomonas spp / microbacterium spp</i>	0 (0.0%)	0 (0.0%)	2 (2.4%)	2 (0.9%)
<i>Klebsiella pneumoniae spp pneumoniae 2</i>	0 (0.0%)	2 (1.7%)	0 (0.0%)	2 (0.9%)
<i>Staphylococcus auricularis</i>	0 (0.0%)	2 (1.7%)	0 (0.0%)	2 (0.9%)
<i>Staphylococcus saprophyticus</i>	0 (0.0%)	2 (1.7%)	0 (0.0%)	2 (0.9%)
<i>Brevibacterium spp</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Candida albicans</i>	0 (0.0%)	0 (0.0%)	1 (1.2%)	1 (0.4%)
<i>Candida tropicalis</i>	0 (0.0%)	0 (0.0%)	1 (1.2%)	1 (0.4%)
<i>Citrobacter koseri / amalonaticus</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Corynebacterium afermentans / coyleae</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Corynebacterium Group G</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Corynebacterium propinquum</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Enterobacter aerogenes</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Pantoea spp 1</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Proteus mirabilis</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Staphylococcus capitis</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Staphylococcus lugdunensis</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Staphylococcus warneri</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
<i>Staphylococcus xylosus</i>	0 (0.0%)	0 (0.0%)	1 (1.2%)	1 (0.4%)
<i>Streptococcus gallolyticus ssp pasteurianus</i>	0 (0.0%)	1 (0.9%)	0 (0.0%)	1 (0.4%)
Total	37 (100%)	115 (100%)	84 (100%)	236 (100%)

Figure 8-7: Graph showing sediment culture isolates identified to species level by API testing, comparing across study groups (rows combined where total isolates 5 or fewer)

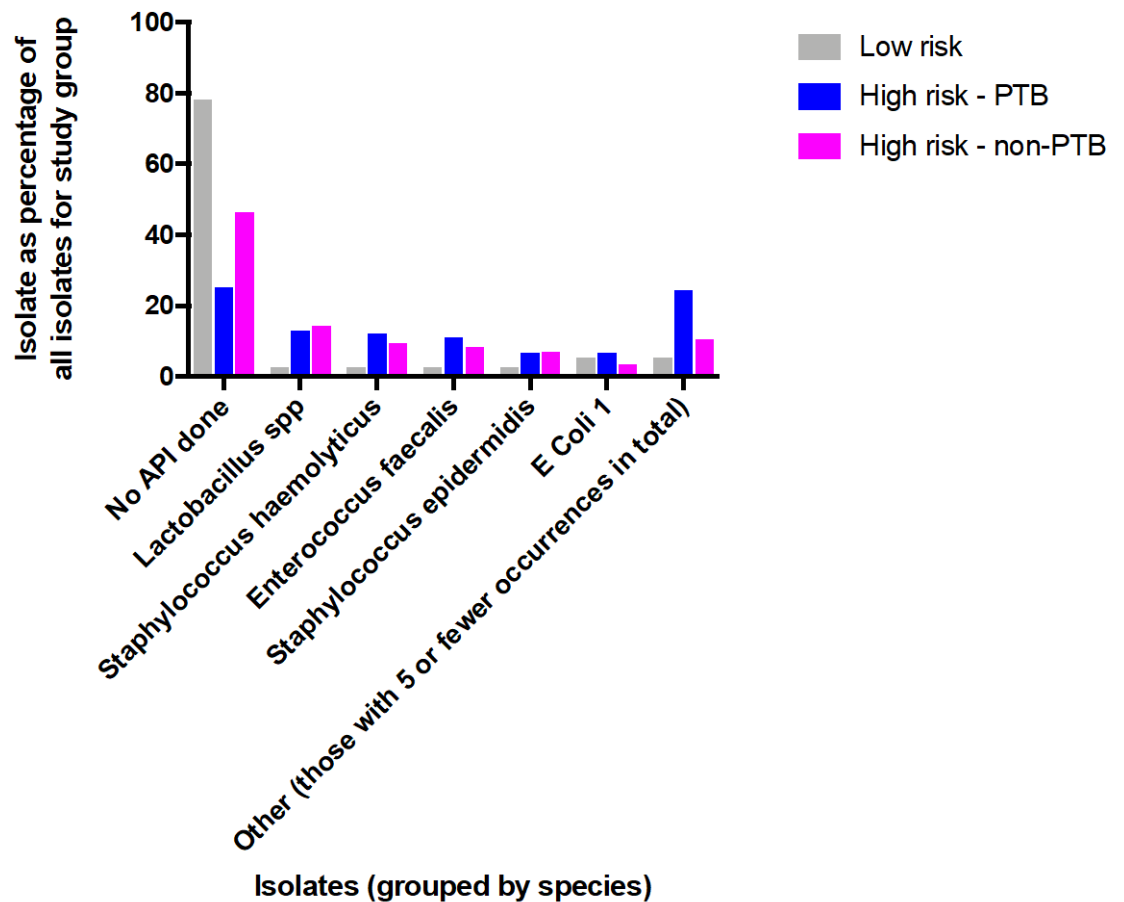
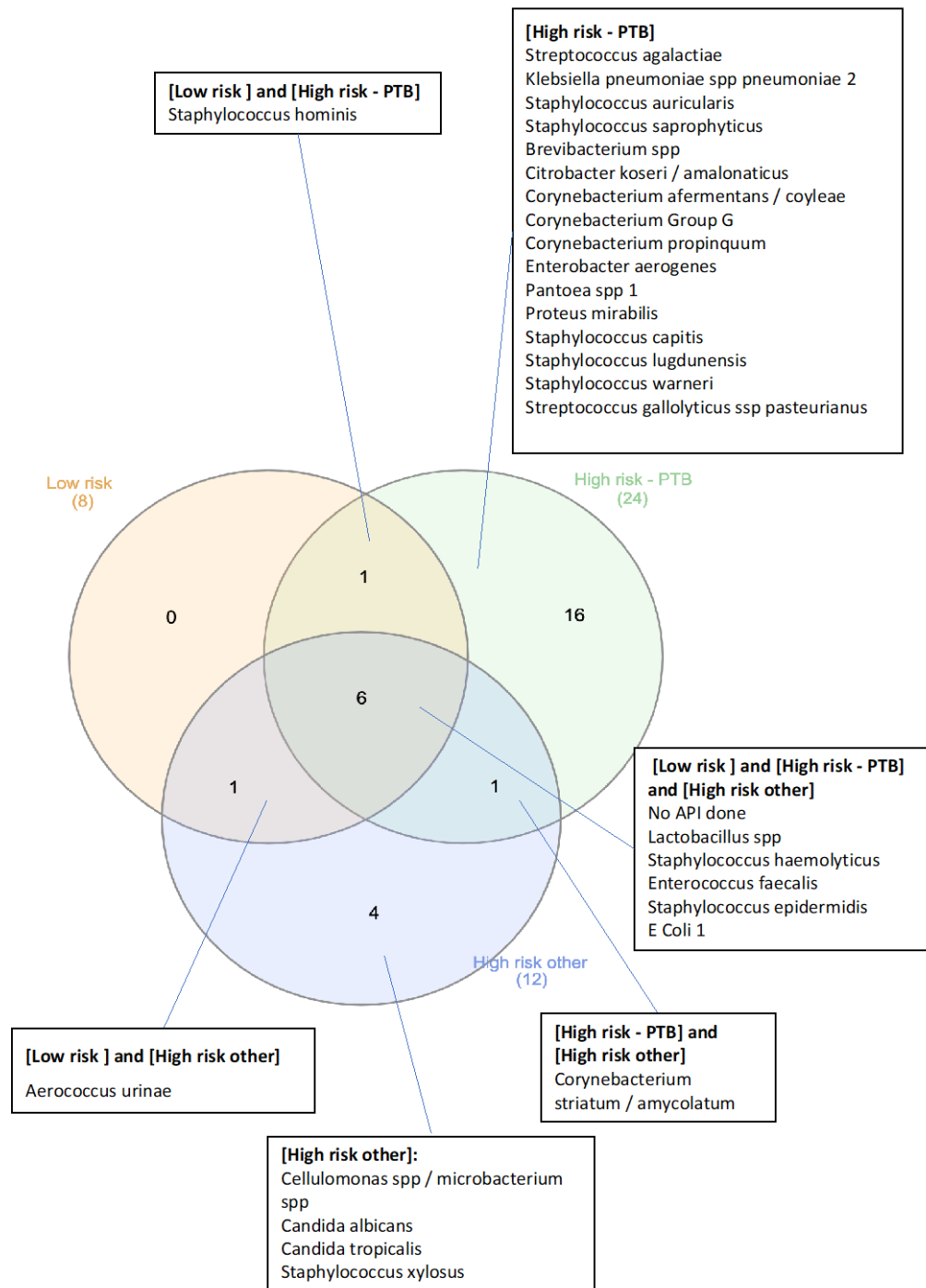


Figure 8-8 is a Venn diagram comparing the overlap of species by study group. This shows that there are 16 species only seen in the HR-PTB group, and zero species only seen in the LR group. The four commonest species mentioned above, along with *Lactobacillus spp* and those that did not have API testing, are the only species that occur in each group.

Figure 8-8: Venn diagram to show species identified by API by study group



8.6.8.2 Discussion

There was bacteriuria in every sample; this is supporting evidence that urine is not sterile.

The lack of difference between study groups suggests enhanced culture does not distinguish between women at high risk of preterm birth, however again it should be noted that the LR group is small and the risk of type II errors is high.

There is a wide range in colony counts suggesting a spectrum of bacteriuria. In contrast, the median number of isolates is 3 for all groups, with relatively small confidence intervals for the larger groups, suggesting polymicrobial urine is common and potentially normal.

There was no difference between groups in terms of genera identified by bench testing, other than the LR group having a higher rate of isolates not identified. This is most likely due to a short period where the culture plates were not analysed in time and the isolates became unidentifiable. Attempts were made to salvage them through reculturing, however there was no growth. This period disproportionately affected LR recruits. An alternative explanation is that LR isolates included unusual genera that were harder to identify, however from review of laboratory records it would appear this is not the case.

In many respects, results from the PTB study echo those from the Abdominal pain study, particularly the finding of bacteriuria in all samples, mostly polymicrobial, with median three isolates. Colony counts were similar, with median 1.9×10^3 CFU/ml.

The genera and species that were common to all study groups again included a mixture of uropathogens and traditional commensals / contaminants, specifically *Lactobacillus* spp, *Staphylococcus haemolyticus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, and *Escherichia coli*. The presence of these did not differentiate between groups.

Sixteen species were unique to the HR-PTB group. They included traditional pathogens including *Klebsiella pneumoniae* spp, *Citrobacter* spp, and *proteus mirabilis*, as well as *Streptococcus agalactiae* and a variety of staphylococci including *Staphylococcus saprophyticus*.

It is tempting to conclude that this hints at a different microbial community in HR-PTB patients. However, given the small number of control patients and high number of isolates in that group that were not identified to species level, it would be an unsafe assumption. It should be emphasised that there was no significant difference overall in the distribution of species between study groups using contingency testing.

The prevalence of *Streptococcus agalactiae* was 2.1%, which appeared lower than the Abdominal pain study, at 16.3%, and was only found in the HR-PTB group. This is a surprising result and perhaps reflects the smaller numbers in this study. It may also be a consequence of the number of API analyses which were not carried out. By genus testing, *Streptococcus spp* were identified in 6.4% of total isolates. One was identified as *Streptococcus gallolyticus ssp pasteurianus*. The rest were not identified, however it is likely that many of these were *Streptococcus agalactiae*, particularly as the chromogenic agar was optimised to identify this. This is backed up by the Abdominal pain study, in which 26/30 (86.7%) *Streptococcus spp* on bench testing were identified as *Streptococcus agalactiae*.

Similar observations apply as to the Abdominal pain study, namely flaws in the enhanced culture approach (which will be discussed fully later), lack of species information for *Lactobacillus spp.*, and use of API testing.

8.6.9 Cytokines – IL-6, IL-8, lactoferrin

8.6.9.1 Methods

80 samples underwent testing for all three urinary cytokines, except one sample from HR-PTB which did not undergo lactoferrin testing.

8.6.9.2 Results

Table 8-23 demonstrates the results. There were no differences between groups in terms of cytokine concentration, cytokine concentration normalised for total protein, or proportion below the lower limit of detection.

The median protein concentration was 49.9µg/ml (95% CI 43.4-60.0, range 18.6-305.1). None were below the lower limit of detection (LLOD).

The median urinary IL-6 concentration was 1.68pg/ml (95% CI 0.8-2.5, range 0.14-56.9). 15/80 (18.8%) were below the LLOD.

The median urinary IL-8 concentration was 25.3pg/ml (95% CI 15.4-37.8, range 0.7-23160). 3/80 (3.8%) were below the LLOD.

The median urinary lactoferrin concentration was 2.21ng/ml (95% CI 2.2-4.7, range 2.2-2138). 40/79 (50.6%) were below the LLOD.

Table 8-23: Urinary cytokines for PTB study

	LR (n=13)	HR-PTB (n=37)	HR-non-PTB (n=30)	Sig.	Total (n=80)
Total protein concentration (µg/ml)					
Median	58.7	45.4	53.3	p=0.627	49.9
(95% CI)	(41.3-115.9)	(41.7-56.3)	(33.9-73.7)	Kruskal-	(43.4-60.0)
(Range)	(19.5–154.5)	(18.6–305.1)	(18.9–140.4)	Wallis	(18.6–305.1)
Protein<LLOD (%)	0/13 (0%)	0/37 (0%)	0/30 (0%)	n/a	0/80 (0%)
(95% CI)	(0-28.3)	(0-11.7)	(0-14.1)		(0-5.7)
IL-6 concentration (pg/ml)					
Median	2.3	1.2	1.6	p=0.601	1.7
(95% CI)	(0.6-5.6)	(0.6-3.1)	(0.5-4.1)	Kruskal-	(0.8-2.5)
(Range)	(0.14–9.9)	(0.14–56.9)	(0.14–15.3)	Wallis	(0.14–56.9)
IL-6<LLOD (%)	1/13 (7.7%)	7/37 (18.9%)	7/30 (23.3%)	Fisher's Exact =1.278 p=0.595	15/80 (18.8%)
(95% CI)	(0.4-37.9)	(8.6-35.7)	(10.6-42.7)		(11.2-29.4)
IL-6 normalised for total protein (ng/µg)					
Median	38	23	32	p=0.708	32
(95% CI)	(10-60)	(13-45)	(12-45)	Kruskal-	(16-42)
(Range)	(4.5–212)	(3.4–187)	(3.7–166)	Wallis	(3.4–212)
IL-8 concentration (pg/ml)					
Median	39.0	24.0	18.7	p=0.320	25.3
(95% CI)	(12.9-140.2)	(12.8-41.0)	(9.4-35.8)	Kruskal-	(15.4-37.8)
(Range)	(0.7–475.2)	(0.7-23160)	(0.7–288.2)	Wallis	(0.7–23160)
IL-8<LLOD (%)	1/13 (7.7%)	1/37 (2.7%)	1/30 (3.3%)	Fisher's exact = 1.125 p=0.561	3/80 (3.8%)
(95% CI)	(0.4-37.9)	(0.1-15.8)	(0.2-19.1)		(1.0-11.3)

Continued next page

	LR (n=13)	HR-PTB (n=37)	HR-non-PTB (n=30)	Sig.	Total (n=80)
IL-8 normalised for total protein (pg/μg) Median (95% CI) (Range)	0.8 (0.2-1.2) (0.04– 8.1)	0.5 (0.4-0.7) (0.04-75.9)	0.3 (0.3-0.5) (0.03–3.6)	p=0.332 Kruskal- Wallis	0.4 (0.4-0.6) (0.03–75.9)
Lactoferrin concentration (ng/ml) Median (95% CI) (Range)	(n=13) 3.23 (2.2-7.2) (2.2-525.8)	(n=36) 3.76 (2.2-6.7) (2.2–2138.0)	(n=30) 2.21 (2.2-4.0) (2.2–19.6)	p=0.326 Kruskal- Wallis	(n=79) 2.21 (2.2-4.7) (2.2–2138.0)
Lactoferrin<LLOD (%) (95% CI)	(n=13) 6/13 (46.2%) (20.4-73.9)	(n=36) 15/36 (41.7%) (26.0-59.1)	(n=30) 19/30 (63.3%) (43.9-79.5)	$\chi^2=3.198$ df=2 p=0.203	(n=79) 40/79 (50.6%) (39.2-62.0)
Lactoferrin normalised for total protein (pg/μg) Median (95% CI) (Range)	(n=13) 54 (36-123) (6 – 8965)	(n=36) 93 (56-152) (13 – 42675)	(n=30) 60 (40-82) (16 – 597)	p=0.144 Kruskal- Wallis	(n=79) 69 (54-85) (13 – 42675)

8.6.9.3 Discussion

There were no between-group differences for any measure. This suggests that IL-6, IL-8 and lactoferrin are not different in women at risk of preterm birth. However, as with the other variables that I measured, there was a wide variance.

8.6.10 Bacterial-associated clue cell proportions

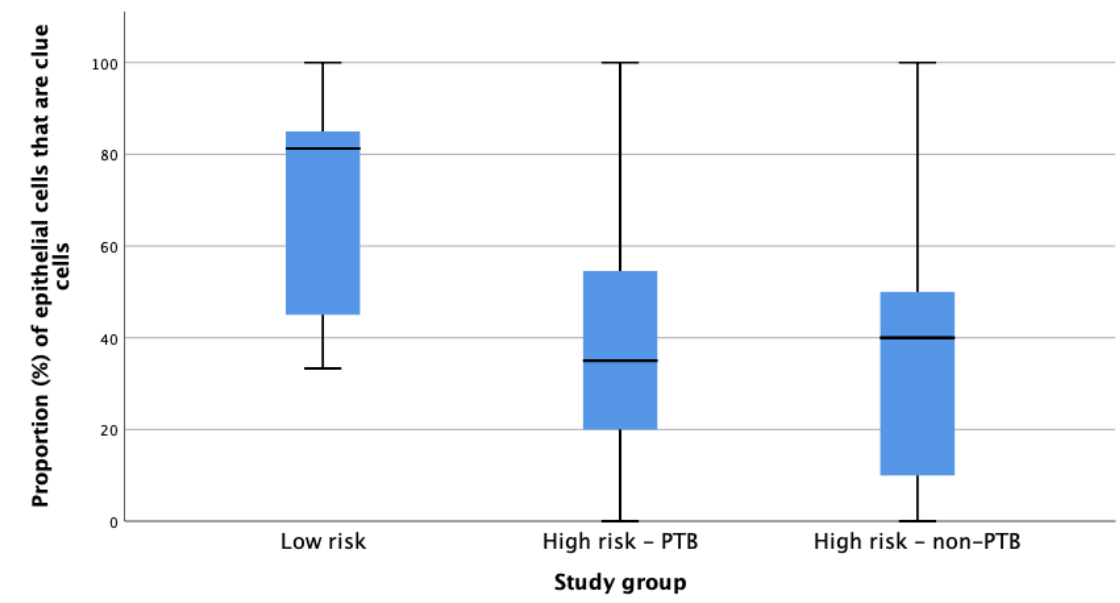
8.6.10.1 Results

Results are presented in Table 8-24 and Figure 8-9. The median proportion (%) of epithelial cells with associated bacteria was 81% (95% CI 39-85) in the LR group, compared with 35% (95% CI 20-50) in HR-PTB and 40% (95% CI 17-46) in HR-non-PTB. There was a difference between groups ($p=0.008$), but the effect size was not significant.

The proportion of epithelial cells with any associated bacteria (i.e., clue cell proportion > 0) was 72/80 (90%, 95% CI 80.7-95.3), which did not differ between groups ($p=0.537$).

Table 8-24: Table comparing bacterial-associated clue cell data for the three study groups

Measure	Low risk (n=13)	High risk - PTB (n=37)	High risk – non-PTB (n=30)	Sig.	Overall (n=80)
Proportion (%) of epithelial cells that are clue cells					
Median	81	35	40	$p=0.008$	40
(95% CI)	(39-85)	(20-50)	(17-46)	Kruskal-	(33-46)
(Range)	(33–100)	(0–100)	(0–100)	Wallis	(0–100)
Proportion of epithelial cells that have any associated bacteria (clue cell proportion > 0)	13/13 (100%)	33/37 (89.2%)	26/30 (86.7%)	Fisher's exact = 1.492	72/80 (90%)
(95% CI)	(71.7-100)	(73.6-96.5)	(68.4-95.6)	$p=0.537$	(80.7-95.3)

Figure 8-9: Proportion (%) of epithelial cells that are clue cells

8.6.10.2 Discussion

Some women at high risk of preterm birth because of previous preterm birth may have chronic UTI that has not been previously recognised. Epithelial cells with adherent bacteria may be a marker of chronic UTI. However, in this small study, it was the low-risk women who had a higher proportion of epithelial cells with associated bacteria, albeit with wide and overlapping confidence intervals.

As mentioned before, epithelial cells with associated bacteria have previously been found to distinguish patients with chronic UTI from controls (Horsley, Malone-Lee et al. 2013), with infected cells detected in 75% of LUTS patients and 17% of healthy controls. In the PTB study there was no between-group difference in the proportion of patients with any infected cells, and overall, 90% (80.7-95.3) of participants had evidence of infected cells. This appears to be a higher rate than in the non-pregnant patients and controls. The Abdominal pain study recorded a clue cell occurrence in 70.7% (95% CI 62.8-77.6) of cells. However, the counting technique used was slightly different in the PTB study, with the first 20 epithelial cells being counted rather than the first 100. Although there was good correlation between these counts, this may explain variation.

The question of whether some women at increased risk of preterm birth may have chronic UTI, recognised or not, warrants future research.

8.7 PTB study discussion

There were important differences between the study groups, in addition to their risk factors for preterm birth. HR-PTB were more likely to have a history of UTI. HR-non-PTB were more likely to be white, lower BMI and nulliparous.

There were no differences in gestation at recruitment, age, smoking, or socio-economic status.

The HR-PTB group included 13 women who already had cerclage at recruitment, and five who went on to have one inserted. There were no cerclages in the other groups. The HR-PTB group were more likely to have progesterone treatment, with no difference in cervical length and higher fetal fibronectin levels (although not clinically meaningful).

Preterm delivery was more common in the HR-PTB and LR groups with no preterm deliveries in the HN-non-PTB group. There were three cases of PPRM in the HR-PTB group. There were no differences in other outcomes, including maternal or neonatal sepsis.

There were no between-group differences in standard tests. HR-PTB patients had 6 MSU samples sent during pregnancy compared with 3 in the other groups, however there were no differences in results with 27.5% patients having at least one positive MSU. Mixed and non-significant growth accounted for 41.6% of samples with no between-group differences. Only 5 samples underwent standard microscopy, so no analysis was done. Positive standard cultures were present in 14.3% LR group compared with 0% HR-PTB and 3.6% HR-non-PTB ($p=0.036$) although variances were wide. 31.6% of samples were mixed or non-significant growth. There were no between-group differences for dipstick.

There were very few differences in alternative tests. LUTS were common with 93.8% reporting any LUTS, however there were no between-group differences.

There were no between-group differences for fresh unspun microscopy or ATP.

Bacteriuria was detected in all samples using enhanced sediment culture.

There were no between-group differences in colony counts or number of species identified (median 3, range 1-7). There appeared to be a list of species that were only detected in HR-PTB patients, however the significance of this is hard to determine in a study of this size. 41.1% of isolates were not identified to species level, which disproportionately affected LR group. 2.1% of isolates were confirmed *Streptococcus agalactiae*, which occurred only in the HR-PTB group, although there were other unidentified *Streptococcus spp* in the other groups. Standard culture did not identify any *Streptococcus agalactiae*.

There were no between-group differences in urinary cytokines although there was a wide variation in results. Clue cell proportions were higher in LR group although confidence intervals were wide and there was no difference in the proportion with any clue cells.

A universal finding was the wide range of results, whether symptoms, host response or quantitative microbiology, again suggesting an underlying pathophysiological process not explained by these groups.

Issues with the PTB study included: (1) it functioning as a pilot study for the Abdominal pain study, with possible effects on fresh microscopy cell counts and identification of species by API; (2) the small size of the low risk control group and the groups in general (3) change or refinement of methods between PTB and Abdominal pain study (4) lack of standard microscopy.

To address the issue of the study being underpowered by its small low risk control group, I explored the use of data from other sources to increase the group size, as described in the following section.

8.8 Urinary pathology in women at risk of preterm birth, using standard and alternative tests; a reworking of the PTB study using additional data

8.8.1 Introduction

In the previous chapter I outlined the rationale for analysing the PTB study results, as recruited. That is, with a small group of low-risk controls, which was limited in size owing to recruitment issues. External feedback suggested that the analysis would benefit from greater numbers in the control group, as a failure to determine differences between groups could be due to small numbers, rather than a true difference. Given the circumstances post-Covid-19 pandemic, and owing to time restrictions, it was not possible to recruit further new participants. However, additional data could be included from low risk women in the Abdominal pain study, or from the earlier student pilot preterm birth studies.

The PTB study I performed was explicitly restricted to women between 13-17 weeks gestation, to reduce effect of gestation on urinary pathology, but also to ensure parity in pregnancy experiences at the point of recruitment, including screening for asymptomatic bacteriuria. Being recruited at 13-17 weeks also meant the period of risk of first trimester miscarriage or termination was minimised, but allowed equal time for second trimester loss or preterm labour to occur.

Women in the high risk groups had to meet criteria for referral to the preterm birth clinic as well as gestation criteria. Of high risk participants in the student pilot studies, 17 met criteria for the high-risk for previous preterm birth group, and have been analysed as part of this (renamed HR-PTB-C), and 33 met criteria for the high risk without previous preterm birth (renamed HR-non-PTB-C).

Women in the low-risk group had to have had at least one previous term delivery and not meet criteria for referral to the preterm birth clinic, as well as meeting gestation criteria. Of normal controls in the Abdominal pain study, 3 met these criteria. Of low risk controls in the student pilot studies, 4 met these

criteria. These have thus been analysed as part of the low risk combined group (LR-C).

The original student pilot low risk group contained a substantial number of participants outside the 13-17 weeks gestation (Table 8-25). It could be argued that there would be little scientific difference between 12 and 13 weeks gestation. However, the low-risk women below 13 weeks were mostly recruited at their booking visit with the midwife. By definition, this is when screening for asymptomatic bacteriuria is performed. Therefore, including these women may bias results, as anyone with asymptomatic bacteriuria at the start of pregnancy would not have had it treated yet. By retaining the 13-17 week rule for this combined analysis, women would be past the point of first trimester screening at the point of recruitment. As none of the studies included data about antibiotic treatment in the pregnancy, this gestational parity between groups is an important element to retain.

Table 8-25: Gestation at recruitment to student pilot study

Weeks gestation	Number recruited to pilot low risk group	Number recruited to pilot high risk – PTB group	Number recruited to pilot high risk non-PTB group
6+	1	0	0
7+	0	0	0
8+	6	0	0
9+	2	0	0
10+	3	0	0
11+	1	0	0
12+	19	1	0
13+	3	1	3
14+	1	3	14
15+	4	6	8
16+	1	2	2
17+	1	0	1
18+	0	2	3
19+	1	0	0
20+	0	0	0
21 or more	2	2	2
Total	45	17	33

8.8.2 Aims

The same as in chapter 8.2, that is to compare the prevalence of urinary pathology in (1) women at risk of preterm birth owing to previous preterm birth (HR-PTB-C), (2) women at risk of preterm birth owing to other risk factors (HR-non-PTB-C) and (3) women at low risk of preterm birth owing to previous term births (LR-C).

Hypothesis:

Women at increased risk for preterm birth may have urinary pathology detected using alternative tests.

Research questions:

Compare the prevalence of urinary pathology using standard and alternative methods:

1. Structured lower urinary tract symptoms assessment using Artemis
2. Standard dipstick, and standard culture
3. Fresh unspun urine microscopy
4. Urinary ATP measurement
5. Sediment culture with counts and identification to *genus* level of cultured organisms
6. Urinary cytokines – IL-6
7. Bacterial-associated clue cell proportions

8.8.3 Methods

Methods were the same as for the PTB study (8.3) except for the elements described below.

8.8.3.1 Personnel

The students who performed the pilot studies did so under the supervision of Professor James Malone-Lee, Professor Anna David, Dr Kiren Gill, Dr Sheela Swamy and Dr Catherine James, between 2012 and 2013. They are listed in Table 8-26. All students were conducting projects as part of the intercalated BSc in Clinical Sciences at UCL.

Table 8-26: Acknowledgement of students who collected the PTB study pilot data

Name	Date	Title of project	Individual focus
Susan Arasaretnam	July-August 2012	Urinary Symptoms in Women at Increased Risk of Preterm Birth	Symptoms (Artemis)
Adam Muse	Nov 2012 – Feb 2013	The Innate Immune Response to Urinary Tract Infection in Women at Risk of Preterm Birth	IL-6
Rwoon Kang	Nov 2012 – Feb 2013	Do women at risk of preterm delivery have evidence of increased incidence of undiagnosed uropathogenic bacteria?	Sediment culture
Greg Dewar	Nov 2012 – Feb 2013	Investigating the prevalence of urinary tract infection and intracellular bacteria in antenatal patients using advanced microscopic techniques	Clue cells

8.8.3.2 Ethics

Ethical approval was obtained under the umbrella of the CLIMB study, as for the other studies in this group (see 6.12).

8.8.3.3 Differences in tests performed

- Sediment culture – this was performed with identification to genus level only
- Urinary cytokines - only IL-6 was studied. No normalisation performed in this re-analysis as protein concentrations appeared different by around two orders of magnitude, despite using the same assay method.
- Clue cells - bacterial-associated clue cell proportions were recorded and have been used, however the raw data used to calculate these proportions, and thus the counting method used, cannot be

determined with certainty. They have been combined with the results here but with some reservations.

- Full microbiological data throughout the index pregnancies was not recorded.

Training and supervision of students in terms of technique was performed by the same people as for Abdominal pain and PTB studies.

8.8.3.4 Eligibility and exclusion criteria

Eligibility included pregnant women (≥ 18 years of age) presenting to the UCLH preterm birth clinic with risk factors for preterm birth, or multiparous women without risk factors attending a routine ANC/US appointment, all at 13-17 weeks of gestation.

When including normal controls from the Abdominal pain study, participants were included in this analysis if they were 13-17 weeks gestation, with at least one previous term delivery, and no risk factors that would meet acceptance criteria for the preterm birth clinic.

Other criteria were as described previously.

8.8.4 Results

8.8.4.1 Demographics

Cases were assigned to the three groups, creating a low risk group (LR-C, $n=20$), a high risk with previous preterm birth (HR-PTB-C, $n=54$), and a high risk without previous preterm birth (HR-non-PTB-C, $n=63$) (Table 8-27).

Table 8-27: Origin of patients in PTB combined study groups

Low risk controls – combined group (LR-C) (n=20)	High risk – PTB – combined group (HR-PTB-C) (n=54)	High risk – non-PTB – combined group (HR-non-PTB-C) (n=63)
Original low risk controls (n=13)	Original HR-PTB group (n=37)	Original HR-non-PTB group (n=30)
Abdo pain study normal controls (n=3)	Student pilot HR-PTB (n=17)	Student pilot (n=33)
Student pilot study low risk controls (n=4)		

The HR-non-PTB-C group contained women with previous LLETZ (n=38), previous cone biopsy (n=13), uterine anomaly (n=6) and other (n=6).

Demographic features are compared in Table 8-28. Similar to the original PTB study, the only difference between the groups is ethnicity, with high-risk-non-PTB-C having fewer non-white participants compared to the other two groups (p=0.009).

Table 8-28: Demographic features for PTB study (combined groups)

Variable	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Gestation at recruitment (days) (95%CI)	116 (110-118)	108.5 (106-114)	110 (106-114)	p=0.194 Kruskal-Wallis	110 (108-114)
Age (years) (95% CI)	37.2 (34.9-38.3)	35.2 (34.0-36.0)	34.0 (33.0-36.0)	p=0.105 Kruskal-Wallis	35.1 (34.0-36.0)
Socio-economic status (proportion in most deprived 50% of deciles) (%) (95% CI)	8/16 (50%) (25.5-74.5)	28/37 (75.7%) (58.5-87.6)	20/30 (66.7%) (47.1-82.1)	$\chi^2=3.369$ p=0.186	56/83 (67.5%) (56.2-77.1)
Ethnicity (proportion of non-white ethnic groups) (%) (95% CI)	7/19 (36.8%) (17.2-61.4)	17/54 (31.5%) (19.9-45.7)	7/63 (11.1%) (5.0-22.2)	$\chi^2=9.333$ p=0.009	31/136 (22.8%) (16.2-30.9)

8.8.4.2 Medical and obstetric risk factors

Table 8-29 shows clinical features of participants.

As would be expected given recruitment criteria for the low risk group, parity differed between groups ($p<0.001$). There was no difference in BMI or smoking status. There was a difference in UTI risk factors ($p=0.007$) with risk factors present in nearly a quarter of those with previous preterm birth.

Table 8-29: Clinical features of participants (medical and obstetric history and risk factors) (combined groups)

Variable	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Nulliparous (%) (95% CI)	0/20 (0%) (0-20.1)	14/54 (25.9%) (15.4-39.9)	43/63 (68.3%) (55.2-79.1)	$\chi^2=38.129$ $p<0.001$	57/137 (41.6%) (33.4-50.4)
Median body mass index (kg/m ²) (95% CI)	(n=19) 24.0 (21.2-27.1)	24.1 (22.9-25.6)	22.3 (21.1-23.6)	$p=0.114$ Kruskal- Wallis	23.3 (22.3-24.3)
UTI risk factors present (%) (95% CI)	0/20 (0%) (0-20.1)	12/52 (23.1%) (13.0-37.2)	4/58 (6.9%) (2.2-17.6)	$\chi^2=9.97$ $p=0.007$	16/130 (12.3%) (7.4-19.5)
Current smokers (%) (95% CI)	1/16 (6.3%) (0.3-32.3)	4/37 (10.8%) (3.5-26.4)	2/30 (6.7%) (1.2-23.5)	Fisher's Exact $p=0.878$	7/83 (8.4%) (3.7-17.1)

Table 8-30 shows clinical information relating only to the preterm birth clinic, i.e. pertaining only to the women at high risk of preterm birth. These qualitatively demonstrate the difference between the two groups in terms of short cervix measurements, and use of progesterone and cervical cerclage.

It should be noted that quantitative fetal fibronectin was introduced to the clinic after the pilot data had been collected and so was not available for these participants.

Table 8-30: preterm birth clinic-specific clinical information and outcomes (combined groups)

Variable	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)
Cervical cerclage this pregnancy (%) (95% CI)	20/54 (37.0%) (24.6-51.3)	0/63 (0%) (0-7.2)
Progesterone treatment this pregnancy (%) (95% CI)	17/54 (31.5%) (19.9-45.7)	4/63 (6.4%) (2.1-16.3)
Scans in PTB clinic (95% CI) (Range)	4 (4-5) (1-9)	2 (2-2) (1-8)
Cervix less than 15mm (%) (95% CI)	3/54 (5.6%) (1.5-16.4)	0/63 (0%) (0-7.2)
Cervix 15 – 25mm (%) (95% CI)	11/54 (20.4%) (11.1-33.9)	6/63 (9.5%) (3.9-20.2)
Shortest ever measured cervix (mm) (95% CI) (Range)	28.0 (27-29) (8-44)	30.0 (29-31) (18-42)
Fetal fibronectin (fFN) test done (%) (95% CI)	48/54 (88.9%) (76.7-95.4)	51/63 (81.0%) (68.7-89.4)
fFN result positive (%) (95% CI)	4/50 (8%) (2.6-20.1)	1/55 (1.8%) (0.1-11)
fFN value (95% CI) (Range)	(n=32) 7.0 (3-14) (0-144)	(n=19) 3.0 (3-5) (2-7)

8.8.4.3 Obstetric and neonatal outcomes

Table 8-31 shows obstetric and neonatal outcomes. Delivery details were missing from 4 participants who were uncontactable for follow-up.

There was a difference in distribution in terms of gestation at delivery, second trimester or preterm birth, spontaneous preterm birth, and days from recruitment to delivery with the HR-PTB-C group most likely to deliver prematurely and HR-non-PTB-C least likely.

The low risk group includes 3 women who delivered preterm and all were spontaneous. Two were previously described (8.4.3). The third went into spontaneous labour at 36+3 weeks.

There were no other differences between groups in terms of maternal and neonatal outcomes, although it should be noted that sepsis and neonatal outcomes other than gestation at delivery were not available for the pilot study participants.

Table 8-31: Obstetric and neonatal outcomes for PTB study (combined groups)

	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Significance
PPROM this pregnancy (%) (95% CI)	0/19 (0%) (0-20.9)	5/50 (10%) (3.7-22.6)	1/62 (1.6%) (0.1-9.8)	Fisher's Exact p=0.120
Gestation at delivery (days) Median (95% CI) (Range)	(n=19) 274 (271-279) (138-290)	(n=52) 269.5 (263-275) (130-294)	(n=61) 279 (276-282) (235-296)	p<0.001 Kruskal-Wallis
PTB or second trimester miscarriage (%) (95% CI)	3/19 (15.8%) (4.2-40.5)	15/53 (28.3%) (17.2-42.6)	2/61 (3.3%) (0.6-12.4)	$\chi^2=13.909$ p=0.001
Spontaneous PTB this pregnancy (%) (95% CI)	3/19 (15.8%) (4.2-40.5)	9/52 (17.3%) (8.7-30.8)	1/60 (1.7%) (0.0-10.1)	$\chi^2=8.48$ p=0.014
Days from recruitment to delivery Median (95% CI) (Range)	(n=19) 159 (152-165) (16-197)	(n=52) 158.5 (150-167) (8-200)	(n=61) 167 (163-172) (100-196)	p=0.002 Kruskal-Wallis
Induction of labour (proportion induced) (%) (95% CI)	0/17 (0%) (0-22.9)	11/52 (21.2%) (11.5-35.1)	10/60 (16.7%) (9.0-29.0)	$\chi^2=4.22$ p=0.121
Caesarean delivery (%) (95% CI)	8/18 (44%) (22.4-68.7)	18/52 (34.6%) (22.3-49.2)	19/61 (31.2%) (20.3-44.4)	$\chi^2=1.092$ p=0.579

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	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Significance
Suspected intrapartum sepsis (%) (95% CI)	2/13 (15.4%) (2.7-46.3)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.120
Suspected postnatal sepsis (%) (95% CI)	1/13 (7.7%) (0.4-37.9)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.389
Admission to NNU (%) (95% CI)	3/13 (23.1%) (6.2-54.0)	6/36 (16.7%) (7.0-33.5)	1/29 (3.5%) (0.2-19.6)	Fisher's Exact p=0.098
Suspected neonatal infection (%) (95% CI)	2/12 (16.7%) (2.9-49.1)	2/36 (5.6%) (1.0-20.0)	0/29 (0%) (0-14.6)	Fisher's Exact p=0.079
Fetal or neonatal loss	1 (miscarriage)	1 (neonatal death)	0	n/a

Discussion

The findings being so similar to the original PTB study mean that the points discussed apply equally here (8.4.4).

The occurrence of spontaneous preterm births in the low risk cohort is challenging to interpret, however it should be remembered again that the group size of 20 is low, and the 95% confidence interval of the proportion with preterm birth includes the national average (3/19, 15.8%, 95% CI 4.2-40.5).

It has been suggested that one of the participants who had a preterm birth should be removed from the cohort. It was discovered several months after recruitment that the patient, whilst reporting she had had a previous term delivery when booking with the midwife, had actually given birth to her first baby at 36+6 weeks. One day off term (37 weeks) would not be considered high risk in clinical practice and certainly would not have fulfilled criteria for referral to the preterm birth clinic. Given the intention of this study to have

clinical face validity, I felt it was appropriate to maintain the patient in the low risk group. Low risk women do have preterm birth and one of the difficulties is to predict who they are. An ideal experiment would be to collate sufficient low risk women, and within them to compare those with preterm birth against those with term birth. However, that would require recruitment of substantial numbers.

8.8.4.4 Full microbiological results in index pregnancy

Standard urine culture information was collected only for four of the additional low risk patients (those from the Abdominal pain study). The data have been reanalysed here for booking urines Table 8-32 and urines throughout pregnancy Table 8-33 with very similar results.

Table 8-32: Table comparing standard culture at booking against study group (combined groups)

MSU result (% of study group)	LR-C (n=16)	HR-PTB- C (n=37)	HR-non- PTB-C (n=30)	Totals (n=83)	Significance
Screening culture negative	4 (25.0%)	18 (48.7%)	12 (40%)	34 (41.0%)	Fisher's Exact Not calculated
Non-significant growth	5 (31.3%)	9 (24.3%)	8 (26.7%)	22 (26.5%)	
Mixed growth	5 (31.3%)	5 (13.5%)	8 (26.7%)	18 (21.7%)	
Positive	2 (12.5%)	5 (13.5%)	2 (6.7%)	9 (10.9%)	
Totals	16 (100%)	37 (100%)	30 (100%)	83 (100%)	

Table 8-33: All standard urine culture results in index pregnancy compared by study group (combined groups)

MSU result (% of study group)	LR-C (n=16)	HR-PTB- C (n=37)	HR-non- PTB-C (n=30)	Totals (n=83)	Significance
Screening culture negative	17 (34.7%)	113 (48.1%)	53 (56.4%)	183 (48.4%)	$\chi^2=10.758$ p=0.096
Non-significant growth	12 (24.5%)	61 (26.0%)	20 (21.3%)	93 (24.6%)	
Mixed growth	10 (20.4%)	39 (16.6%)	15 (16.0%)	64 (16.9%)	
Positive	10 (20.4%)	22 (9.4%)	6 (6.4%)	38 (10.1%)	
Totals	49 (100%)	235 (100%)	94 (100%)	378 (100%)	

Discussion

Given only four additional participants were included in one study group, the findings are not substantially different and the previous discussion would apply here (8.5.3).

8.8.4.5 Comparing the prevalence of urinary pathology using standard and alternative methods

In this section I present data comparing the prevalence of urinary pathology in pregnant women with (1) high risk of preterm birth owing to previous preterm birth (HR-PTB-C), (2) high risk of preterm birth owing to other risk factors (HR-non-PTB-C) or (3) low risk for preterm birth with previous term births (LR-C), using standard and alternative methods.

Methods for all standard and alternative tests are as described in chapter 6, unless otherwise specified.

8.8.4.6 Structured assessment of lower urinary tract symptoms

Table 8-34 demonstrates LUTS scores for the PTB study combined groups. As before, there was a wide range in total LUTS and in grouped LUTS. There were no differences between the three study groups for total LUTS or any of the categories of LUTS.

Individual symptom scores were not available for the pilot study participants so analysis to this level was not possible.

Table 8-34: LUTS for PTB study (combined groups)

	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Significance	Overall (n=137)
Total LUTS (max 40)					
Median	4	6	5	p=0.657 Kruskal-Wallis	5
(95% CI)	(3-10)	(5-7)	(4-8)		(5-6)
(Range)	(0-17)	(0-32)	(0-23)		(0-32)
Any LUTS (%)	19/20 (95%)	51/54 (94.4%)	60/63 (95.2%)	Fisher's Exact p=1.000	130/137 (94.9%)
(95% CI)	(73.1-99.7)	(83.7-98.6)	(85.8-98.8)		(89.4-97.7)
Frequency (number of episodes)		(n=53)			(n=136)
Median	5.5	7.5	7.5	p=0.100 Kruskal-Wallis	7.5
(95% CI)	(4.5-8.5)	(5.5-9.5)	(6.5-8.5)		(6.5-8.5)
(Range)	(2.5-11.5)	(2.5-20.5)	(3.5-20.5)		(2.5-20.5)
Nocturia (number of episodes)					
Median	1.0	1.5	1.5	p=0.387 Kruskal-Wallis	1.5
(95% CI)	(0.5-2.0)	(1.0-2.0)	(1.0-2.0)		(1.0-1.5)
(Range)	(0-6.0)	(0-6.5)	(0-6.5)		(0.0-6.5)
Any daytime incontinence (%)	3/19 (15.8%)	19/51 (37.3%)	15/60 (25%)	$\chi^2=3.788$ p=0.150	37/130 (28.5%)
(95% CI)	(4.2-40.5)	(24.5-51.9)	(15.1-38.1)		(21.1-37.2)
Any night incontinence (%)	0/20 (0%)	5/49 (10.2%)	1/59 (1.7%)	Fisher's Exact p=0.129	6/128 (4.7%)
(95% CI)	(0-20.1)	(3.8-23)	(0.1-10.3)		(1.9-10.4)
Stress incontinence (max 7)					
Median	0.0	0.0	0.0	p=0.141 Kruskal-Wallis	0.0
(95% CI)	(0-0)	(0-1)	(0-1)		(0-1)
(Range)	(0-2)	(0-5)	(0-7)		(0-7)
Any stress incontinence	5/20 (25%)	26/54 (48.2%)	27/63 (42.9%)	$\chi^2=3.216$ p=0.200	58/137 (42.3%)
(95% CI)	(9.6-49.4)	(34.5-62.0)	(30.7-55.9)		(34.0-51.2)

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	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Significance	Overall (n=137)
Overactive bladder symptoms (max 12) Median (95% CI) (Range)	2.0 (0-3) (0-8)	1.5 (0-3) (0-10)	2.0 (1-2) (0-7)	p=0.875 Kruskal-Wallis	2.0 (1-2) (0-10)
Any overactive bladder symptoms (%) (95% CI)	14/20 (70%) (45.7-87.2)	34/54 (63.0%) (48.7-75.4)	44/63 (69.8%) (56.8-80.4)	$\chi^2=0.710$ p=0.701	92/137 (67.2%) (58.5-74.8)
Voiding symptoms (max 8) Median (95% CI) (Range)	1.5 (1-3) (0-5)	1.5 (1-2) (0-7)	2.0 (1-3) (0-8)	p=0.534 Kruskal-Wallis	2.0 (1-2) (0-8)
Any voiding symptoms (%) (95% CI)	15/20 (75%) (50.6-90.4)	42/54 (77.8%) (64.1-87.5)	49/63 (77.8%) (65.2-86.9)	Fisher's Exact p=0.924	106/137 (77.4%) (69.3-83.9)
Pain symptoms (max 13) Median (95% CI) (Range)	0.5 (0-2) (0-6)	1.0 (0-2) (0-13)	1.0 (0-2) (0-9)	p=0.795 Kruskal-Wallis	1.0 (0-1) (0-13)
Any pain symptoms (%) (95% CI)	10/20 (50%) (27.9-72.2)	28/54 (51.9%) (38.0-65.5)	34/63 (54.0%) (41.0-66.4)	$\chi^2=0.114$ p=0.945	72/137 (52.6%) (43.9-61.1)

As before, there were no differences between groups for LUTS, despite the difference in preterm birth rates. Whilst this may still reflect the relatively small group sizes with wide confidence intervals, it may be interpreted as reflecting a lack of urinary pathology contributing to pathophysiology of preterm birth. It is possible that within these groups, there are a subsection of women for whom urinary pathology does contribute to preterm birth pathophysiology, and more nuanced methods may be required to tease these out.

8.8.4.7 Dipstick

Table 8-35 compares dipstick results. These were similar to the original PTB study analysis. 65/137 (47.5%) had a dipstick positive for nitrite or at least tract of leucocyte or protein. Only 2/136 (1.5%) samples were positive for nitrite. There were no between group differences.

Table 8-35: Dipstick results for PTB study (combined groups)

Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Leucocyte esterase (≥ trace) (%) (95% CI)	4/16 (25%) (8.3-52.6)	16/54 (29.6%) (18.4-43.8)	25/62 (40.3%) (28.3-53.5)	$\chi^2=3.305$ $p=0.192$	45/136 (33.1%) (25.4-41.8)
Blood (≥ trace) (%) (95% CI)	2/20 (10%) (1.8-33.1)	9/54 (16.7%) (8.4-29.8)	11/63 (17.5%) (9.4-29.5)	Fisher's Exact $p=0.817$	22/137 (16.1%) (10.6-23.5)
Protein (≥ trace) (%) (95% CI)	6/20 (30.0%) (12.8-54.3)	16/54 (29.6%) (18.4-43.8)	15/63 (23.8%) (14.4-36.5)	$\chi^2=0.606$ $p=0.739$	37/137 (27.0%) (20.0-35.4)
Nitrite (%) (95% CI)	0/20 (0%) (0-20.1)	0/54 (0%) (0-8.3)	2/62 (3.2%) (0.6-12.2)	Fisher's Exact $p=0.635$	2/136 (1.5%) (0.3-5.8)
Dipstick positive for leu/pro/nit (%) (95% CI)	7/20 (35%) (16.3-59.1)	24/54 (44.4%) (31.2-58.5)	34/63 (54.0%) (41.0-66.4)	$\chi^2=2.512$ $p=0.285$	65/137 (47.5%) (38.9-56.1)

8.8.4.8 Standard microscopy and culture

Table 8-36 shows standard culture results. Microscopy and species information were not recorded for the student pilot study. Standard culture results were reported with screening culture negative and non-significant growth amalgamated into one 'negative' group.

With very similar findings to the original PTB study, positive cultures were present in LR-C group for 3/19 (15.8%, 95% CI 4.2-40.5), HR- PTB-C group for 0/51 (0%, 95% CI 0-8.7) and HR-non-PTB-C group for 2/62 (3.2%, 95% CI 0.6-12.2). This was different between groups ($p=0.011$) although confidence intervals were wide.

Table 8-36: Standard microscopy and culture for PTB study (combined groups)

Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Significance	Overall (n=80)
Culture positive >10 ⁵ CFU/ml (%) (95% CI)	3/19 (15.8%) (4.2-40.5)	0/51 (0%) (0-8.7)	2/62 (3.2%) (0.6-12.2)	Fisher's Exact $p=0.011$	5/132 (3.8%) (1.4-9.1)
Mixed growth of uncertain significance (%) (95% CI)	3/19 (15.8%) (4.2-40.5)	4/51 (7.8%) (2.5-19.8)	1/62 (1.6%) (0.08-9.8)	Fisher's Exact $p=0.048$	8/132 (6.1%) (2.9-12.0)

8.8.4.9 Fresh unspun urine microscopy

Table 8-37 shows fresh unspun microscopy results. These are very similar to the original PTB study results. There were no differences between groups.

Table 8-37: Fresh unspun microscopy for PTB study (combined groups)

Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Median white cell count (WBC μl^{-1}) (95% CI) (Range)	0 (0-24) (0-340)	2 (0-4) (0-122)	4 (2-6) (0-4800)	Kruskal-Wallis p=0.353	4 (2-4) (0-4800)
Any white cells (%) (95% CI)	9/20 (45%) (23.8-68.0)	30/54 (55.6%) (41.5-68.8)	42/62 (67.7%) (54.5-78.7)	$\chi^2=3.843$ p=0.146	81/136 (59.6%) (50.8-67.8)
Median red cell count (RBC μl^{-1}) (95% CI) (Range)	0 (0-14) (0-720)	0 (0-2) (0-660)	0 (0-2) (0-1280)	Kruskal-Wallis p=0.766	0 (0-2) (0-1280)
Any red cells (%) (95% CI)	8/20 (40%) (20.0-63.6)	23/54 (42.6%) (29.5-56.7)	28/62 (45.2%) (32.7-58.2)	$\chi^2=0.187$ p=0.911	59/136 (43.4%) (35.0-52.1)
Median epithelial cell count (EPC μl^{-1}) (95% CI) (Range)	7 (2-18) (0-64)	10 (4-14) (0-800)	8 (4-12) (0-320)	Kruskal-Wallis p=0.681	8 (6-10) (0-800)
Any epithelial cells (%) (95% CI)	15/20 (75%) (50.6-90.4)	47/54 (87.0%) (74.5-94.2)	57/63 (90.5%) (79.8-96.1)	Fisher's Exact p=0.213	119/137 (86.9%) (79.8-91.8)

8.8.4.10 ATP measurement

Table 8-38 compares ATP measurements. ATP (measured in relative light units, RLU) was detected in every sample, ranging from 537 to 24523. There was no difference between groups (p=0.380).

Table 8-38: ATP measurements for PTB study (combined groups)

Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Median ATP(RLU) (95% CI) (Range)	3756 (2158-6499) (537-16502)	4949 (3533-7915) (608-24523)	4967 (3733-5667) (710-19985)	Kruskal-Wallis p=0.365	4473 (3896-5567) (537-24523)

8.8.4.11 Sediment culture with counts and identification to species level of cultured organisms

Table 8-39 shows enhanced (sediment) culture results. Bacteriuria was detected in 136/137 (99.3%) samples. The median number of isolates was 3 (range 1-7). There were no between-group differences.

Table 8-39: Sediment culture results for PTB study (combined groups)

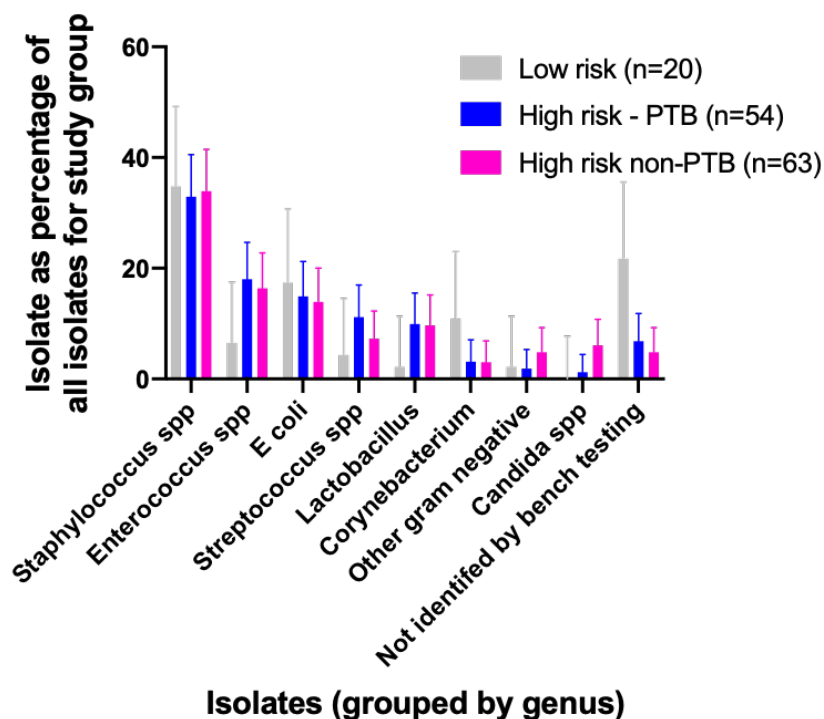
Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Total colony counts	749		573		704
Median CFU/ml	(52-4011)	1482	(258-1094)	p=0.115	(410-1556)
(95% CI)	(2-1.3x10 ⁶)	(369-3195)	(0-1.0x10 ⁷)	Kruskal-Wallis	(0-1.0x10 ⁷)
(Range)		(2-1.0 x10 ⁶)			
Colony count for dominant isolate					
Median CFU/ml	701	1410	562	p=0.103	592
(95% CI)	(41-2640)	(309-22555)	(223-962)	Kruskal-Wallis	(309-1280)
(Range)	(2-1.3x10 ⁶)	(2-1.0 x10 ⁶)	(0-1.0x10 ⁷)		(0-1.0x10 ⁷)
Number of isolates					
Median	3	3	2	p=0.297	3
(95% CI)	(2-3)	(2-3)	(2-3)	Kruskal-Wallis	(2-3)
(Range)	(1-6)	(1-7)	(0-6)		(0-7)
Proportion with any bacteriuria (%)	20/20 (100%)	54/54 (100%)	2/63 (98.4%)	Fisher's Exact	136/137 (99.3%)
(95% CI)	(80.0-100)	(91.7-100)	(90.3-99.9)	p=0.999	(95.4-100.0)

Table 8-40 and Figure 8-10 show the genera of isolates as identified by bench testing, compared by study group. The most commonly identified genera by bench testing were *Staphylococcus spp*, *Enterococcus spp*, *E. coli*, and *Lactobacillus spp*. This top four are the same as the original PTB study.

Table 8-40: Comparison of genus of isolates as identified by bench testing with study group (combined groups)

	LR-C (n=20) (% of all LR isolates)	HR- PTB-C (n=54) (%) of all HR- PTB isolates)	HR-non- PTB-C (n=63) (% of all HR-non- PTB isolates)	Totals (n=137) (% of all isolates)	Significance*
<i>Staphylococcus</i> <i>spp</i>	16 (34.8%)	53 (32.9%)	56 (33.9%)	125 (33.6%)	Condensed table: $\chi^2=27.1$ df=12 p=0.008 Excluding non- identified isolates: $\chi^2=13.24$ df=10 p=0.211
<i>Enterococcus</i> <i>spp</i>	3 (6.5%)	29 (18.0%)	27 (16.4%)	59 (15.9%)	
<i>Escherichia coli</i> <i>spp</i>	8 (17.4%)	24 (14.9%)	23 (13.9%)	55 (14.8%)	
<i>Streptococcus</i> <i>spp</i>	2 (4.3%)	18 (11.2%)	12 (7.3%)	32 (8.6%)	
<i>Lactobacillus</i> <i>spp</i>	1 (2.2%)	16 (9.9%)	16 (9.7%)	33 (8.9%)	
<i>Corynebacterium</i> <i>spp</i>	5 (10.9%)	5 (3.1%)	5 (3.0%)	15 (4.0%)	
Other gram negative <i>spp</i>	1 (2.2%)	3 (1.9%)	8 (4.8%)	12 (3.2%)	
<i>Candida spp</i>	0 (0%)	2 (1.2%)	10 (6.1%)	12 (3.2%)	
Not identified by bench testing	10 (21.7%)	11 (6.8%)	8 (4.8%)	29 (7.8%)	
Total isolates	46 (100%)	161 (100%)	165 (100%)	372 (100%)	

Figure 8-10: Comparison of sediment culture isolates identified to genus level by bench testing, using combined dataset



Median colony counts appear lower than in the original PTB study. Note that student total CFU counts are likely to be undercounted – they often did not report the numbers of yeast, lactobacillus or the smallest number isolate. This may have led to systematic undercounting.

The student pilot data did not contain identification to species level. However, the genus level identification was similar in proportions identified to the original PTB study.

8.8.4.12 Cytokines – IL-6

Protein concentrations for normalisation were not available for the pilot study participants so normalisation was not performed. Table 8-41 demonstrates the results. There were no differences between groups in terms of IL-6 concentration.

The median urinary IL-6 concentration was 2.2pg/ml (95% CI 1.7-2.9, range 0.14-736). 16/137 (11.7%) were below the LLOD.

Table 8-41: Urinary cytokines for PTB study (combined groups)

	LR-C (n=20)	HR-PTB-C (n=54)	HR-non- PTB-C (n=63)	Sig.	Total (n=137)
IL-6 concentration (pg/ml) Median (95% CI) (Range)	(n=20) 2.6 (0.9-4.1) (0.14–9.9)	(n=54) 1.6 (0.8-2.9) (0.14–56.9)	(n=63) 2.4 (1.7-4.4) (0.14–736)	p=0.221 Kruskal- Wallis	(n=137) 2.2 (1.7-2.9) (0.14–736)
IL-6<LLOD (%) (95% CI)	2/20 (10%) (1.8-33.1)	7/54 (13.0%) (5.8-25.5)	7/63 (11.1%) (5.0-22.2)	Fisher's Exact p=0.936	16/137 (11.7%) (7.0-18.6)

8.8.4.13 Bacterial-associated clue cell proportions

Results are presented in Table 8-42. The median proportion (%) of epithelial cells with associated bacteria was 42% (95% CI 15-85) in the LR-C group, compared with 23% (95% CI 10-35) in HR-PTB-C and 12% (95% CI 4-25) in HR-non-PTB-C. There was a difference between groups (p=0.042), but the effect size was not significant.

The proportion of epithelial cells with any associated bacteria (i.e., clue cell proportion > 0) was 101/136 (74.3%, 95% CI 65.9-81.2), which did not differ between groups (p=0.537).

Table 8-42: Table comparing bacterial-associated clue cell data for the three study groups (combined groups)

Measure	LR-C (n=20)	HR-PTB-C (n=54)	HR-non-PTB-C (n=63)	Sig.	Overall (n=137)
Proportion (%) of epithelial cells that are clue cells					
Median	42	23	12	p=0.042	20
(95% CI)	(15-85)	(10-35)	(4-25)	Kruskal-	(10-28)
(Range)	(0-100)	(0-100)	(0-100)	Wallis	(0-100)
Proportion of epithelial cells that have any associated bacteria (clue cell proportion > 0)	17/20 (85%)	40/54 (74.1%)	44/62 (71.0%)	$\chi^2=1.560$ p=0.458	101/136 (74.3%)
(95% CI)	(61.1-96.0)	(60.1-84.6)	(57.9-81.5)		(65.9-81.2)

The proportion of clue cells appeared lower in this cohort than in the original PTB study. However, the overall patterns were similar, including the highest proportion of clue cells in the low risk group.

It should be noted that the method of counting clue cells in the pilot study was the 'count to 100' method, compared with the 'count to 20' method used in the original PTB study. It is not clear what difference, if any, this would contribute to these findings.

8.8.5 Combined data PTB study discussion and conclusions

Many of the findings were the same using the additional data. These included the differences between study groups in terms of risk factors (history of UTI, parity, ethnicity) and lack of differences (gestation at recruitment, age, BMI and smoking status). As before, there were substantial differences between the high risk group with previous preterm birth, and the high risk group without previous preterm birth, with the former more likely to have cervical cerclage, progesterone, and to deliver preterm.

In terms of the standard tests, there remained no differences between groups in terms of dipstick positivity. Standard culture positive or mixed growth were higher in the low risk group, again. However, the confidence intervals were broad, given the small numbers in the group, and larger numbers would be needed to detect a meaningful difference.

Regarding the alternative tests, there were no between-group differences in symptoms, fresh unspun microscopy, urinary ATP, sediment culture (colony counts, number of isolates or distribution of genus), or IL-6. There was a difference in clue cell proportions, which appeared higher in the low risk group.

Again, a wide range of results was noted across all groups, whether this related to symptoms, host response or quantitative microbiology. This suggests an underlying pathophysiology not explained by these groups.

This attempt to improve on the weaknesses of the PTB study was again restricted by the small size of the low risk group. A power calculation performed for the MSU-CSU study, based on total colony counts, showed that a sample size of 20 subjects in each group would suffice (0). By this standard, it would appear that there is truly no difference in total colony counts between the three groups.

There are methodological flaws, previously described (8.7) but in some cases exacerbated by the inclusion of the pilot study data. These include the lack of full microbiology data throughout the pregnancies, and a lack of detail in the tests, particularly identification of isolates to species level, analysis of cytokines other than IL-6, and normalisation for protein. However, by including additional data, and in particular expanding the high risk groups substantially, with no change in conclusions, this provides important negative findings.

The alternative tests in this study were unable to differentiate between study groups based on clinical risk factors for preterm birth even when additional participants were included.

8.9 PTB study conclusion

I described the arguments associating preterm birth with UTI at the outset of this thesis; however there are gaps in our knowledge of how urinary pathology

relates to preterm birth. In the PTB study I set out to establish if women at risk of preterm birth because of previous preterm birth have urinary pathology not detected by standard tests, compared with women at risk because of other risk factors, or low risk women multiparous women. This comparison was performed using standard and alternative tests, using parallel methods as the Abdominal pain study, with all participants recruited between 13-17 weeks gestation.

By the very nature of its study design, there were expected demographic and clinical differences between the study groups, with women with previous preterm birth receiving greater interventions to prevent preterm birth such as progesterone or cervical cerclage, and higher rates of preterm birth. Interestingly women with previous preterm birth were more likely to report previous UTI history.

The groups differed by standard culture, with a higher proportion of positive cultures at the time of recruitment in the low risk group. There were no substantial between-group differences in alternative tests, other than enhanced culture, where there were some species only detected in the previous preterm birth group, although numbers were too small for this to be conclusive.

A similar finding to the Abdominal pain study was that most women had organisms identified by sediment culture, and this was often polymicrobial. Again, the range of results for all alternative tests was very broad suggesting a spectrum of urinary pathology not explained by these study groups.

The greatest methodological problem for this study was the small number of participants in the low risk group. To address this, appropriate PTB pilot study participants, and Abdominal pain study normal controls meeting PTB study inclusion criteria, were included for a re-analysis. While limited in scope as the full range of alternative tests were not included, this had similar findings to the original study. In particular this reinforced the negative finding that urinary pathology, while prevalent across all groups, was not more prevalent in those with previous preterm birth. Unfortunately the modified analysis remained underpowered to detect differences between the low risk group and the two

high risk groups. Despite this, the lack of between-group differences suggests that if there is urinary pathology underlying increased risk of preterm birth, it was not elicited by this study design.

Preterm birth is a clinical syndrome with multiple phenotypes; it may be that preterm birth associated with urinary pathology is a small sub-set of cases and would not emerge from a study of this size or design. Future research on alternative urinary tests may need to focus on longitudinal sampling, with sample size calculated according to the outcome of preterm birth rather than purely on risk factors.

One criticism of the work so far, in both Abdominal pain and PTB studies, is the use of a mid-stream urine specimen and the difficulty in determining what is contamination. In the next chapter I describe the findings of the MSU-CSU study which set out to explore this issue.

9 The effect of urine sampling method on standard and alternative tests of urinary pathology in pregnancy: a prospective study ('MSU-CSU study')

9.1 Introduction

An issue that has dogged UTI research is contamination. I wanted to build on previous studies comparing catheter specimens with voided samples, in pregnancy. I was keen to study the alternative tests used in the Abdominal pain and PTB studies, to understand more about the relationship between MSU and CSU samples; this gave rise to the MSU-CSU study.

Aims

To determine the prevalence of urinary pathology detected in (1) voided mid-stream samples of urine (MSU) and (2) paired catheter specimens of urine (CSU).

Hypothesis

Tests for urinary pathology may have different findings in CSUs compared with MSUs, particularly regarding the risk of contamination of voided urine samples

Research questions

- (3) Describe the cohort in terms of likelihood of urinary pathology using the following methods:
 - a. Structured lower urinary tract symptoms assessment using Artemis
 - b. Quality of life assessment
- (4) Compare the prevalence of urinary pathology in voided and catheter specimens using standard and alternative methods:
 - a. Standard dipstick, microscopy and culture
 - b. Fresh unspun urine microscopy
 - c. Urinary ATP

- d. Sediment culture with counts and identification to species level of cultured organisms
- e. Urinary cytokines – IL-6, IL-8, lactoferrin
- f. Bacterial-associated clue cell counts
- g. Uroplakin-positive epithelial cell proportions

9.2 Methods

Methods for the MSU-CSU study were the same as described in chapter 6 except for the elements described below.

9.2.1 Study design

This was a prospective study comparing standard and alternative urinary tests in catheter specimens of urine (CSU) with matched midstream urine specimens of urine (MSU) in pregnant women undergoing catheterisation for clinical indications. This is demonstrated in Figure 9-1.

Figure 9-1: Diagram to summarise study design and methods for the MSU-CSU study

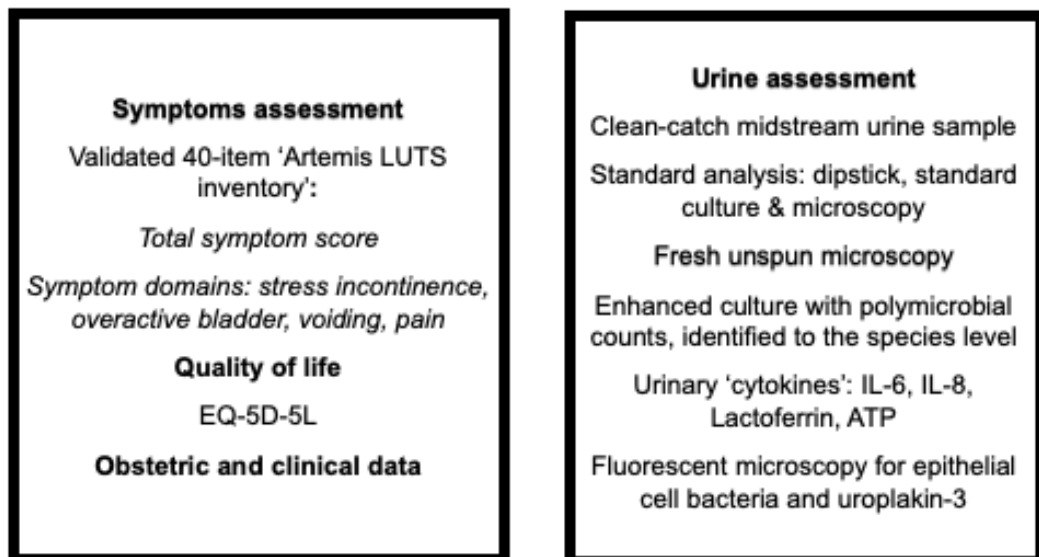
Prospective recruitment of women undergoing catheterisation, in two groups:



All participants provided an MSU sample immediately prior to catheterization, and a catheter specimen, immediately following catheterisation:



Descriptive analysis of clinical data and paired comparison of urine tests:



9.2.2 Recruitment

Eligibility criteria: Any pregnant woman (≥ 18 years of age) being catheterised for elective clinical indications.

Exclusion criteria: Women having catheters for emergency indications or intrapartum.

Deviation from original planned protocol:

Initially it was planned to recruit participants from elective caesarean section lists or elective cervical cerclage lists. Owing to unpredictability of cerclage

cases only two cerclage participants were recruited. They are included here but cannot be analysed separately.

Methods of case ascertainment and control selection: Patients acted as their own controls with a planned comparison of voided and catheter specimens of urine. A secondary analysis of voided samples against other voided samples in the related studies (Abdominal pain and PTB) was planned.

Participant sampling:

To reduce recruitment bias, we aimed for consecutive recruitment during the lead recruiter's (NL) recruitment periods. In total 36 patients were approached to participate; 2 declined and 34 patients were recruited.

To facilitate patient flow and reduce impact on the clinical service, as well as to ensure consistency in sample preparation, NL performed all the catheterisations following a standard aseptic technique. It should be noted that median time between collection of MSU and CSU specimens was 2.5 hours (interquartile range 1.5-3.0).

9.2.3 Sampling methods

MSU: Participants were given the same instructions and equipment for their MSU sample as the Abdominal pain and PTB studies (see 6.5). They were asked to void during their immediate pre-operative preparations.

CSU: NL performed all catheterisations following standard aseptic technique at the start of the elective procedure. A CSU was taken immediately (directly from the catheter, not from the collection bag). Catheterisation and urine sampling was performed prior to routine prophylactic antibiotics.

Median time between collection of the MSU and CSU specimens was 2.5 hours (interquartile range 1.5-3.0).

Sample handling for both MSUs and CSUs followed the same careful procedures as described in section 6.6.

9.2.4 Data collection

Recruitment to this study occurred from June 2015 until August 2015 inclusive.

JC conceived the study. NL designed the study under supervision from JC and co-supervisors, using the model and data collection proformas of the Abdominal pain study. NL recruited patients, collected initial clinical data, collected samples, performed unspun fresh microscopy, dipstick, ATP, sediment culture to genus (by bench test) level, and epifluorescent microscopy analysis of urothelial cells. JC completed clinical data collection, and performed urinary cytokine analysis and analysis of isolates to species level by API. Analysis presented below has been analysed by JC, including the two cerclage patients, who were not included in NL's dissertation.

9.3 Results

9.3.1 Demographics, group characteristics and outcomes

First, I present the demographic and obstetric data for the MSU-CSU study, shown in Table 9-1 (demographics), Table 9-2 (clinical features), and Table 9-3 (obstetric and neonatal outcomes).

Of the 34 patients in the study, 32 were undergoing elective caesarean ('caesarean cases'), and 2 cervical cerclage ('cerclage cases'). No significance testing was carried out as there were only two Cerclage cases.

Median gestation at recruitment of caesarean cases was 272.5 days (range 248–288); median gestation of cerclage cases was 91.0 days (range 88–94). Median age was 34.5 years (95% CI 31-38). 19/34 (55.9%) patients were non-white ethnicity.

Median BMI was 25.2 (95% CI 23.1-28.0). 11/34 (32.4%) patients were nulliparous. 2/34 (5.9%) were current smokers. 1/34 (2.9%) had a multiple pregnancy. 4/34 had risk factors for preterm birth, 2 caesarean cases (previous preterm birth and multiple pregnancy) and the 2 cerclage cases. 10/34 reported previous UTI (all caesarean cases), including 4 with a history of recurrent UTI.

Caesarean cases delivered on the day of sampling; median sample-to-delivery time for cerclage cases was 190 days. There were 2 preterm births, in the caesarean group only. These were medically-indicated.

None had antenatal, intrapartum or postpartum infection. Three babies had suspected neonatal infection, and six were admitted for neonatal care (all caesarean cases).

Table 9-1: Demographic data for MSU-CSU study

Variable	Caesarean cases (n=32)	Cerclage cases (n=2)
Gestation at recruitment (days)		
Median	272.5	91.0
(95% CI)	(269-273)	(88-94)
(Range)	(248-290)	(88-94)
Age (years at recruitment)		
Median	35.0	30.0
(95% CI)	(31.0-38.0)	(28.0-32.0)
(Range)	(23.0-44.0)	(28.0-32.0)
Socio-economic status		
Bottom 50%	20/31	2/2
(%)	(64.5%)	(100%)
(95% CI)	(45.4-80.2)	(19.8-100)
Ethnicity (proportion of non-Caucasian ethnic groups)		
(%)	19/32	0/2
(95% CI)	(59.4%)	(0%)
	(41-76)	(0-80)

Table 9-2: Clinical features of participants for MSU-CSU study

Variable	Caesarean cases (n=32)	Cerclage cases (n=2)
Parity (proportion nulliparous)		
(%)	10/32	1/2
(95% CI)	(31.3%)	(50%)
	(17-50)	(3-97)
Body mass index (kg/m ²)		
Median	25.0	28.2
(95% CI)	(21.9-28.0)	(28-28.4)
(Range)	(18.4-38.5)	(28.0-28.4)
Preterm birth risk factors		
(%)	2/32	2/2
(95% CI)	(6.3%)	(100%)
	(1-22)	(20-100)
Previous UTI history		
(%)	10/32	0/2
(95% CI)	(31.3%)	(0%)
	(17-50)	(0-80)
Current smoker		
(%)	2/32	0/2
(95% CI)	(6.3%)	(0%)
	(1-22)	(0-80)
Multiple pregnancy		
(%)	1/32	0/2
(95% CI)	(3.1%)	(0%)
	(0-18)	(0-80)

Table 9-3: Obstetric and neonatal outcome data for MSU-CSU study

Variable	Caesarean cases (n=32)	Cerclage cases (n=2)
Gestation at delivery (days)		
Median	272.5	281.0
(95% CI)	(267-274)	(271-291)
(Range)	(248-288)	(271-291)
Preterm birth (any)	2/32	0/2
(%)	(6.3%)	(0%)
(95% CI)	(1-22)	(0-80)
	<i>(medically-indicated)</i>	
Days from recruitment to delivery		
Median	0	190
(95% CI)	(0-0)	(183-197)
(Range)	(0-0)	(183-197)
Birthweight (g)	(n=33)	(n=2)
Median	3210	3785
(95% CI)	(3000-3580)	(3250-4320)
Mode of delivery	32/32 caesarean	1/2 caesarean 1/2 instrumental
Induction of labour	0/32	1/1
Intrapartum sepsis	0/32	0/2
(%)	(0%)	(0%)
(95% CI)	(0-80)	(0-80)
Postpartum sepsis	0/32	0/2
(%)	(0%)	(0%)
(95% CI)	(0-80)	(0-80)
Neonatal sepsis	3/31	0/2
(%)	(9.7%)	(0%)
(95% CI)	(3-27)	(0-80)
Admission to neonatal care	6/33	0/2
(%)	(18.2%)	(0%)
(95% CI)	(8-36)	(0-80)
Apgars at 1 minute	(n=33)	(n=2)
Median	9	9
(95% CI)	(9-9)	(9-9)
Apgars at 5 minutes	(n=33)	(n=2)
Median	10	9.5
(95% CI)	(10-10)	(9-10)
Sex (proportion male)	14/33	1/2
(%)	(42.4%)	(50%)
(95% CI)	(26-61)	(3-97)

Only two patients were recruited from cerclages, owing to them being added to the theatre list at late notice, at times that were awkward from the point of view of immediately carrying out the laboratory analysis of urine, compared with elective caesarean lists which were more predictable.

More than a quarter had a history of UTI. This may increase the likelihood of urinary pathology, allowing more scope for comparison of voided and catheter specimens.

It is important to note that none of the patients had spontaneous preterm births, pyelonephritis or sepsis episodes.

9.3.2 Details of all standard urine cultures reported throughout the index pregnancy

Next, I reviewed all microbiological results throughout the pregnancy. Table 9-4 shows that all participants had a booking culture reported. Of these none were positive. 18/34 (52.9%) were screening culture negative, 10/34 (29.4%) non-significant growth, and 6/34 (17.6%) mixed growth.

Table 9-4: Table comparing standard culture at booking against study group

MSU result	Caesarean cases (n=32) (%)	Cerclage cases (n=2) (%)	Overall (n=34) (%)
Screening culture negative	16 (50)	2 (100)	18 (52.9)
Non-significant growth	10 (31.3)	0 (0)	10 (29.4)
Mixed growth	6 (18.8)	0 (0)	6 (17.6)
Positive	0 (0)	0 (0)	0 (0)
Totals	32 (100)	2 (100)	34 (100)

Table 9-5 shows that 110 standard MSU cultures were reported throughout 34 pregnancies. This includes the 32 that were sent as part of the study (but not the CSU cultures). Of these, 50/110 (45.5%) were screening culture negative, 28/110 (25.5%) were no-significant growth, 18/110 (16.4%) were mixed growth, and 14/110 (12.7%) were positive. Groups were not statistically compared owing to small numbers but are included for illustration.

Table 9-5: Table comparing all standard MSU results in index pregnancy against study group

MSU result	Caesarean cases (n=32) (%)	Cerclage cases (n=2) (%)	Overall (n=34) (% of total)
Screening culture negative	43 (42.6)	7 (77.8)	50 (45.5)
Non-significant growth	26 (25.7)	2 (22.2)	28 (25.5)
Mixed growth	18 (17.8)	0 (0)	18 (16.4)
Positive	14 (13.9)	0 (0)	14 (12.7)
Totals	101 (100)	9 (100)	110 (100)

Table 9-6 shows all standard urine cultures at participant level. Median number of cultures reported during the index pregnancy was 2.5 (95% CI 2-3, range 1-8). 9/34 (26.5%, 95% CI 13.5-44.7) had positive cultures, all in the Caesarean group. Of the 14 positive cultures, 3 were *Enterococcus spp.*, 3 were *Escherichia coli*, 8 were *Streptococcus agalactiae*, and 3 were *Candida spp.*

Table 9-6: Full standard culture results throughout index pregnancy for MSU-CSU study

Microbiological parameter	Caesarean cases (n=32)	Cerclage cases (n=2)	Overall (n=34)
Number of cultures reported (total=110) Median (95% CI) (Range)	2.5 (2-3) (1-8)	4.5 (2-7) (2-7)	2.5 (2-3) (1-8)
Number of screening culture negative Median (95% CI) (Range)	1.0 (1-2) (0-5)	3.5 (2-5) (2-5)	1.0 (1-2) (0-5)
Number of non-significant growth Median (95% CI) (Range)	1.0 (0-1) (0-3)	1.0 (0-2) (0-2)	1.0 (0-1) (0-3)
Number of mixed growth Median (95% CI) (Range)	0 (0-1) (0-3)	0 (0-0) (0-0)	0.0 (0-1) (0-3)
Number of positive cultures Median (95% CI) (Range)	0 (0-0) (0-5)	0 (0-0) (0-0)	0.0 (0-0) (0-5)
Proportion with any positive cultures this pregnancy (%) (95% CI)	9/32 (28.1%) (14.4-47.0)	0/2 (0%) (0-80.2)	9/34 (26.5%) (13.5-44.7)

Booking cultures were not positive for any participant in this group, however thanks to the small sample size, the 95% CI was 0-12.6% which would encompass the rates seen in the previous studies and in the published literature. 16/34 (47.1%) were mixed or non-significant growth, similar to previous studies, and similar to the proportion seen in the 110 samples sent throughout the pregnancies (including only MSU samples, not the additional CSUs). Approximately 1/10 had a positive culture at some point during the pregnancy.

In total, 110 cultures were reported for 34 patients; this means 42 cultures were reported in addition to booking and participation in this study. Again, understanding the implications of such a common test is important.

I went on to review all the other microbiology reported during each pregnancy.

9.3.3 Details of all other microbiology investigations reported during the index pregnancy

Table 9-7 shows all other microbiological results. No participants had blood cultures sent. 11/34 (32.4%) had vaginal swabs sent, of which one showed *Streptococcus agalactiae* and one *Escherichia coli spp.* There was one faeces culture (negative) and 34/34 (100%) patients had MRSA testing. No other culture tests were sent including no wound, respiratory or placental swabs. One placenta was sent for histopathology and was reported as mild chorioamnionitis.

Table 9-7: Table to show other microbiological results compared with study group for MSU-CSU study

Test	Caesarean cases (n=32)	Cerclage cases (n=2)	Overall (n=34)
Any blood cultures sent during index pregnancy** (%)	0/32 (0%)	0/2 (0%)	0/34 (0%)
Any vaginal swabs sent in index pregnancy (%)	9/32 (28.1%)	2/2 (100%)	11/34 (32.4%)
Any GBS positive swabs (%)	1/9 (11.1%)	0/2 (0%)	1/11 (9.1%)
Any positive swabs reported as non-GBS, non- <i>Candida spp</i> (%)	1/9 (11.1%)	0/2 (0%)	1/11 (9.1%)
Faeces culture (%, all <i>negative</i>)	1/32 (3.1%)	0/2 (0%)	1/34 (2.9%)
MRSA (%, all <i>negative</i>)	32/32 (100%)	2/2 (100%)	34/34 (100%)
Chorioamnionitis / funisitis on placental histology (%)	1/1 (100%)	0/0 (0%)	1/1 (100%)

No blood cultures were sent. No other micro was sent including no wound swabs, respiratory swabs or placental swabs.

No participants had blood cultures sent, which correlates with a lack of sepsis outcomes in these patients. All patients had MRSA testing, which is appropriate for a cohort having operative procedures. One participant had a placenta showing signs of mild chorioamnionitis, again, the lack of placentas being sent for histopathology correlates with low levels of suspected sepsis in this cohort.

9.4 Compare the prevalence of urinary pathology using standard and alternative methods

9.4.1 Structured lower urinary tract symptoms and quality of life assessment for MSU-CSU study

9.4.1.1 Methods

As this was a within-patient study, there was no comparison of LUTS to be made. However, it was important to assess LUTS in this cohort, to be able to interpolate findings from this study to other settings. Although there were only two cerclage cases, I have separated out their findings, as these are patients at a very different gestation, with specific pathologies requiring cerclage. I have, however, not performed any statistical comparisons, as these would be meaningless. I have also presented the overall findings, as all MSU-CSU comparisons are made using the combined dataset.

9.4.1.2 Results

LUTS scores using Artemis for MSU-CSU study are described in Table 9-8. Median LUTS score was 3/40 (95% CI 1-5), frequency 9 (95% CI 6.5-10.5) and nocturia 2.3 (95% CI 1.5-3.5). Incontinence occurred in 13/34 (38.2%) in daytime and 5/34 (14.7%) at night. The commonest LUTS group was overactive bladder symptoms.

Table 9-8: Structured lower urinary tract symptoms assessment for MSU-CSU study

Measure	Caesarean cases (n=32)	Cerclage cases (n=2)	Overall (n=34)
Total LUTS /40			
Median	3.0	2.5	3.0
(95% CI)	(0-7)	(2-3)	(1-5)
(Range)	(0-17)	(2-3)	(0-17)
Frequency (number of episodes)			
Median	9.0	11.5	9.0
(95% CI)	(6.5-10.5)	(3.5-19.5)	(6.5-10.5)
(Range)	(3.5-20.5)	(3.5-19.5)	(3.5-20.5)
Nocturia			
Median	2.8	1.0	2.3
(95% CI)	(1.5-4.0)	(0.5-1.5)	(1.5-3.5)
(Range)	(0-6.5)	(0.5-1.5)	(0.0-6.5)
Any daytime incontinence			
Median	13/32	0/2	13/34
(95% CI)	(40.6%)	(0%)	(38.2%)
(Range)	(24.2-59.2)	(0-80.2)	(22.7-56.4)
Any night incontinence			
Median	5/32	0/2	5/34
(95% CI)	(15.6%)	(0%)	(14.7%)
(Range)	(5.9-33.6)	(0-80.2)	(5.6-31.8)
Stress incontinence symptoms/7			
Median	0.0	0.5	0.0
(95% CI)	(0-1)	(0-1)	(0-1)
(Range)	(0-4)	(0-1)	(0-4)
Overactive bladder symptoms/12			
Median	1.0	0.5	1.0
(95% CI)	(0-2)	(0-1)	(0-2)
(Range)	(0-6)	(0-1)	(0-6)
Voiding symptoms/8			
Median	0.0	1.5	0.0
(95% CI)	(0-2)	(1-2)	(0-2)
(Range)	(0-6)	(1-2)	(0-6)
Pain symptoms/13			
Median	0.0	0.0	0.0
(95% CI)	(0-0)	(0-0)	(0-0)
(Range)	(0-5)	(0-0)	(0-5)

Next, I assessed quality of life scores, as shown in Table 9-9. Median "your health today" score was 80.0% (95% CI 70-85) and median QoL index value was 0.88 (95% CI 0.80-0.88).

Table 9-9: Quality of life assessments for MSU-CSU study

Measure	Caesarean cases (n=32)	Cerclage cases (n=2)	Overall (n=34)
Your health today (self-scored percentage)			
Median	80.0	75.0	80.0
(95% CI)	(70-90)	(70-80)	(70-85)
(Range)	(50–100)	(70–80)	(50–100)
EQ-5D-5L index value			
Median	0.84	0.94	0.88
(95% CI)	(0.74-0.88)	(0.88-1.00)	(0.80-0.88)
(Range)	(0.36–1.00)	(0.88–1.00)	(0.36–1.00)

9.4.1.3 Discussion

LUTS and quality of life scores will be formally compared with the other study results in chapter 10.

9.4.2 Standard dipstick for MSU-CSU study

9.4.2.1 Results

Results are shown in Table 9-10. There were significant between-group differences for leucocyte esterase, where 30/34 MSU samples were positive compared with 14/34 CSU samples ($p < 0.001$), and overall dipstick positive for leucocyte / protein / nitrite, with 31/34 MSU samples positive compared with 24/34 CSU samples ($p = 0.039$). There was no difference in the proportion positive for blood, protein, nitrite or glucose.

Table 9-10: Urinary dipstick findings for MSU and CSU samples

Measure	MSU (n=34)	CSU (n=34)	Significance
Leucocyte esterase (trace or more) (%) (95% CI)	30/34 (88.2%) (71.6-96.2)	14/34 (41.2%) (25.1-59.2)	McNemar test p<0.001
Blood (trace or more) (%) (95% CI)	12/34 (35.3%) (20.3-53.5)	15/34 (44.1%) (27.6-61.9)	McNemar test p=0.549
Protein (trace or more) (%) (95% CI)	17/34 (50%) (32.8-67.3)	16/34 (47.1%) (30.2-64.6)	McNemar test p=1.000
Nitrite (%) (95% CI)	0/34 (0%) (0-12.6)	0/34 (0%) (0-12.6)	n/a
Glucose (%) (95% CI)	0/34 (0%) (0-12.6)	0/34 (0%) (0-12.6)	n/a
Dipstick positive for leu/pro/nit (%) (95% CI)	31/34 (91.2%) (75.2-97.7)	24/34 (70.6%) (52.3-84.3)	McNemar test p=0.039

It is clinically relevant if an MSU is more likely to show leucocyte esterase than a CSU, as in a sick (septic) patient, only a CSU may be possible. Dipstick is instant so likely to influence immediate management.

Understanding whether this effect reflects contamination or differential sampling of shed urothelial cells is critical to interpretation of the result.

Similarity between protein measurements is relevant to management of pre-eclampsia.

9.4.3 Standard microscopy and culture for MSU-CSU study

9.4.3.1 Results

Table 9-11 shows standard microscopy and culture for paired MSU-CSU samples. Only 13 MSU and 2 CSU samples underwent microscopy.

Table 9-11: Standard microscopy and culture for MSU and CSU samples

Measure	MSU (n=34)	CSU (n=34)	Significance
White cell count (any white cells seen) (%) (95% CI)	(n=13) 9/13 (69.2%) (38.9-89.6)	(n=2) 0/2 (0%) (0-80.2)	n/a
Red blood cell count (any red blood cells seen) (%) (95% CI)	(n=13) 4/13 (30.8%) (10.4-61.1)	(n=2) 1/2 (50%) (2.7-97.3)	n/a
Epithelial cell count (any epithelial cells seen) (%) (95% CI)	(n=13) 12/13 (92.3%) (62.1-99.6)	(n=2) 0/2 (0%) (0-80.2)	n/a
Culture positive (>10 ⁵ CFU/ml) (%) (95% CI)	(n=33) 9/33 (27.3%) (13.9-45.8)	(n=33) 2/33 (6.1%) (1.1-21.6)	McNemar test p=0.039

MSU and CSU samples were not statistically compared for standard microscopy since only 2 CSU samples underwent standard microscopy.

There was a difference in standard culture results with 9/33 MSU results reported as positive (27.3%) compared with 2/33 (6.1%) CSUs (p=0.039, McNemar test).

One CSU showed no significant growth, compared with 6 MSUs; no CSUs showed mixed growth, compared with 3 MSUs. Table 9-12 compares standard culture in detail for paired MSU and CSU samples. It demonstrates that of all the positive MSU results, only one was confirmed in the CSU sample (GBS); there was one negative MSU which showed *E. coli* in the CSU sample.

Table 9-12: Comparison of standard culture for paired MSU and CSU samples

MSU culture result	Corresponding CSU culture result
<i>Candida</i> (n=2)	Screening culture negative (x2)
<i>E. coli</i> (n=2)	Screening culture negative (x2)
<i>Enterococcus spp</i> (n=3)	No significant growth (x1) Screening culture negative (x2)
GBS (n=2)	GBS (x1) Screening culture negative (x1)
Mixed growth of uncertain significance (n=3)	Screening culture negative (x3)
No significant growth (n=6)	Screening culture negative (x6)
Screening culture negative (n=15)	<i>E. coli</i> (x1) Screening culture negative (x14)
No sample received (n=1)	No sample received

9.4.3.2 Discussion

The differential findings in MSU versus CSU may reflect a difference in contamination or a difference in sampling. Importantly, MSU and CSU samples were obtained within a short period of each other, and prior to routine prophylactic antibiotics, so there was no differential antibiotic exposure to explain the differences. It is often assumed that CSUs avoid the problem of contamination, however the inability of the CSU to detect bacteriuria that the MSU detects may represent underdiagnosis. The detection of *E. coli* in CSU and not in MSU might provide evidence against the contamination argument. Next, I compared alternative tests.

9.4.4 Fresh unspun urine microscopy

9.4.4.1 Results

Table 9-13 compares cell counts from MSU and CSU samples. Median white cell count for MSU samples was $65\mu\text{l}^{-1}$ (95% CI 42-90) compared with $46\mu\text{l}^{-1}$ (95% CI 34-60) for CSU ($p=0.008$). Figure 9-2 shows paired comparisons demonstrating lower white cell counts in CSUs. All samples had white cells present.

Median red cell count for MSUs was $4\mu\text{l}^{-1}$ (95% CI 2-10) compared with $7\mu\text{l}^{-1}$ (95% CI 2-22, $p=0.0029$). Figure 9-3 demonstrates higher red cell counts in CSUs. Red cells were present in 53/68 (77.9%, 95% CI 66-87) with no between-group difference ($p=1.0$).

Median epithelial cell count for MSUs was $41\mu\text{l}^{-1}$ (95% CI 22-64) compared with $22\mu\text{l}^{-1}$ (95% CI 14-30, $p=0.0057$). Figure 9-4 demonstrates lower epithelial cell counts in CSUs. Most samples (65/68, 95.6%, 95% CI 87-99) had epithelial cells, with no between-group difference ($p=1.0$).

Table 9-13: Fresh unspun microscopy cell counts comparing MSU and CSU samples

Measure	All MSU (n=34)	All CSU (n=34)	Significance
White cell count Median μl^{-1} (95% CI) (Range)	65 (42-90) (22-1370)	46 (34-60) (8-200)	p=0.008
Any white cells (%) (95% CI)	34/34 (100%) (87.4-100)	34/34 (100%) (87.4-100)	n/a
Red blood cell count Median μl^{-1} (95% CI) (Range)	4 (2-10) (0-96)	7 (2-22) (0-2990)	p=0.0029
Any red blood cells (%) (95% CI)	26/34 (76.5%) (58.4-88.6)	27/34 (79.4%) (61.6-90.7)	p=1.000
Epithelial cell count Median μl^{-1} (95% CI) (Range)	41 (22-64) (0-222)	22 (14-30) (0-164)	p=0.0057
Any epithelial cells (%) (95% CI)	32/34 (94.1%) (79.0-99.0)	33/34 (97.1%) (83.0-99.9)	p=1.000

Figure 9-2: Paired white cell counts comparing MSU and CSU in the same subjects (logarithmic scale)

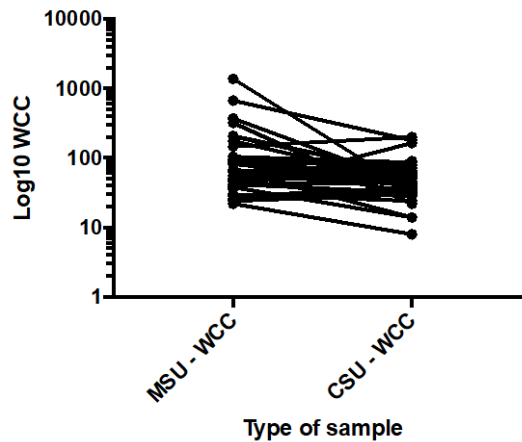


Figure 9-3: Paired red cell counts comparing MSU and CSU in the same subjects (logarithmic scale)

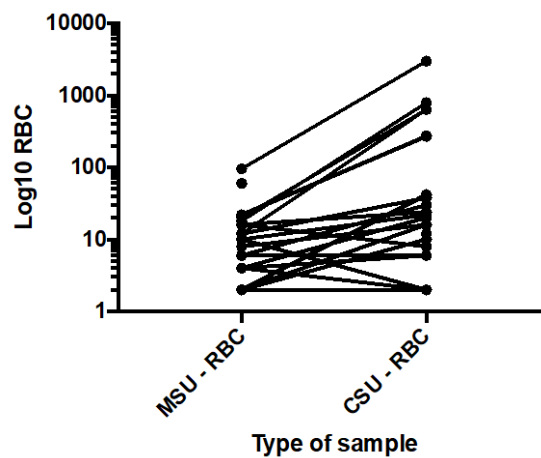
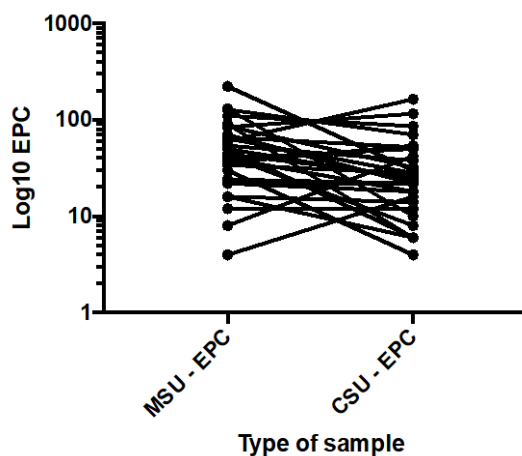


Figure 9-4: Paired epithelial cell counts comparing MSU and CSU in the same subjects (logarithmic scale)



White cells and epithelial cells were higher in the MSU samples, whereas red cells were higher in the CSU samples. Distributions were significantly different, although confidence intervals for the medians overlapped.

Red cell counts may be higher in CSUs because of trauma caused by catheterisation.

White cells may have been higher in MSUs because of contamination from vaginal secretions, and epithelial cells higher because of the same, or contamination from perineal skin. However, epithelial cells were still present in CSUs, which would seem unlikely if caused by contamination. Alternatively, white and epithelial cells may originate from urinary sediment (Collins, Sathiananthamoorthy et al. 2020), and the catheter tip may sample this differently to a voided sample.

This is a small sample. However, even with non-parametric tests there were significant differences between the two sample groups. Not all MSU-CSU paired results were in the same direction, particularly for epithelial cells; this may reflect a sampling issue or may show random variation.

The concept that CSUs are better to avoid contamination is not supported by this finding. If a dichotomous result, with cells present or absent, is required, it is reassuring that there was no difference between the two sampling methods.

Next, I compared ATP.

9.4.5 ATP measurement – methods

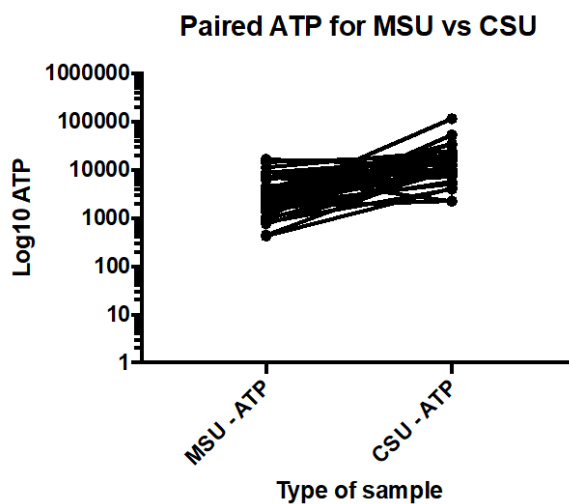
9.4.5.1 Results

Table 9-14 shows that ATP in MSUs was lower than in CSUs (MSU 2618 (95% CI 1678-4509) versus 12323 (95% CI 9582-20620), $p < 0.001$). This is supported visually by Figure 9-5 which showed that although there was overlap in the distributions, most patients had an increase from MSU to CSU.

Table 9-14: Comparison of ATP for MSU and CSU samples

Measure	MSU (n=34)	CSU (n=34)	Significance
ATP (Median RLU) (95% CI) (Range)	2618 (1678-4509) (426–16432)	12323 (9582-20620) (2220–114483)	$p < 0.001$

Figure 9-5: Paired ATP comparing MSU and CSU samples (logarithmic scale)



ATP was higher in CSUs. ATP is thought to function as a biomarker for UTI as outlined previously; particularly since it is released from urothelial cells infected by *E. coli* and causes the release of interleukin-8 (Save and Persson 2010). However, white cell and epithelial cell counts were lower in CSUs. ATP is released from urothelial cells in response to mechanical stretch and electrical

stimulation (Kumar, Chapple et al. 2004). Catheterisation trauma, or altered voiding dynamics with catheterisation, may provide a non-infective explanation for ATP release.

The difference between ATP measured in MSU and CSU would have implications if ATP was found to have clinical utility for infective urinary pathology.

9.4.6 Enhanced sediment culture for MSU and CSU samples.

9.4.6.1 Results

Table 9-15 summarises sediment culture results. MSUs had higher median colony counts (MSU 8088 CFU/ml, 95% CI 3755-76032 versus CSU 0 CFU/ml, 95% CI 0-8, $p<0.001$, see Figure 9-6). MSUs had higher median numbers of different isolates (MSU 3, 95% CI 2-4 versus CSU 0, 95% CI 0-1, $p<0.001$, see Figure 9-7). 100% MSUs had bacteriuria versus 44.1% CSUs ($p<0.001$).

Table 9-15: Sediment culture results for MSU versus CSU

Measure	MSU (n=34)	CSU (n=34)	Significance
Total colony counts			
Median CFU/ml	11377	0	$p<0.0001$
(95% CI)	(3755-76032)	(0-8)	
(Range)	(10-1099395)	(0-61323)	
Colony count for dominant species			
Median CFU/ml	11047	0	$p<0.0001$
(95% CI)	(3508-71488)	(0-14)	
(Range)	(10-1099200)	(0-56059)	
Number of isolates			
Median	3	0	$p<0.0001$
(95% CI)	(2-4)	(0-1)	
(Range)	(1-5)	(0-3)	
Any bacteriuria	34/34	15/34	$p<0.0001$
(%)	(100%)	(44.1%)	
(95% CI)	(87.4-100)	(27.6-61.9)	

Figure 9-6: Sediment culture paired total colony counts for MSU versus CSU samples

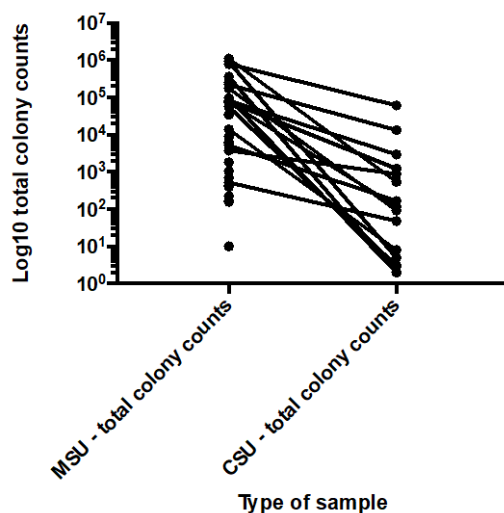
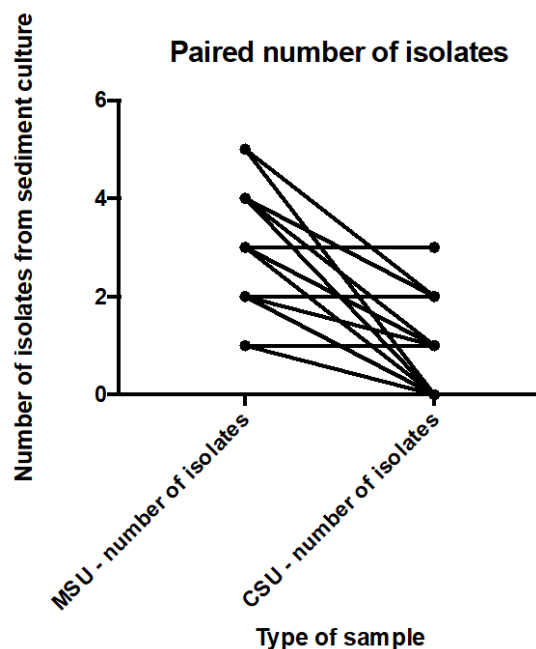


Figure 9-7: Sediment culture paired number of isolates for MSU versus CSU samples



Next, I compared the isolates, identified to genus level using API, from MSU and CSU samples using enhanced culture, as shown in Table 9-16 and Figure 9-8.

The three commonest genera in MSUs were *Staphylococcus spp*, *Escherichia spp*, and *Enterococcus spp*. The three commonest genera in CSUs were *Streptococcus spp*, *Staphylococcus spp*, and *Enterococcus spp*. *Escherichia spp* was not identified in CSU samples. *Lactobacillus spp*, *Candida spp* and *Corynebacterium spp* were found in both MSUs and CSUs.

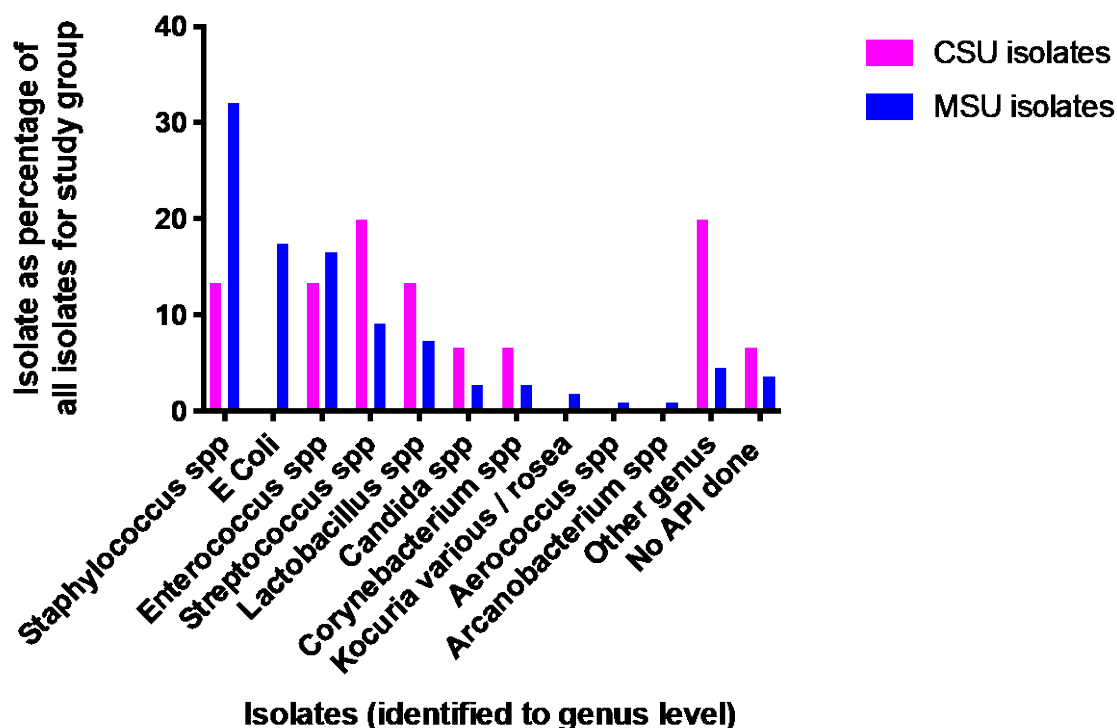
There was no statistical difference in distribution between MSU and CSU isolates (χ^2 , $p=0.050$). This was calculated with the top four most common genus categories with the rest collapsed into one category to make the test valid.

Table 9-16: Ten most common isolates (genus) identified by sediment culture (MSU-CSU study)

Genus	MSU total number of isolates (% of MSU)	CSU total number of isolates (% of CSU)	Total isolates (% of total)
<i>Staphylococcus spp</i>	35 (32.1)	2 (13.3)	37 (29.8)
<i>Escherichia spp</i>	19 (17.4)	0 (0)	19 (15.3)
<i>Enterococcus spp</i>	18 (16.5)	2 (13.3)	20 (16.1)
<i>Streptococcus spp</i>	10 (9.2)	3 (20.0)	13 (10.5)
<i>Lactobacillus spp</i>	8 (7.3)	2 (13.3)	10 (8.1)
<i>Candida spp</i>	3 (2.8)	1 (6.7)	4 (3.2)
<i>Corynebacterium spp</i>	3 (2.8)	1 (6.7)	4 (3.2)
<i>Kocuria various / rosea</i>	2 (1.8)	0 (0)	2 (1.6)
<i>Aerococcus spp</i>	1 (0.9)	0 (0)	1 (0.8)
<i>Arcanobacterium spp</i>	1 (0.9)	0 (0)	1 (0.8)
<i>Other genus</i>	5 (4.6)	3 (20.0)	7 (5.6)
<i>No API done</i>	4 (3.7)	1 (6.7)	5 (5.6)
	109 (100)	15 (100)	124 (100)

χ^2 (using top four genus, others collapsed into single category): $\chi^2=9.486$,
 $p=0.050$

Figure 9-8: Ten most common isolates (genus) identified by sediment culture (MSU-CSU study)



Next, I compared the isolates, identified to species level using API, for MSU versus CSU samples, as in Table 9-17 and Figure 9-9. The three commonest species in MSUs were *Staphylococcus haemolyticus*, *Escherichia coli*, and *Enterococcus faecalis*. The commonest species in CSUs were *Staphylococcus haemolyticus*, *Enterococcus faecalis* and *Lactobacillus spp.*; there were eight other single isolates. *Streptococcus agalactiae* made up 7/97 (7.2%) MSU isolates and 1/12 (8.3%) CSU isolates.

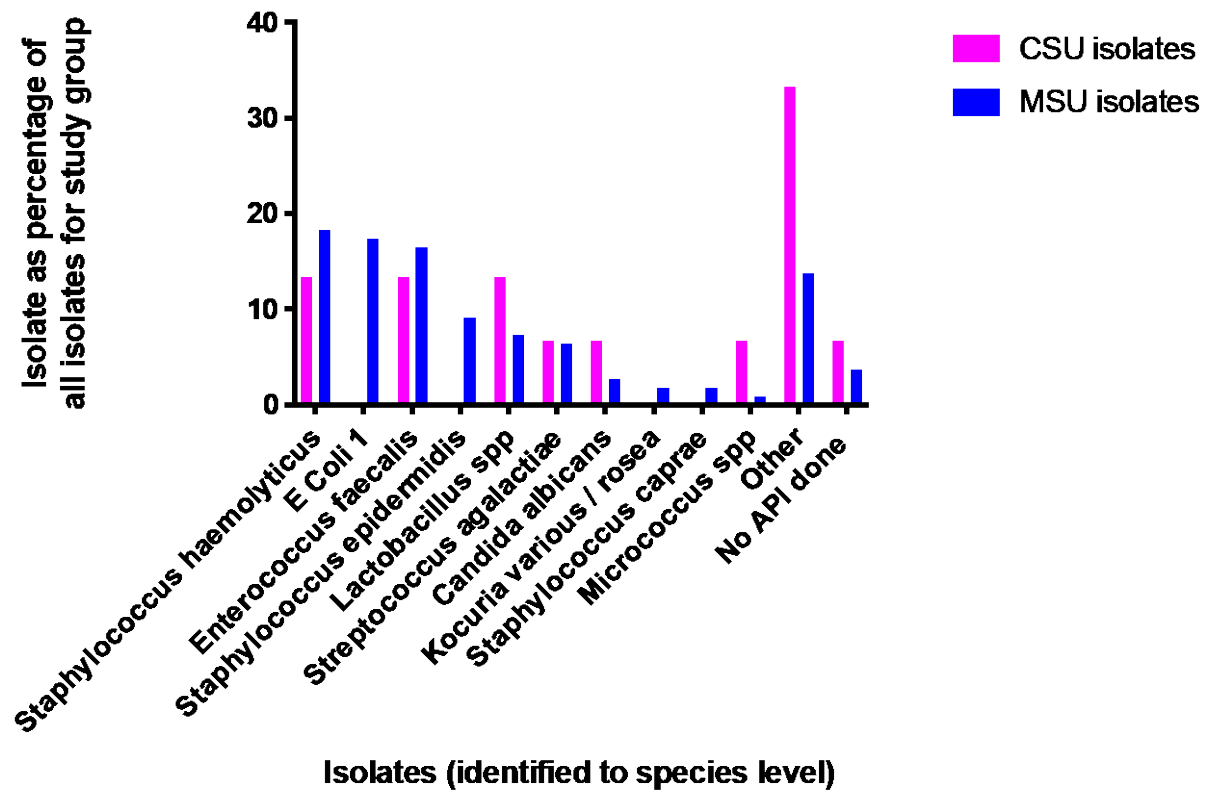
There was no statistical difference in distribution between MSU and CSU isolates ($\chi^2=7.604$, $p=0.107$). This was again calculated with the top four most common genus categories with the rest collapsed into one category to make the test valid.

Table 9-17: Ten most common species identified by sediment culture for MSU versus CSU samples

Species	MSU total number of isolates (% of MSU)	CSU total number of isolates (% of CSU)	Total isolates (% of total)
<i>Staphylococcus haemolyticus</i>	20 (18.3)	2 (13.3)	22 (17.7)
<i>Escherichia Coli 1</i>	19 (17.4)	0 (0)	19 (15.3)
<i>Enterococcus faecalis</i>	18 (16.5)	2 (13.3)	20 (16.1)
<i>Staphylococcus epidermidis</i>	10 (9.1)	0 (0)	10 (8.1)
<i>Lactobacillus spp</i>	8 (7.3)	2 (13.3)	10 (8.1)
<i>Streptococcus agalactiae</i>	7 (6.4)	1 (6.7)	8 (6.4)
<i>Candida albicans</i>	3 (2.8)	1 (6.7)	4 (3.2)
<i>Kocuria various / rosea</i>	2 (1.8)	0 (0)	2 (1.6)
<i>Staphylococcus caprae</i>	2 (1.8)	0 (0)	2 (1.6)
<i>Micrococcus spp</i>	1 (0.9)	1 (6.7)	2 (1.6)
Other	15 (13.8)	5 (33.3)	18 (14.5)
No API done	4 (3.7)	1 (6.7)	5 (4.0)
Totals	109 (100)	15 (100)	124 (100)

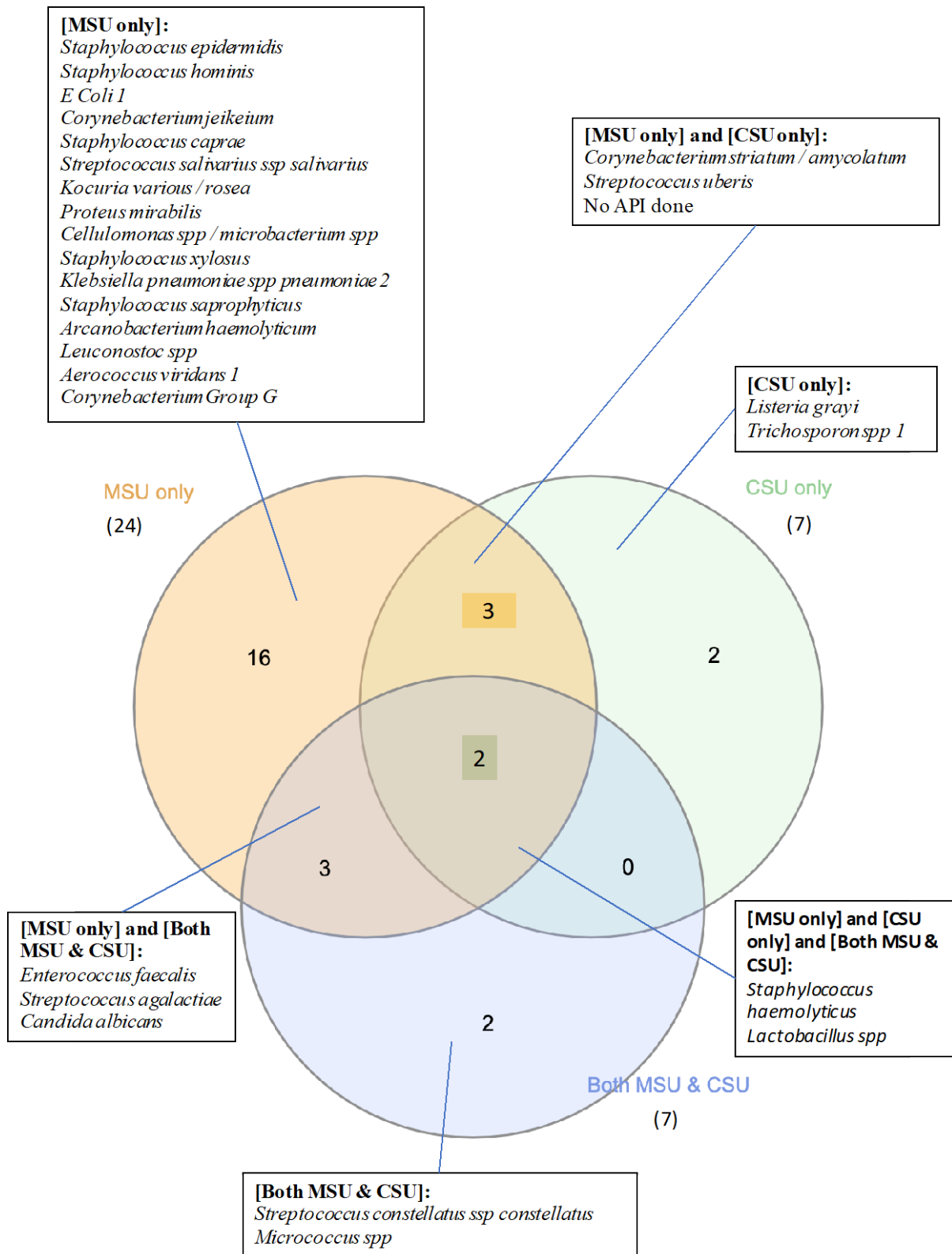
χ^2 (using top four genus, others collapsed into single category): $\chi^2 7.604$,
 $p=0.107$

Figure 9-9: Ten most common isolates (species) identified by sediment culture (MSU-CSU study)



Next, I compared species identified in MSU, CSU, or both MSU and CSU samples (see Figure 9-10). This showed many species found only in MSU samples, including *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus saprophyticus*.

Figure 9-10: Venn diagram to show species identified in MSU, CSU, or both MSU and CSU samples



Next, I compared paired MSU and CSU samples (see Table 9-18). 14/34 participants had growth in CSU, which included 18 isolates. For ten isolates, there was a match with the paired MSU. For seven isolates, there was no match with the paired MSU

Table 9-18: Comparison of isolates from paired CSU and MSU samples.

14/34 participants had growth in CSU sample (18 CSU isolates)	Isolates involved	Notes
CSU isolate matches an isolate from MSU (n=10)	<i>Lactobacillus spp</i> x 3 <i>Micrococcus spp</i> <i>Candida albicans</i> <i>Enterococcus faecalis</i> x 2 <i>Staphylococcus haemolyticus</i> <i>Streptococcus constellatus spp constellatus</i> <i>Streptococcus agalactiae</i>	For one sample with three organisms grown in CSU, two were identified in MSU. For one sample with two organisms in CSU, both were identified in MSU
CSU isolate does not match an isolate from MSU (n=7)	<i>Lactobacillus spp</i> x 2 <i>Staphylococcus haemolyticus</i> <i>Streptococcus uberis</i> <i>Corynebacterium striatum / amycolatum</i> <i>Listeria grayi</i> <i>Trichosporon spp</i> 1	For one sample with two organisms grown in CSU, one was identified in MSU and one was not
No API done (n=1)		
Total (n=18)		

Table 9-19 compares standard and enhanced culture for CSUs. 30/34 standard CSUs were negative which makes comparison by category of result difficult. However, one-third of those reported negative, and all three with a report of any growth, had bacteriuria by enhanced culture.

Table 9-19: Comparison of standard culture and enhanced culture for CSUs

CSU standard culture result	CSU enhanced culture result		
	Median colony count /CFU/ml (95% CI)	Median number of isolates (95% CI)	Proportion with bacteriuria by enhanced culture (%)
Negative (n=30)	0 (0-1.6)	0 (0-1)	10/30 (33.3%)
Non-significant growth (n=0)	n/a	n/a	n/a
Mixed growth (n=1)	118	1	1/1 (100%)
Positive (n=2)	8 and 61322	1 and 2	2/2 (100%)
No result (n=1)	48	1	1/1 (100%)

There were differences in colony counts and diversity of species, with both higher in MSUs than CSUs. This is, again, consistent either with contamination, or with differential sampling of the urinary sediment by CSUs. While some MSU-CSU pairs showed matching isolates, there were others where CSU isolates differed to MSU isolates. There were typical 'contaminants' in some CSUs, such as *Lactobacillus spp.* This is against the contamination argument.

It has been commented that enhanced culture results for MSU and CSUs seem similar to nearby bacterial flora. If this were MSUs alone (as with the previous studies described in this thesis), it would be hard to disentangle contamination from urinary microbiome. However, a freshly passed CSU is likely to be less contaminated and this provides support that the tests are identifying urothelial-cell associated organisms in the urinary sediment.

When CSUs were compared for standard culture and enhanced culture, enhanced culture was more likely to identify bacteriuria. This also supports

enhanced culture identifying bacteria in urinary sediment rather than contaminants.

GBS was found in 6 MSUs and a matched CSU-MSU pair. Similarly, by standard culture, GBS was found in 1 MSU and a matched CSU-MSU pair. Although numbers are small, CSU may not be as good at detecting GBS as MSU.

It is of note that *Enterococcus faecalis* and *Streptococcus agalactiae* were both identified in paired MSU-CSU samples. This contrasts with a previously-discussed study of non-pregnant women with symptoms of acute cystitis, in which voided samples were compared with contemporaneous urethral catheter samples (Hooton, Roberts et al. 2013). In that study, gram negative rods showed close correlation between CSU and MSU samples, whereas enterococci and Group B streptococci did not, with these gram positives rarely being detected in CSU samples. In that study the authors assumed the CSU represented the true bladder bacteriuria, and that MSU was affected by contamination. This assumption will be addressed later (9.5).

There were no samples in which the CSU colony count was higher than MSU. An alternative hypothesis was that something had changed in the patient between voiding the sample, and the CSU being taken, such as prophylactic antibiotics given for the operation, or antisepsis procedures as part of the catheterisation.

It should be noted that catheterisation occurred before prophylactic antibiotics were administered as part of the operation. A subset of five patients had CSUs collected pre- and post-antibiotic administration. There was no difference in total colony count in the two samples (Wilcoxon signed rank, $z=-1.0$, $p=1.0$).

9.4.7 Cytokines – IL-6, IL-8, lactoferrin

9.4.7.1 Results

The results are shown in Table 9-20 and Figure 9-11.

There was a difference in protein concentration, with higher median protein in CSUs (97.2µg/ml, 95% CI 51.2-82.7 for MSU versus 65.7µg/ml, 95% CI 56.5-140.4 for CSU, $p=0.017$).

There was no difference in IL-6 concentration between MSU and CSU samples, whether it was corrected for protein or not. The proportion of IL-6 below LLOD was higher for CSUs.

There was a difference in IL-8 concentration which remained when normalised for total protein. This was higher for MSU samples.

There was a difference in lactoferrin concentration which remained when normalised for total protein; this was also higher for MSU samples.

Table 9-20: Comparison of urinary cytokines for MSU and CSU samples

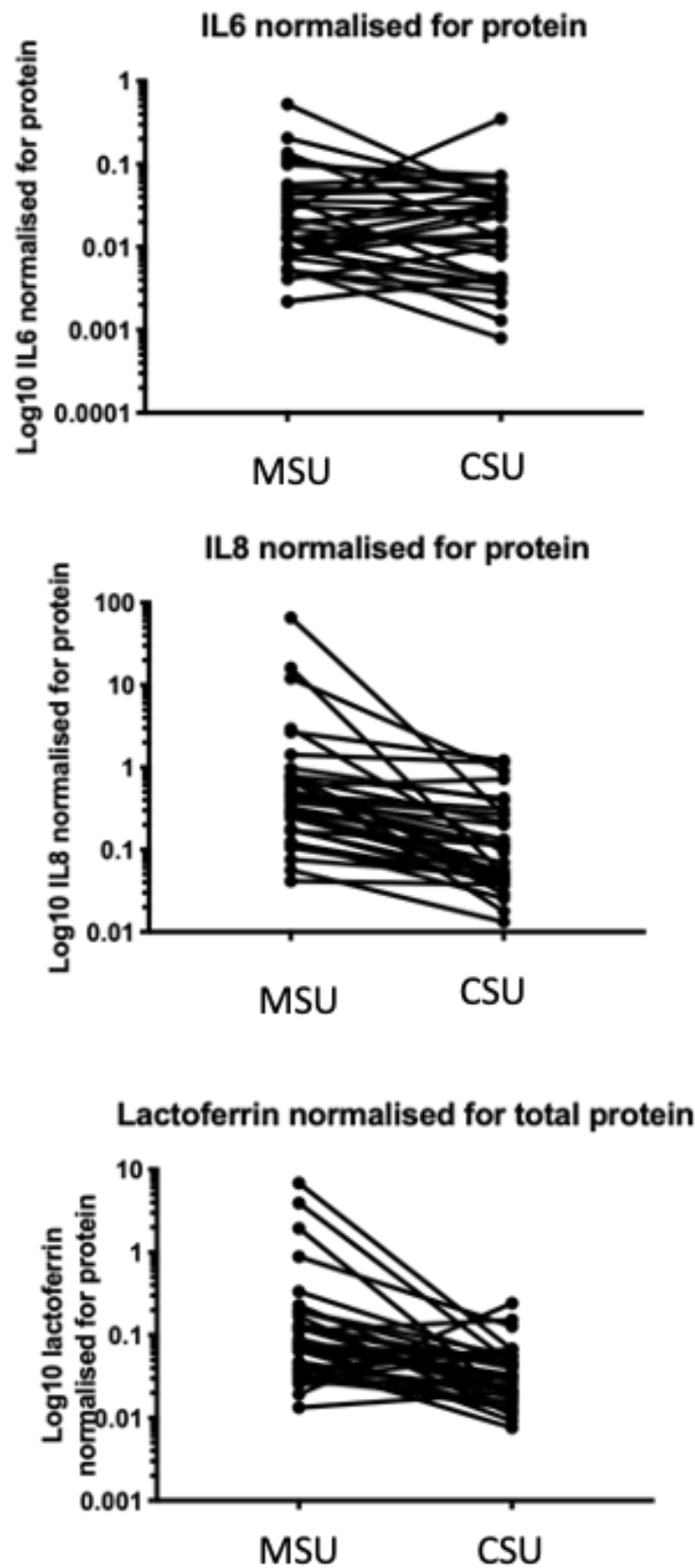
	MSU (n=34)	CSU (n=34)	Significance
Total protein concentration (µg/ml)			
Median	65.7	97.2	p=0.017
(95% CI)	(51.2-82.7)	(56.5-140.4)	WSR
(Range)	(27.4–231.3)	(33.0–499.2)	
Proportion with protein below lower limit of detection	0/34	0/34	McNemar -not valid
(%)	(0%)	(0%)	
(95% CI)	(0-12.6)	(0-12.6)	
IL-6 concentration (pg/ml)			
Median	1.6	1.7	p=0.824
(95% CI)	(0.5-2.8)	(0.9-2.7)	WSR
(Range)	(0.14–34.9)	(0.14–102.5)	
Proportion with IL-6 below lower limit of detection	5/34	8/34	McNemar p<0.001
(%)	(14.7%)	(23.5%)	
(95% CI)	(5.6-31.8)	(11.4-41.6)	
IL-6 normalised for total protein (ng/µg)			
Median	15	14	p=0.221
(95% CI)	(9-36)	(4-33)	WSR
(Range)	(2–525)	(1–351)	
IL-8 concentration (pg/ml)			
Median	29.2	9.4	p=0.0006
(95% CI)	(17.7-47.9)	(4.3-19.1)	WSR
(Range)	(1.1–4272)	(0.71–212)	
Proportion with IL-8 below lower limit of detection	0/34	1/34	McNemar -not valid
(%)	(0%)	(2.9%)	
(95% CI)	(0-12.6)	(0.2-17.1)	
IL-8 normalised for total protein (pg/µg)			
Median	0.4	0.1	p<0.0001
(95% CI)	(0.3-0.7)	(0.0-0.2)	WSR
(Range)	(0.04–65.9)	(0.01–1.2)	

Continued next page

	MSU (n=34)	CSU (n=34)	Significance
Lactoferrin concentration (ng/ml)			
Median	4.7	2.21	p=0.0010 WSR
(95% CI)	(2.21-8.4)	(2.21-2.21)	
(Range)	(2.21-443.8)	(2.21-13.7)	
Proportion with lactoferrin below lower limit of detection	12/34	24/34	McNemar p=0.815
(%)	(35.3%)	(70.6%)	
(95% CI)	(20.3-53.5)	(52.3-84.3)	
Lactoferrin normalised for total protein (pg/μg)			
Median	78	31	p<0.0001
(95% CI)	(45-122)	(20-46)	
(Range)	(13-6847)	(8-243)	

Statistical comparisons using Wilcoxon signed rank (WSR) or McNemar test.

Figure 9-11: Comparison of urinary cytokines, normalised for protein, for paired MSU and CSU samples



Cytokine concentrations differed between MSU and CSU samples. Whereas ATP was higher in CSUs, IL-8 and lactoferrin were higher in MSUs. This relationship held when normalised for total protein, which was higher in CSU samples. IL-6 did not differ between sample types other than proportion below LLOD (higher in CSUs). IL-8 was detected in 33/34 CSU samples and all the MSU samples.

The finding that protein concentration was higher in CSU samples is interesting. This did not emerge from comparison of dipstick protein. This may have implications for pre-eclampsia screening and diagnosis. Currently urinary protein-creatinine ratio (uPCR) is used first-line for diagnosis. I did not measure creatinine concentrations and so cannot comment on this relationship.

It is interesting to wonder why protein was higher in CSU samples, whereas IL-8 and lactoferrin were lower in CSU samples, and the proportion with IL-6 below LLOD was lower.

The methods used for urinary cytokine analysis were robust as described before. It is interesting that so many samples were below LLOD for lactoferrin; this suggests either that levels were low, or that the test was not sensitive enough.

9.4.8 Bacterial-associated clue cell proportions for MSU and CSU samples

9.4.8.1 Methods

See chapter 6.16.8 for methods. It should be remembered that this was slightly different in the MSU-CSU study compared with the other studies. Firstly, a combined uroplakin-3/WGA/DAPI stain was used so that clue cell counts were performed in confirmed urothelial cells. Secondly, there was no upper limit on cell counts which are given per slide. Occasionally this led to proportions of clue cells of more than 100% as clue cells and urothelial cells were counted separately.

9.4.8.2 Results

Table 9-21 compares bacterial-associated clue cells in MSU and CSU samples. Uroplakin-3 positive epithelial cells were higher for MSU samples with 396/slide (95% CI 216-744) versus 58/slide (95% CI 35-113) ($p=0.003$). The proportion of uroplakin-3 positive epithelial cells was 0.92 (95% CI 0.85-0.98) for MSU and 0.93 (95% CI 0.88-1.00) for CSU with no difference ($p=0.910$).

The proportion of uroplakin-3 positive cells that had associated bacteria (i.e., were clue cells) was higher in MSU than CSU samples ($p=0.004$). There was no difference in the proportion of samples with any clue cells.

Table 9-21: Bacterial-associated clue cells in MSU and CSU samples

Measure	MSU (n=34)	CSU (n=34)	Significance
Number of uroplakin-3 positive epithelial cells (median) (95% CI) (Range)	396 (216-744) (11-2124)	58 (35-113) (0-2052)	$p=0.003$ Wilcoxon signed rank
Proportion of epithelial cells that are uroplakin-3 positive (median) (95% CI) (Range)	0.92 (0.85-0.98) (0.67-1.3)	0.93 (0.88-1.00) (0.0-1.2)	$p=0.910$ Wilcoxon signed rank
Proportion of uroplakin cells that are clue cells (median) (95% CI) (Range)	0.96 (0.78-0.98) (0.05-1.0)	0.86 (0.39-0.95) (0.003-1.0)	$p=0.004$ Wilcoxon signed rank
Proportion with any clue cells (%) (95% CI)	30/33 (90.9%) (74.5-97.6)	24/33 (72.7%) (54.2-86.1)	McNemar test $p=0.07$

9.4.8.3 Discussion

This data supports the hypothesis that the majority of epithelial cells in the sample were urothelial in origin rather than contamination. The absolute numbers of urothelial cells in MSU samples were higher than CSU samples

which supports the idea that the amount of urothelial cell sampling by CSU is less than MSU samples.

The proportion of clue cells was higher for MSU samples, although with widely overlapping confidence intervals, and the proportion with any clue cells appeared higher in MSU samples but this did not reach statistical significance. It is not clear why clue cell proportions would be higher in MSU samples.

The method used to count cells was slightly different to that used in the previous two studies. In this study the researcher counted all the cells present on the slide, leading to, in some cases, numbers above 1000. Epithelial cells and uroplakin-3 positive cells were counted separately, which led to in some cases the total numbers of uroplakin-3 cells being higher than epithelial cells. Technically this should not be possible. This suggests either that some cells did not stain appropriately with both DAPI and UP-3, or that there was intra-person variability in counting. This effect was seen for both MSU and CSU samples, so the hope would be that any bias affected both groups equally.

Slides were labelled in such a way that the researcher could not identify if the slide was MSU or CSU. This ensured that counting was blind and supports a comparison between the two sample types.

9.5 Overall discussion and conclusion

In summary, the MSU-CSU study cohort was mostly women at or near term having elective caesareans, with two women in second trimester having cervical cerclage. There were no cases of maternal sepsis or pyelonephritis. Two patients delivered preterm, neither due to spontaneous preterm labour. Symptom scores, other than frequency and nocturia, were relatively low compared to the previous two studies.

Standard culture was more likely to be positive for MSUs. Dipstick leucocyte esterase was more likely to be positive in MSUs, with no other differences.

Fresh microscopy showed higher white cell and epithelial cell counts in MSUs. Red cell counts were higher in CSUs. ATP was higher in CSUs.

Enhanced culture showed higher colony counts in MSUs and more different isolates. Fewer than half CSUs had bacteriuria compared with all MSUs. There were many species identified in MSU samples that were not seen in CSU samples. Paired MSU and CSU sample isolates did not map perfectly, with some appearing in one and not the other.

Urinary cytokines IL-8 and lactoferrin were higher in MSU samples with no difference in IL-6 other than proportion below LLOD. Total protein was higher in CSU samples.

The number of uroplakin-3 positive epithelial cells in MSU samples was higher than CSU, but with no difference in the proportion of these cells compared with total epithelial cells, which was high in both. The proportion of uroplakin-3 positive clue cells was high in both, but higher in MSU samples.

These tests were used to compare MSU and CSU samples, to understand more about how MSU and CSU samples might differ and explore the concept of contamination in voided samples. The main hypotheses outlined at the beginning were firstly that CSU samples would have no contamination from perineal skin, and secondly that CSUs differently sample the urine in the bladder compared with MSUs, particularly the urinary sediment.

The finding that standard and enhanced culture are more likely to be positive for MSU samples does not differentiate between these two hypotheses.

However, the uroplakin-3 staining does, as the majority of epithelial cells in the urine are urothelial in origin, with higher absolute levels in voided samples compared with catheterised samples; this would be consistent with differential sampling of a sediment containing shed urothelial cells where voided samples pass more of this sediment compared with a catheter tip that may miss the sediment. The fact that the proportion of uroplakin-3 positive epithelial cells was not different is against the contamination theory – presumably if MSUs were more contaminated, the proportion of uroplakin-3-positive epithelial cells would be lower.

This is consistent with a recent study in patients with chronic LUTS using the same technique of uroplakin-3 staining. MSUs and CSUs were compared for 60 patients (Collins, Sathiananthamoorthy et al. 2020). While the total epithelial cell counts were higher in MSUs, the proportions of uroplakin-3 positive epithelial cells were not, suggesting contamination did not contribute to the higher epithelial cell counts. In this study, colony counts from both standard and enhanced cultures were lower in CSUs than MSUs. This supported the hypothesis that the urinary sediment was sampled differently according to the sampling method (see Figure 9-12).

Figure 9-12: Diagram of urinary sediment in CSU and MSU sampling

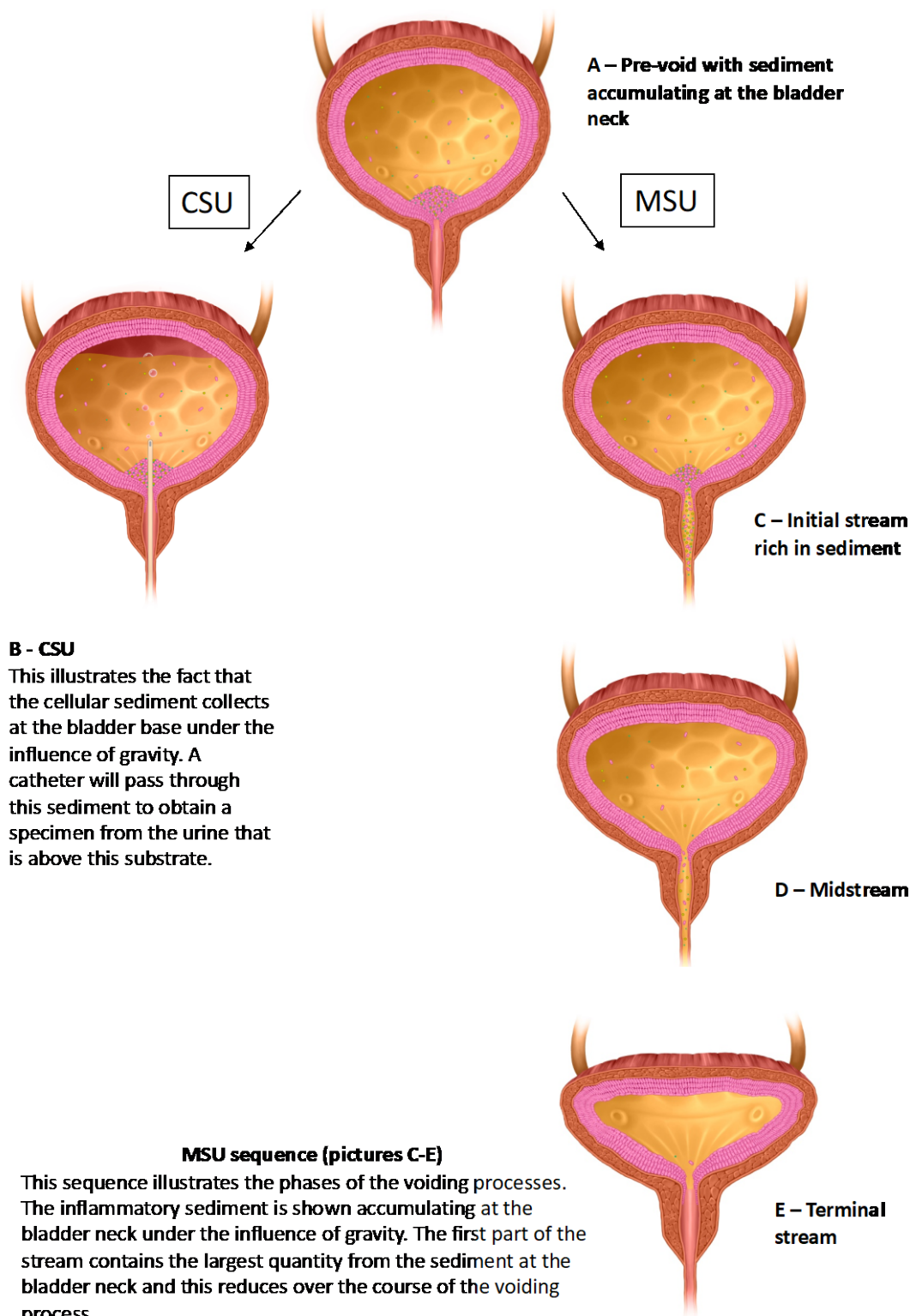


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The data presented here support other older studies discussed in the introduction, suggesting that CSUs were associated with similar isolates to MSUs including typical vaginal commensals (Khasriya, Khan et al. 2010) (Khasriya, Sathiananthamoorthy et al. 2013) (Hooton, Roberts et al. 2013).

A CSU-based comparison of enhanced culture and molecular sequencing methods in 51 pregnant women, did detect what would typically be considered contamination using both methods, in that it detected *Lactobacillus spp.* and *Gardnerella spp.* (Jacobs, Thomas-White et al. 2017). This again suggests these are not contaminants.

High ATP in CSUs contrasts with the lower levels of bacteriuria and inflammatory markers. This suggests ATP levels were not driven by sediment. It may suggest a physical role for ATP release, such as cellular trauma from catheterisation, or altered urothelial stretch from a catheterised void.

The cohort of participants is a combination of women having elective caesareans at term and without apparent UTI symptoms, and two women having cervical cerclage at the start of second trimester. This cannot be representative of all pregnant women. However, it provides a starting point for further research to understand this topic.

It was novel to use these alternative tests to compare voided and catheter specimens. The possibility that the CSU is a different specimen, not necessarily one avoiding contamination, rather one sampling a different part of the urine, is a challenging one. If true, then it has implications for clinicians who use CSUs to investigate for UTI in pregnancy, for example when a patient in labour is catheterised then develops a pyrexia.

So far, I have explored standard and alternative tests in pregnancy in the setting of abdominal pain in pregnancy, risk factors for preterm birth, and needing a catheter for medical reasons. I have done this using very similar or identical methods. To understand how these tests relate to each other, and what we can learn from these relationships about urinary pathology in pregnancy, these data were pooled. I shall present this in the next chapter.

10 Pooled results from combining the three studies

10.1 Introduction

So far, I have addressed two of the four aims of this thesis, that is: (1) to compare the prevalence of urinary pathology in pregnancy in different clinical settings using standard and alternative tests; (2) to compare urinary specimen sampling techniques. The third aim, to be addressed now, was to compare:

- a) Alternative tests with standard tests
- b) Both test sets with clinical outcomes.

The three studies (Abdominal pain study, PTB study, and MSU-CSU study) adopted similar methods so that the data from the three studies could be pooled for this analysis. There are arguments for and against pooling data from observational studies. Pooling data allows greater numbers of participants to be included. However, if biases are present, these will remain present in pooled data. It is important that studies from which data are pooled are comparable. These three related studies were conducted at a single institution over the same time period. Pooling data is justifiable because urinary tests are generally applied to all pregnant women, regardless of their risk factors.

Hypotheses:

Alternative urinalysis methods may be superior to standard tests in pregnancy.

Research questions:

- (1) Describe the relationships between standard and alternative tests
- (2) Compare the performance of alternative methods against the gold standard culture
- (3) Compare the performance of standard and alternative methods in warning of complications:
 - a. Pyelonephritis
 - b. Preterm birth below 37 weeks

10.2 Methods

Before pooling data, I compared studies in terms of demographics and risk factors. The purpose of presenting these data is not to statistically compare them as that would be not be clinically meaningful. Instead it is to describe and assess the clinical heterogeneity of participants included in the pooled analysis. This is to support future generalisability.

Heterogeneity can be clinical (arising from different patient populations and treatment approaches), methodological (arising from different study designs and risks of bias), or statistical (a greater difference in outcomes between studies than would be expected from chance alone, which could arise from clinical or methodological variation) (Melsen, Bootsma et al. 2014).

No calculation of heterogeneity statistics (such as I^2 statistic), was performed (von Hippel 2015), as described previously (6.8). Heterogeneity would normally not be a desirable feature of a meta-analysis. However, in this situation, in terms of tests that would be applied across a pregnant population, heterogeneity may be a positive feature in terms of generalisability. It reduces the likelihood of an enhanced sample and is likely to be more representative of the general obstetric population being described.

Combined data is presented for each demographic variable, risk factor and outcome, to describe the study participants.

Urinary test data from the three previous studies were combined. CSU samples were excluded because the CSU is markedly different to the MSU, as explained in Chapter 10. Non-parametric statistical tests were applied. Relationships were described using scatter plots, and correlations using Spearman rank correlation. A Bland-Altman analysis was used to measure agreement. Comparisons between dichotomous outcome measures were calculated with Mann Whitney U or Chi Square / Fisher's Exact tests. Multiple regression was used to model the contribution of risk factors (independent variables) to outcomes (dependent variables).

10.3 Results

10.3.1 Overview of demographic, risk factors and clinical outcomes

Table 10-1 describes demographic and risk factors for the three studies and overall, for the pooled dataset. This shows that they included diverse women, with and without risk factors for preterm birth and UTI, and varying in BMI, ethnicity and socio-economic status. Smoking and multiple pregnancy rates were low across all three studies.

Sampling technique, experimental methods and clinical data collection strategies for each study were intentionally similar, minimising methodological heterogeneity. The main differences were in recruitment criteria, which could lead to clinical heterogeneity. However, by recruiting women in early second trimester (PTB study), women having elective caesareans (MSU-CSU study), and women presenting with pain or acute problems in second or third trimester (Abdominal pain study) with gestation-matched controls, participants represented the full breadth of two trimesters along with varied demographic and risk factors. In this way, while there is some clinical heterogeneity, this may be a positive feature in terms of understanding urinary tests that could be applied to diverse pregnant women.

Table 10-1: Demographics and risk factors – pooled summary data

Demographic feature	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
Gestation at recruitment (days, median) (95% CI) (Range)	190.0 (180-206) (98-287)	114 (111-117) (92-125)	272.5 (269-273) (88-290)	174 (143-183) (88-290)
Age (years, median) (95% CI) (Range)	33.5 (32.3-34.3) (19.5-43.6)	36.2 (35.0-37.3) (27.0-49.6)	34.5 (31.0-38.0) (23.0-44.0)	34.3 (33.6-35.0) (19.5-49.6)
Socio-economic status (top 5 most deprived deciles) (%) (95% CI)	111/156 (71.2%) (63.3-78.0)	55/80 (68.8%) (57.3-78.4)	22/33 (66.7%) (48.1-81.5)	166/236 (70.3%) (64.0-76.0)
Ethnicity (proportion of non- white ethnic groups) (%) (95% CI)	49/147 (33.3%) (25.9-41.6)	25/80 (31.3%) (21.6-42.7)	19/34 (55.9) (38.1-72.4)	93/261 (35.6%) (29.9-41.8)
Parity (proportion nulliparous) (%) (95% CI)	89/159 (56.0%) (47.9-63.8)	26/80 (32.5%) (22.7-44.0)	11/34 (32.4%) (18.0-50.6)	126/273 (46.2%) (40.2-52.3)
Body mass index (kg/m ²) Median (95% CI) (Range)	23.5 (22.8-24.3) (15.0-38.6)	23.8 (22.2-25.4) (17.6-40.2)	25.2 (23.1-28.0) (18.4-38.5)	23.7 (23.1-24.5) (15.0-40.2)
PTB risk factors (excluding multiple pregnancy) (%) (95% CI)	14/159 (8.8%) (5.1-14.6)	67/80 (83.8%) (73.5-90.7)	4/34 (11.8%) (3.8-28.4)	85/273 (31.1%) (25.8-37.1)
Previous UTI history Proportion (%) (95% CI)	17/159 (10.7%) (6.5-16.8)	13/73 (17.8%) (10.2-28.9)	10/34 (29.4%) (15.7-47.7)	40/266 (15.0%) (11.1-20.0)
Current smoker (%) (95% CI)	7/157 (4.5%) (2.0-9.3)	7/80 (8.8%) (3.9-17.8)	2/32 (6.3%) (1.1-22.2)	16/271 (5.9%) (3.5-9.6)
Multiple pregnancy (%) (95% CI)	5/159 (3.1%) (1.2-7.6)	0/80 (0%) (0-5.7)	1/34 (2.9%) (0.2-17.1)	6/273 (2.2%) (0.9-5.0)
MSU positive at booking (%) (95% CI)	20/156 (12.8%) (8.2-19.3)	8/80 (10%) (4.7-19.3)	0/34 (0%) (0-12.6)	28/270 (10.4%) (7.1-14.8)

Outcomes from the three studies have been described previously and are summarised along with pooled summary data in Table 10-2.

These pooled data demonstrate an overall preterm birth rate of 23/268 (8.6%, 95% CI 5.6-12.8). This is consistent with the national preterm birth rate, of between 7-8% between 2010-2019 (ONS 2020).

Table 10-3 shows the distribution of gestation at delivery according to study group; it appears that late miscarriage and extreme prematurity were more common in the PTB study, as would be expected in a cohort at high risk for preterm birth.

These data describe a largely healthy cohort, with only a small number of cases of suspected sepsis, identified because blood cultures were done. In 18/273 (6.6%, 4.1-10.4) pregnancies, blood cultures were sent, all intrapartum or post-partum. There were no cases of pyelonephritis. I was therefore not able to compare standard and alternative tests against the outcome of pyelonephritis.

Table 10-2: Clinical outcomes – pooled summary data

Outcome	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
Gestation at delivery (days, median) (95% CI) (Range)	276.0 (274-280) (223-298)	(n=77) 274.0 (272-277) (130-295)	272.5 (268-274) (248-291)	(n=267) 275 (273-276) (130-298)
Preterm birth – any (%) (95% CI)	10/156 (6.4%) (3.3-11.8)	11/78 (14.1%) (7.6-24.3)	2/34 (5.8%) (1.0-21.1)	23/268 (8.6%) (5.6-12.8)
Preterm birth – spontaneous (%) (95% CI)	5/155 (3.2%) (1.2-7.8)	6/78 (7.7%) (3.2-16.6)	0/34 (0%) (0-12.6%)	11/267 (4.1%) (2.2-7.5)
PPROM (%) (95% CI)	2/155 (1.3%) (0-5)	3/76 (4.0%) (1-12)	0/34 (0%) (0-13.3)	5/265 (1.9%) (0.7-5)
Days from recruitment to delivery (median) (95% CI) (Range)	86.0 (69-97) (0-184)	161.0 (158-165) (8-185)	0.0 (0-0) (0-197)	101 (91-123) (0-197)
Induction of labour	42/155 (27.1%) (20.4-34.9)	15/74 (20.3%) (12.2-31.5)	1/33 (3.0%) (0.2-18)	57/262 (21.8%) (17-27)
	SVD 63/155 (40.6%) (32.9-48.8)	SVD 42/76 (55.3%) (43.5-66.5)	SVD 0/34 (0%) (0-12.6)	SVD 105/265 (39.6%) (33.7-45.8)
Mode of delivery (%) (95% CI)	Instrumental 33/155 (21.3%) (15.3-28.7)	Instrumental 2/76 (2.6%) (0.5-10.1)	Instrumental 1/34 (2.9%) (0.2-17.1)	Instrumental 36/265 (13.6%) (9.8-18.4)
	CS 59/155 (38.1%) (30.5-46.2)	CS 32/76 (42.1%) (31.1-54.0)	CS 33/34 (97.1%) (83.0-99.9)	CS 124/265 (46.8%) (40.7-53.0)
Sex (proportion male) (%) (95% CI)	93/160 (58.1%) (50-66)	41/74 (55.4%) (43-67)	15/35 (42.9) (27-60)	149/270 (55.2) (49-61)

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Outcome	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
Birthweight (g)	(n=160)	(n=74)	(n=35)	(n=269)
Median	3400	3309	3240	3350
(95% CI)	(3310-3460)	(3070-3370)	(3010-3580)	(3266-3420)
	(1890-4580)	(744-4341)	(1780-4320)	(744-4580)
Apgars at 1 minute	(n=156)	(n=66)	(n=35)	(n=257)
Median	9	9	9	9
(95% CI)	(9-9)	(9-9)	(9-9)	(9-9)
	(1-10)	(5-10)	(4-10)	(1-10)
Apgars at 5 minutes	(n=156)	(n=66)	(n=33)	(n=257)
Median	10	10	10	10
(95% CI)	(10-10)	(10-10)	(10-10)	(10-10)
	(5-10)	(7-10)	(9-10)	(5-10)
Antenatal sepsis (possible or likely) (%)	0/156	0/75	0/34	0/265
(95% CI)	(0%)	(0%)	(0%)	(0%)
	(0-3)	(0-6)	(0-13.3)	(0-2)
Intrapartum sepsis (possible or likely) (%)	10/154	4/75	0/34	14/263
(95% CI)	(6.5%)	(5.3%)	(0%)	(5.3%)
	(3.3-11.9)	(2-14)	(0-13.3)	(3-9)
Postnatal sepsis (possible or likely) (%)	15/154	3/75	0/34	18/263
(95% CI)	(9.7%)	(4.0%)	(0%)	(6.8%)
	(5.7-15.8)	(1-12)	(0-13.3)	(4-11)
Neonatal sepsis (possible or likely) (%)	17/150	3/74	3/33	23/257
(95% CI)	(11.0%)	(4.1%)	(9.1%)	(9.0%)
	(6.8-17.3)	(1-12)	(2-25)	6-13)
Admission to NNU (%)	17/154	8/73	6/35	31/262
(95% CI)	(11.0%)	(11.0%)	(17.1%)	(11.8%)
	(6.8-17.8)	(5.2-21.0)	(7-34)	(8-17)
Pyelonephritis (%)	0/156	0/75	0/34	0/265
(95% CI)	(0%)	(0%)	(0%)	(0%)
	(0-3)	(0-61)	(0-13)	(0-2)

Table 10-3: Gestation at delivery for preterm birth, according to study group

	Abdominal pain study (n=156)	PTB study (n=78)	MSU-CSU study (n=34)	Pooled data (n=268)
Late miscarriage	0	2	0	2
PTB < 28 weeks	0	2	0	2
PTB < 34 weeks	2	4	0	6
PTB < 37 weeks	8	3	2	13
Total	10	11	2	23

10.3.2 Combined results from the three studies

I pooled standard and alternative test results from all three studies, and have summarised combined totals in Table 10-4. As with demographic and outcome data, I have not compared studies, rather I present it to demonstrate the heterogeneity present within the dataset. Most results have overlapping ranges which is supportive for pooling the data.

Comparison of each pooled result against published literature will be described later in chapter 11.5.

Table 10-4: Summary of test results across different studies

	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
Total LUTS score - median / 40 (95% CI) (range)	6.0 (5.0-7.0) (0-24)	5.0 (4.0-7.0) (0-32)	3.0 (1.0-7.0) (0-17)	5.0 (5.0-6.0) (0-32)
Quality of life 'Your health today' (95% CI) (range)	(n=152) 80 (75-80) (20-100)	(n=11) 85 (80-100) (50-100)	(n=34) 80 (70-85) (50-100)	(n=197) 80 (75-80) (20-100)
Dipstick (leu/pro/nit) positive) (%) (95% CI)	109/159 (68.6%) (60.6-75.6)	45/80 (56.3%) (44.7-67.2)	31/34 (91.2%) (75.2-97.7)	185/273 (67.8%) (61.8-73.2)
Nitrite positive (%) (95% CI)	1/159 (0.6%) (0.03-4.0)	0/80 (0%) (0-5.7)	0/34 (0%) (0-12.6)	1/273 (0.4%) (0.02-2.4)
Standard culture positive (%) (95% CI)	10/157 (6.4%) (3.3-11.7)	3/76 (4.0%) (1.0-11.9)	9/33 (27.3%) (13.9-45.8)	22/266 (8.3%) (5.4-12.4)
Standard microscopy any white cells (%) (95% CI)	68/97 (70.1%) (59.8-78.8)	2/5 (40%) (7.3-83.0)	10/13 (76.9%) (46.0-93.8)	80/115 (69.6%) (60.2-77.6)
Fresh microscopy any white cells (%) (95% CI)	139/159 (87.4%) (81.0-92.0)	42/80 (52.5%) (41.1-63.7)	34/34 (100%) (87.4-100)	215/273 (78.8%) (73.3-83.4)
Fresh microscopy white cell count (median WBCμl⁻¹) (95% CI) (range)	14 (10-24) (0-1190)	2 (0-4) (0-190)	65 (42-90) (22-1370)	14 (8-20) (0-1370)
Standard microscopy any red cells (%) (95% CI)	(n=97) 16/97 (16.5%) (10-25.7)	(n=5) 3/5 (60%) (17.1-92.7)	(n=13) 4/13 (30.8%) (10.4-61.1)	(n=115) 23/115 (20.0%) (13.4-28.7)
Fresh microscopy any red cells (%) (95% CI)	77/159 (48.4%) (40.5-56.5)	34/80 (42.5%) (31.7-54.1)	26/34 (76.5%) (58.4-88.6)	137/273 (50.2%) (44.1-56.3)

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	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
Fresh microscopy red cell count (median RBCμl^{-1}) (95% CI) (range)	0 (0-2) (0-7520)	0 (0-2) (0-740)	4 (2-10) (0-96)	1 (0-2) (0-7520)
Standard microscopy any epithelial cells (%) (95% CI)	94/97 (96.9%) (90.6-99.2)	5/5 (100%) (46.3-100)	12/13 (92.3%) (62.1-99.6)	111/115 (96.5%) (90.8-98.9)
Fresh microscopy any epithelial cells (%) (95% CI)	140/159 (88.1%) (81.7-92.5)	68/80 (85.0%) (74.9-91.7)	32/34 (94.1%) (79.0-99.0)	240/273 (87.9%) (83.0-91.4)
Fresh microscopy epithelial cell count (median EPCμl^{-1}) (95% CI) (range)	14 (8-18) (0-184)	8 (4-12) (0-800)	41 (22-64) (0-222)	12 (10-16) (0-800)
ATP (median RLU) (95% CI) (range)	6717 (5513-7900) (679-48800)	5772 (4827-7915) (608-24523)	2618 (1678-4509) (426-16432)	6034 (5159-6921) (426-48800)
Any bacteriuria by enhanced culture (%) (95% CI)	157/159 (98.7%) (95.1-99.8)	80/80 (100%) (94.3-100)	34/34 (100%) (87.4-100)	271/273 (99.3%) (97.1-99.9)
Enhanced culture total colony counts (median CFU/ml) (95% CI) (range)	1220 (720-1945) (0-1.4x10 ⁶)	1920 (537-2969) (1.6-1.3 x10 ⁶)	11377 (3755-76032) (9.6-1.1 x10 ⁶)	1747 (1010-2900) (0-1.4 x10 ⁶)
Number of isolates (95% CI) (range)	3 (3-3) (0-7)	3 (2-3) (1-7)	3 (3-4) (1-5)	3 (3-3) (0-7)
Total protein (median $\mu\text{g/ml}$) (95% CI) (range)	77.7 (67.4-92.7) (15.6-348.5)	50.0 (43.4-60.0) (18.6-305.1)	65.7 (52.2-82.7) (27.4-231.3)	66.5 (58.7-73.7) (15.6-348.5)
IL-6 (median pg/ml) (95% CI) (range)	1.6 (1.2-2.3) (0.1-281.4)	1.7 (0.8-2.5) (0.1-56.9)	1.6 (0.5-2.8) (0.1-34.9)	1.6 (1.2-2.1) (0.1-281.4)

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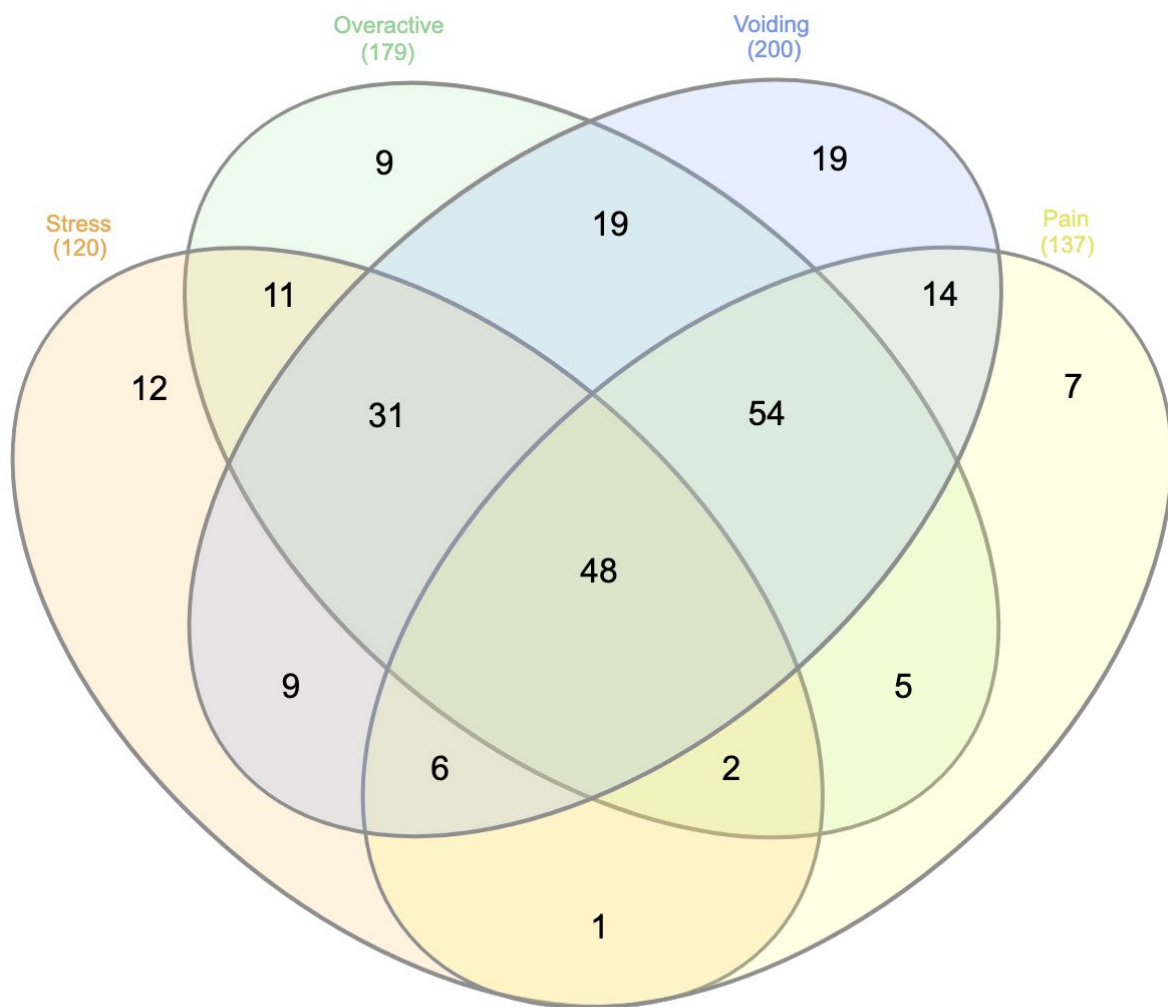
	Abdominal pain study (n=159)	PTB study (n=80)	MSU-CSU study (n=34)	Pooled data (n=273)
IL-6 / total protein (median ng/g) (95% CI) (range)	20 (15-27) (2-1380)	32 (16-42) (3-210)	15 (9-36) (2-530)	22 (17-27) (2-1380)
IL-8 (median pg/ml) (95% CI) (range)	46.4 (22.0-61.6) (0.7-2782)	25.3 (15.4-37.8) (0.7-23160)	29.2 (17.7-47.9) (1.1-4272)	32.9 (22.0-41.7) (0.7-23160)
IL-8 / total protein (median ng/g) (95% CI) (range)	450 (300-760) (10-28200)	440 (350-580) (30-75900)	440 (280-740) (40-65900)	450 (360-560) (10-75900)
Lactoferrin (ng/ml) (95% CI) (range)	(n=159) 4.2 (2.2-5.8) (2.2-896)	(n=79) 2.2 (2.2-4.7) (2.2-2138)	(n=34) 4.6 (2.2-8.4) (2.2-444)	(n=272) 3.8 (2.2-5.1) (2.2-2138)
Lactoferrin / total protein (g/g) (95% CI) (range)	(n=159) 70 (50-90) (10-20890)	(n=79) 70 (50-90) (10-42680)	(n=34) 80 (40-120) (10-6850)	(n=272) 70 (60-80) (10-42680)
Presence of clue cells (%) (95% CI)	111/157 (70.7%) (62.8-77.6)	72/80 (90%) (80.7-95.3)	33/33 (100%) (87.0-100)	217/270 (80.4%) (75.0-84.8)
Proportion of epithelial cells that are clue cells (median) (95% CI) (range)	(n=157) 21.1 (14.0-27.8) (0-100)	(n=80) 40 (33.3-46.2) (0-100)	(n=33) 98 (88-100) (0-100)	Unable to combine as different measurement methods

For a detailed examination of combined LUTS scores, I constructed a Venn diagram showing how subgroups of LUTS were combined for each participant (Figure 10-1).

This shows that of the 273 respondents, 48 reported symptoms across all four symptom groups of the questionnaire. This was the second commonest combination; the most common combination being overactive/voiding/pain (n=54). The least common combinations were stress/pain (n=1) and overactive/pain/stress (n=2). All combinations were noted. This demonstrates

the variety of LUTS that may be present in pregnancy in this cohort and highlights the problems with categorisation.

Figure 10-1: Venn diagram showing the relationships between groups of LUTS across 3 prospective studies



Having established the background demographics, risk factors, outcomes and simple results for the entire dataset, I compared alternative tests against standard tests.

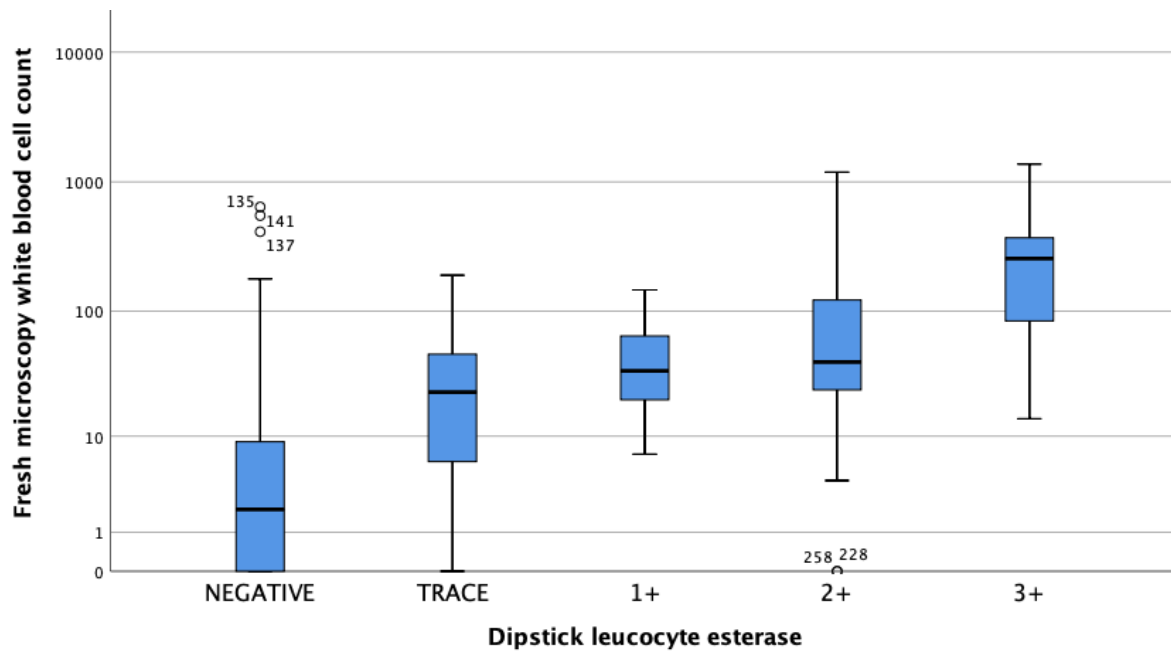
10.3.3 Comparison of alternative tests with standard tests

First, I compared tests looking at white cell counts: dipstick leucocyte esterase, standard microscopy, and fresh unspun microscopy. Note that standard microscopy was only performed in 117/273 (42.9%) cases. Table 10-5 and Figure 10-2 compare fresh microscopy white cell counts against dipstick leucocyte esterase. The urinary dipsticks show some correlation with white cell counts. The white cell counts do not discriminate the dipstick categories.

Table 10-5: Comparison of dipstick leucocyte esterase versus fresh unspun microscopy white cell counts

		Fresh unspun microscopy white cell count (WBC μl^{-1})			Number of cases (n=273)
		Median	95% CI	Range	
Dipstick leucocyte esterase	Negative	2	2-4	0-640	123
	Trace	23	14-32	0-190	70
	1+	34	22-56	7-146	33
	2+	40	26-100	0-1190	33
	3+	262	26-520	14-1370	14

Figure 10-2: Boxplot comparing dipstick leucocyte esterase versus fresh microscopy white cell count

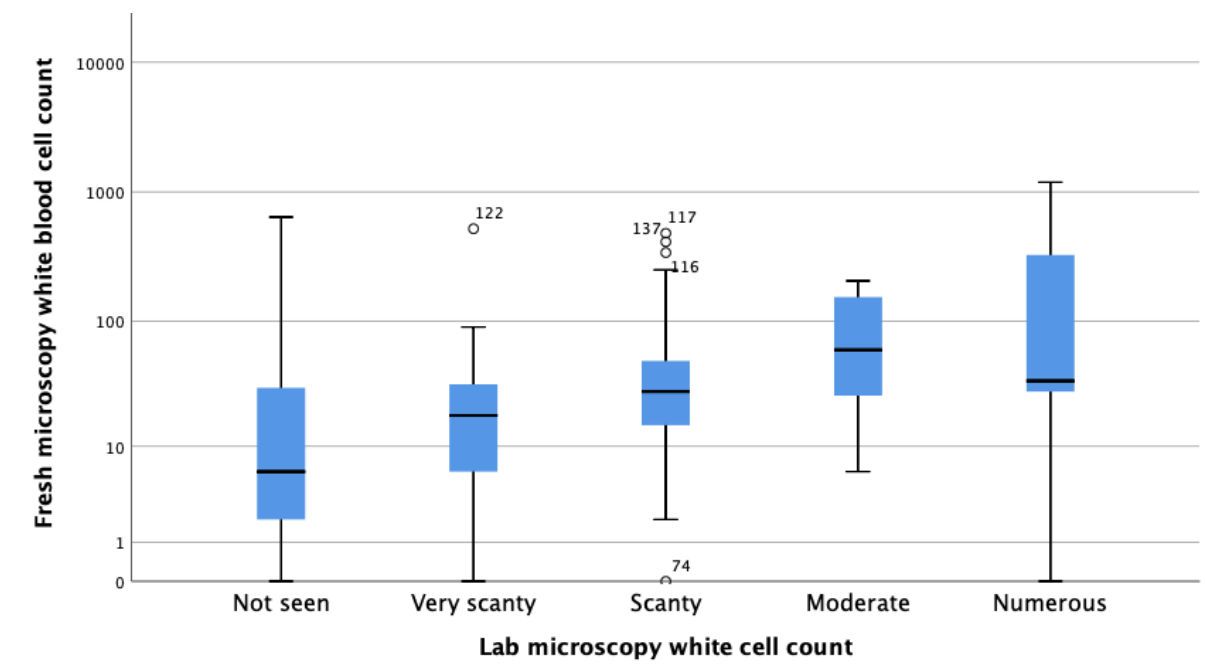


These data show that dipstick for leucocyte esterase is not sensitive; negative dipstick had median white cell count of 2, ranging from 0 to 640. The dipstick does show specificity. A result of specificity; 3+ was never associated with a zero pyuria. This has clinical implications; dipstick is often used to 'rule out' an infection which is not appropriate. If positive the probability of pyuria is high. A test that is specific is useful in gauging the efficacy of a treatment.

Table 10-6 and Figure 10-3 compare standard microscopy and fresh unspun microscopy for white cell counts. This showed a similar relationship.

Table 10-6: Comparison of standard lab microscopy reports with fresh unspun microscopy white cell counts

		Fresh unspun microscopy white cell count (WBC μl^{-1})			Number of cases (n=273)
		Median	95% CI	Range	
Standard lab microscopy white cell count	Not seen	6	2-16	0-640	36
	Very scanty	18	8-26	0-520	25
	Scanty	28	16-40	0-480	35
	Moderate	60	26-188	6-206	14
	Numerous	34	0-1190	0-1190	5
	<i>Not done</i>	8	<i>4-14</i>	<i>0-1370</i>	<i>158</i>

Figure 10-3: Boxplot comparing standard lab microscopy with fresh microscopy for white cell count

Standard lab microscopy underestimated the fresh microscopy result reflecting vulnerability to processing delays. Microscopy is not a routine, which accounts for the smaller number of data points. Neither dipstick leucocyte esterase, nor

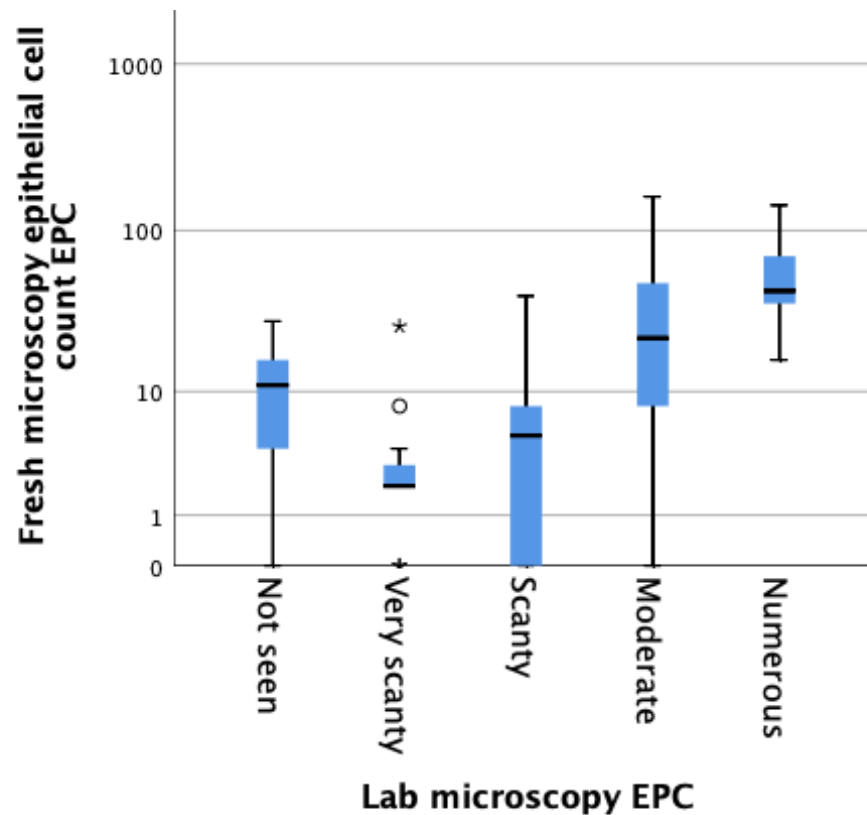
standard lab microscopy, are accurate substitutes for fresh unspun microscopy.

Next, I compared epithelial cell counts by standard and fresh unspun microscopy (Table 10-7 and Figure 10-4) in 117 samples and they did correlate but the standard microscopy was reported using categories. In the absence of fresh unspun microscopy, standard microscopy epithelial cell counts may have a role in assessing epithelial cell load in the urine.

Table 10-7: Comparison of standard microscopy epithelial cell counts versus fresh microscopy

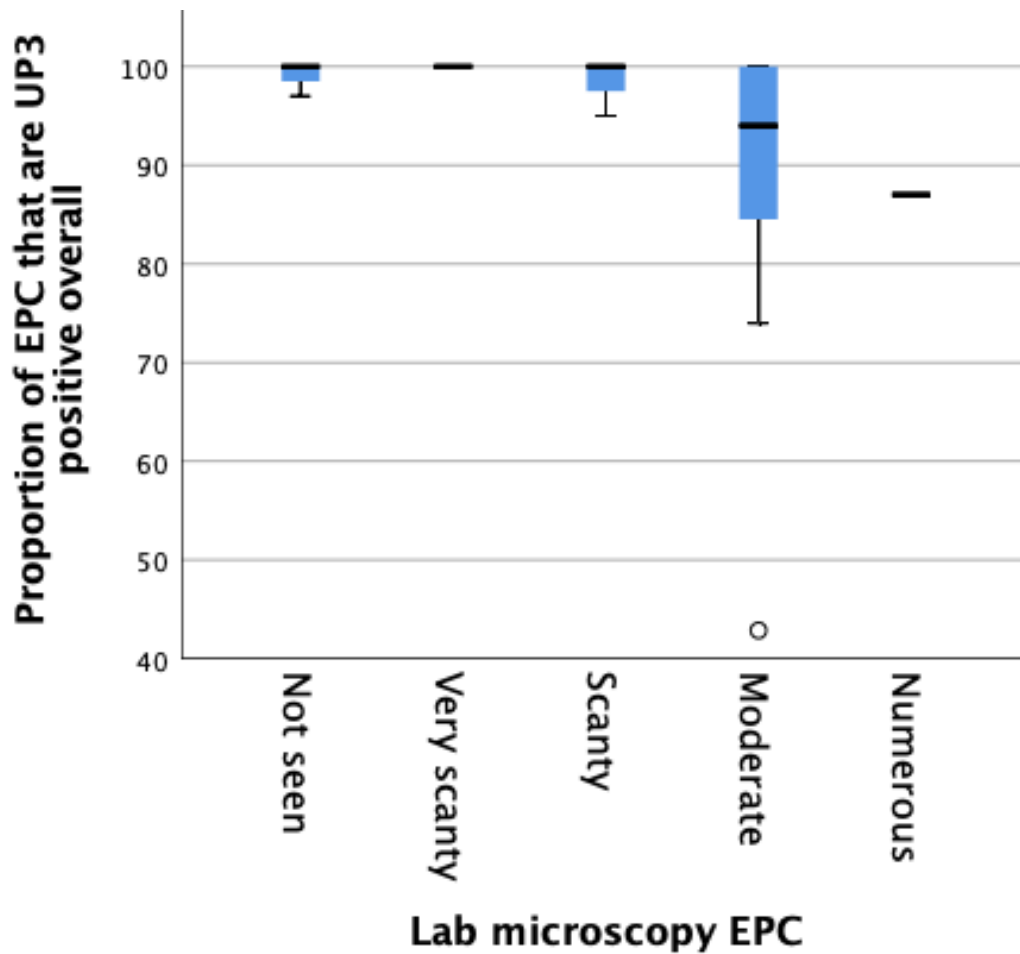
		Fresh unspun microscopy epithelial cell count (EPC μ l ⁻¹)			Number of cases (n=273)
		median epithelial cell count	95% CI	Range	
Standard lab microscopy epithelial cell count	Not seen	9	0-16	0-16	4
	Very scanty	2	0-8	0-26	11
	Scanty	5	0-8	0-40	22
	Moderate	22	16-36	0-160	62
	Numerous	43	36-76	16-142	16
	<i>Not done</i>	<i>12</i>	<i>8-14</i>	<i>0-800</i>	<i>158</i>

Figure 10-4: Boxplot comparing standard lab microscopy with fresh unspun microscopy for epithelial cell counts (logarithmic conversion)



I compared standard lab microscopy epithelial cell counts against fluorescent microscopy uroplakin-3 positive epithelial cell proportions (Figure 10-5), for 84 samples. There was no difference between distributions (Kruskal-Wallis=5.766, $p=0.217$). This shows that contamination of epithelial cells with non-uroplakin-3 positive cells was not related to the quantity of epithelial cells noted at standard microscopy. This is important as higher epithelial cell counts may be erroneously considered as evidence of contamination.

Figure 10-5: Comparison of standard microscopy epithelial cell counts versus uroplakin-3 positive epithelial cell proportions

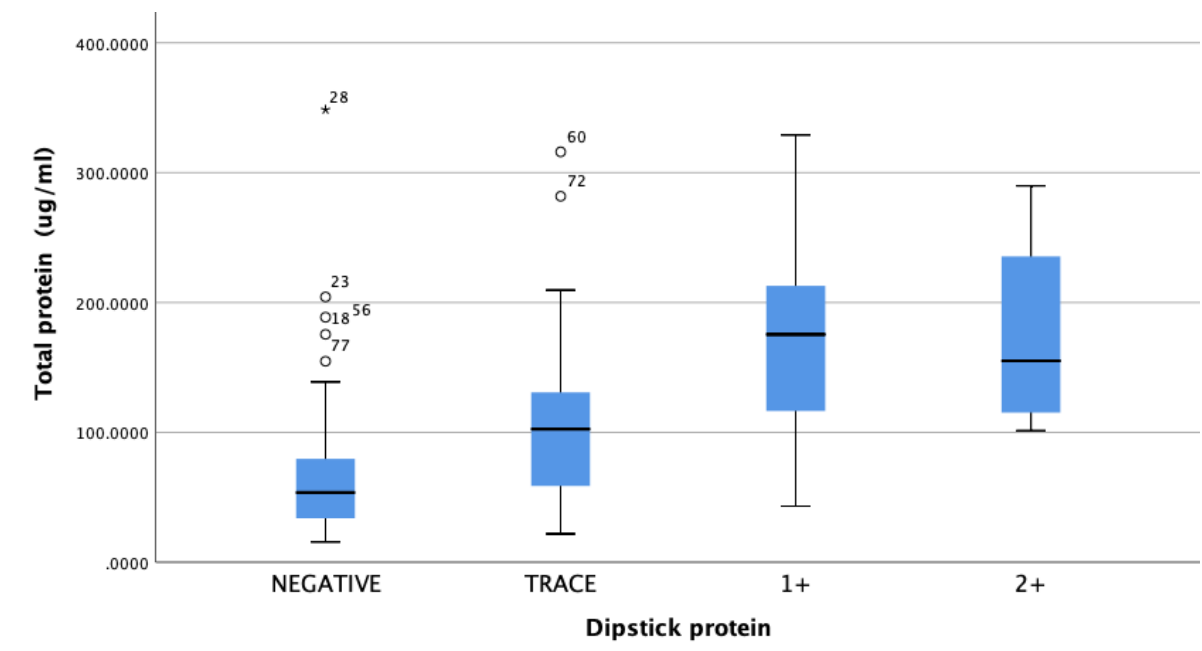


I also compared standard microscopy epithelial cell counts against clue cell proportions, for 104 samples. There was no difference between distributions (Kruskal-Wallis=4.535, $p=0.338$). This demonstrates the abundance of urothelial cell shedding was not related to evidence of cell parasitisation.

Next, I compared dipstick protein with total protein concentration (Table 10-8 and Figure 10-6). These data correlated. In our study there were no samples with dipstick protein of 3+ or 4+.

Table 10-8: Comparison of dipstick protein versus total protein concentration

		Protein concentration ($\mu\text{g/ml}$)			Number of cases (n=273)
		Median	95% CI	Range	
Dipstick protein	Negative	53.7	46-59	16-349	166
	Trace	102.6	71-115	22-316	82
	1+	175.4	117-213	43-329	21
	2+	155.1	101-290	101-499	4
	3+	n/a	n/a	n/a	0

Figure 10-6: Boxplot comparing dipstick protein with total protein concentration

Around 50% of urinary protein arises from sediment (Barratt and Topham 2007). Dipstick is unspun and should reflect this. A comparison of dipstick protein against sediment culture colony counts showed a correlation between these (Table 10-9). In contrast, urinary protein was measured using centrifuged urine so sediment cells should have been removed. When

comparing centrifuged protein against sediment culture colony counts there was no correlation (Spearman's rho, $r^2=0.083$, $p=0.172$).

Table 10-9: Comparison of sediment culture colony counts against dipstick protein

		Sediment culture colony counts (CFU/ml)			Number of cases (n=273)
		Median	95% CI	Range	
Dipstick protein	Negative	1157	669-2416	0-1390319	166
	Trace	3272	1051-8351	0-1280000	82
	1+	2806	398-19363	26-631087	21
	2+	421944	39-1099394	39-1099394	4
	3+	n/a	n/a	n/a	0

I did not formally compare dipstick nitrite against standard culture since only one sample was nitrite positive (standard culture was positive with *E. coli*).

Next, I compared standard culture at recruitment with standard culture throughout the rest of the pregnancy (Table 10-10); these were differently distributed ($p<0.001$). In particular, there were 30.8% non-significant or mixed growth at recruitment, compared with 46.6% through the rest of pregnancy.

Table 10-10: Comparison of standard culture at recruitment with those through rest of pregnancy

	Standard culture at recruitment (%) (95% CI)	Standard culture throughout rest of pregnancy (%) (95% CI)	Totals (%) (95% CI)
Screening culture negative	162 (60.9%) (54.7-66.8)	339 (44.1%) (40.5-47.7)	501 (48.4%) (45.3-51.5)
Non-significant growth	49 (18.4%) (14.1-23.7)	201 (26.1%) (23.1-29.4)	250 (24.2%) (21.6-26.9)
Mixed growth	33 (12.4%) (8.8-17.1)	158 (20.5%) (17.8-23.6)	191 (18.5%) (16.2-21.0)
Positive culture	22 (8.3%) (5.4-12.4)	71 (9.2%) (7.3-11.6)	93 (9.0%) (7.4-10.9)
Totals	266 (100%)	769 (100%)	1035 (100%)

$$\chi^2=23.722, p<0.001$$

This difference may have arisen for multiple reasons. However, an important difference is urine sampling method – this was a standard pregnancy void through the rest of pregnancy, compared with the study void which had instructions about how to do a clean-catch midstream sample, a wipe to clean the labia, and a large pot. It can be argued that mid-stream clean catch samples miss the sediment, and so may miss urinary pathology (Collins, Sathiananthamoorthy et al. 2020). However, if it is believed that non-significant and mixed growth represent at best a confusing result that requires repeating, then an enhanced void method may reduce costs and confusion.

Next, I compared standard culture with enhanced culture (Table 10-11). There was no difference in distributions (Fishers Exact, $p=1.00$).

Table 10-11: Comparison of standard cultures versus presence of bacteriuria by sediment culture

	Standard lab culture negative	Standard lab culture positive	Total
No bacteriuria present	2	0	2
Bacteriuria present	242	22	264
Total	244	22	266

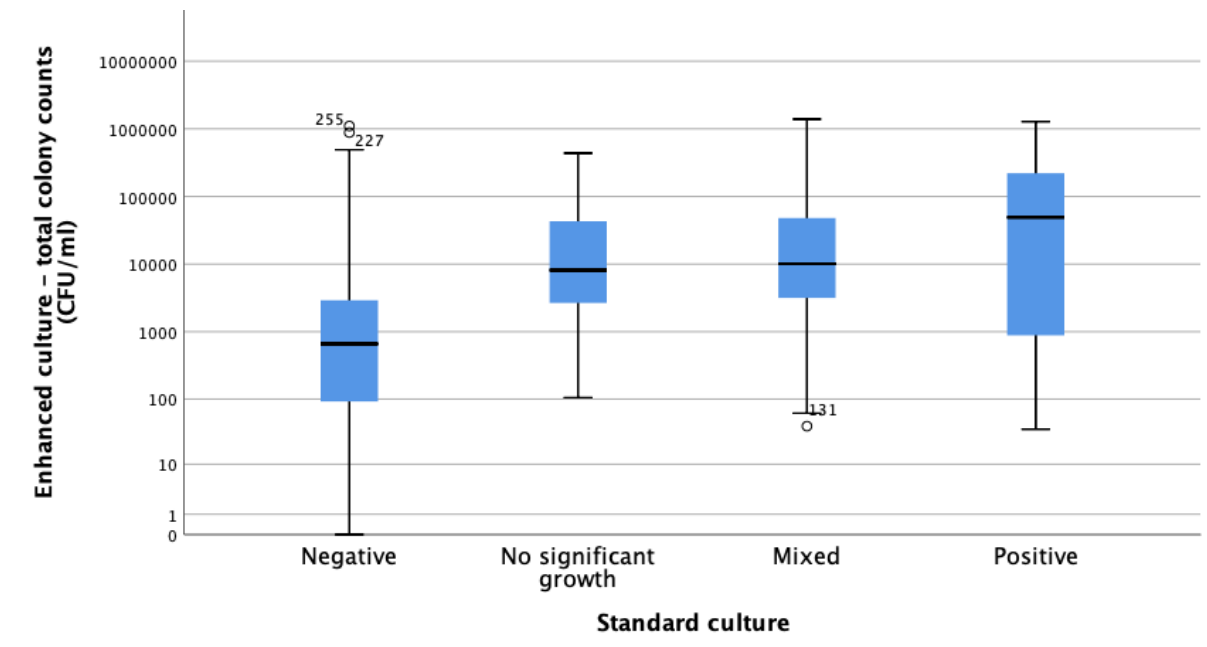
Fisher's Exact test $p=1.00$

Next, I compared standard culture against colony counts by enhanced culture (Table 10-12 and Figure 10-7). There was some correlation although the standard culture used categories.

Table 10-12: Comparison of standard culture versus colony counts by sediment culture

		Enhanced culture colony counts (CFU/ml)			Total cases (n=266)
		Median	95% CI	Range	
Standard lab culture	Negative	661	401-1019	0-1,099,395	162
	No significant growth	8123	2987-21628	106-435,782	49
	Mixed growth	10117	3407-36360	39-1,390,319	33
	Positive	51464	884-220480	35-1,280,000	22

Figure 10-7: Boxplot comparing standard culture categories with enhanced culture total colony counts



Next, I compared standard culture against the number of different isolates by enhanced culture (Table 10-13). The median number of isolates identified by enhanced culture was three for all categories of standard culture, including positive cultures which reported a single organism.

Table 10-13: Comparison of standard culture versus number of isolates by sediment culture

		Enhanced culture - number of isolates				Total (n=266)
		Median	95% CI	Range	Mixed growth (>1 isolates) (%) (95% CI)	
Standard lab culture	Negative	3	3-3	0-6	143/162 (88.3%) (82.1-92.6)	162
	No significant growth	3	3-4	1-7	48/49 (98.0%) (87.8-99.9)	49
	Mixed growth	3	3-4	2-6	33/33 (100%) (87.0-100)	33
	Standard lab culture positive	3	1-3	1-6	16/22 (72.7%) (49.6-88.4)	22

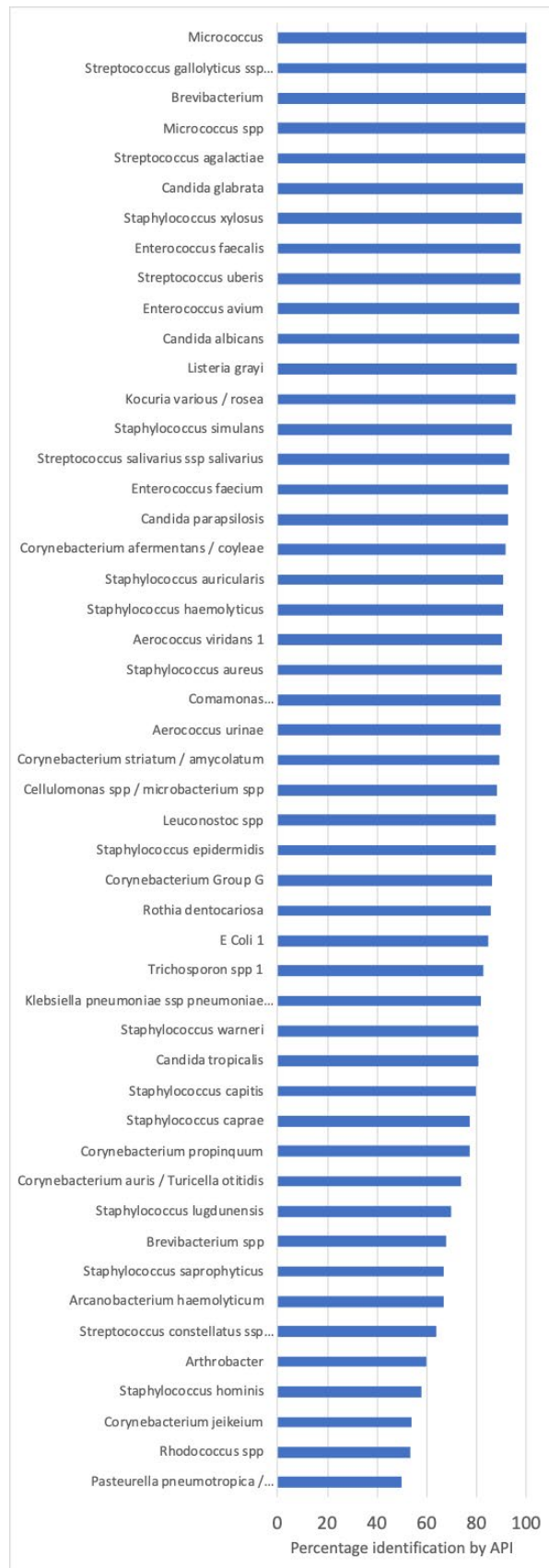
It is interesting that standard culture mapped quantitatively onto enhanced culture, but that polymicrobial growth was very common in all categories of standard culture. This fits with the belief that urine is not sterile. It makes it hard to understand why a positive standard culture (with apparently only one pathogen identified) should be treated differently in the clinic from a mixed growth standard culture, other than the increased colony count.

The accuracy of bench genus assessment was assessed in the PTB study by comparison against API genus identification. The accuracy of API species identification can be estimated using information supplied by the manufacturer, specifically the identification percentage (probability of species identification). These are summarised here by study group (Table 10-14) and by species (Figure 10-8). Some species had a very high level of accuracy of >99% (e.g. *Streptococcus agalactiae*); others were accepted with a lower accuracy if the manufacturer described the identification as at least acceptable.

Table 10-14: Accuracy of API identification by study group

	Abdominal pain study	PTB study	MSU-CSU study	Overall
Identification by API testing - mean % (standard deviation)	89.8 (15.6)	89.4 (12.9)	92.3 (11.7)	91.6 (14.4)

Figure 10-8: Accuracy of API identification by species using identification percentage (probability of identification according to manufacturer)



Next, I compared the isolates identified in standard culture against the isolates identified by sediment culture, shown in Table 10-15. This shows that there were some cases in full agreement, including 5/22 (22.7%) cases where *E. coli* was the sole organism in both standard and enhanced culture, and one case of *E. coli* standard culture where no *E. coli* was identified at sediment culture. In all other cases the organism identified in standard culture was present in the polymicrobial growth of the enhanced culture. However other isolates were present, and the organism from standard culture was not always the most numerous colony in the enhanced culture.

Table 10-15: Comparison of standard culture isolates versus sediment culture isolates

Standard culture result CFU/ml	Total colony count (CFU/ml)	Number of isolates	Enhanced culture
			Isolates identified (<u>Underlined</u> denotes identified by bench tests only, not API testing)
10^5 <i>E. coli</i>	1280000	1	<i>E. coli</i> 1
10^5 <i>E. coli</i>	1280000	1	<i>E. coli</i> 1
10^5 <i>E. coli</i>	472005	2	<u><i>E. coli</i></u> , <i>Lactobacillus spp.</i>
10^4 - 10^5 <i>E. coli</i>	35723	1	<i>E. coli</i> 1
10^5 <i>E. coli</i>	5554	1	<i>E. coli</i> 1
10^5 <i>E. coli</i>	884	2	<i>S. agalactiae</i> , <i>Lactobacillus spp</i>
10^5 <i>E. coli</i>	884	3	<i>Corynebacterium</i> group G, <i>S. agalactiae</i> , <i>E. coli</i> 1
10^5 <i>E. coli</i>	158	3	<i>E. coli</i> 1, <i>Coryneform jeikeium</i> , <i>E. faecalis</i>
10^5 <i>E. coli</i>	35	1	<u><i>E. coli</i></u>
10^5 <i>E. faecalis</i>	95862	2	<i>E. faecalis</i> , <i>E. coli</i> 1
10^5 <i>E. faecalis</i>	82295	5	<u>Other</u> , <i>E. faecalis</i> , <i>S. haemolyticus</i> , <i>Micrococcus spp</i> , <i>E. coli</i> 1
10^5 <i>E. faecalis</i>	74342	4	<i>E. faecalis</i> , <i>S. haemolyticus</i> , <i>S. saprophyticus</i> , <i>E. coli</i> 1
10^5 <i>E. faecalis</i>	17606	3	<i>E. faecalis</i> , <i>S. haemolyticus</i> , <i>Citrobacter koseri</i> / <i>amalonaticus</i>
10^5 <i>E. faecalis</i>	275	3	<u><i>Enterococcus spp.</i></u> , <u><i>Staphylococcus spp.</i></u> , <u>Other</u>
10^5 <i>E. faecalis</i>	203	4	<i>E. faecalis</i> , <u><i>Streptococcus spp.</i></u> , <i>S. haemolyticus</i> , <i>E. coli</i> 1
10^5 <i>S. agalactiae</i>	921138	3	<i>S. agalactiae</i> , <i>Lactobacillus spp</i> , <i>Kocuria various / rosea</i>
10^5 <i>S. agalactiae</i>	789229	4	<i>S. agalactiae</i> , <i>Streptococcus constellatus ssp constellatus</i> , <i>Lactobacillus spp</i> , <i>Klebsiella pneumoniae spp pneumoniae 2</i>
10^5 <i>S. agalactiae</i>	67206	3	<i>S. agalactiae</i> , <i>S. aureus</i> , <i>Klebsiella pneumoniae spp pneumoniae 1</i>
10^5 <i>S. agalactiae</i>	24595	3	<i>Lactobacillus spp</i> , <i>S. agalactiae</i> , <i>Corynebacterium</i> group G
10^5 <i>C. albicans</i>	220480	1	<i>Candida albicans</i>
10^5 <i>C. albicans</i>	76032	2	<u><i>Candida spp.</i></u> , <i>E. coli</i> 1
10^5 <i>Citrobacter koseri</i>	280	5	<i>Citrobacter koseri</i> / <i>amalonaticus</i> , <i>E. faecalis</i> , <i>S. haemolyticus</i> , <i>Citrobacter freundii</i> , <i>E. coli</i> 1

This shows that the standard culture and the enhanced culture were related. It supports the hypothesis that including the urinary sediment enhances the standard culture rather than replacing it with something else. It discredits the idea that a UTI must be defined by a single growth of an organism, given that the same sample generated alternative culture results when handled differently.

Next, I compared detection of *Streptococcus agalactiae* (GBS) in standard and enhanced culture, as detecting this pathogen has important implications in obstetrics as described previously. I included standard cultures throughout the pregnancy, and standard vaginal swabs throughout the pregnancy, for comparison (Table 10-16). Standard culture at the time of recruitment detected GBS in 1.5% (95% CI 0.5-4.0) compared with enhanced culture which detected 11.7% (95% CI 8.3-16.3). Standard culture throughout pregnancy detected GBS at least once in 3.7% (1.9-6.8). Swabs were carried out in 166 women, of which 27/166 (16.3%, 95% CI 11.2-23.0) showed GBS. Including all those women who were not swabbed, GBS was detected in 27/273 (9.9%, 95% CI 6.7-14.2).

Table 10-16: Detection of *Streptococcus agalactiae* (GBS) by enhanced culture compared to other methods

Method of detection	GBS detected
Enhanced culture (%) (95% CI)	32/273 (11.7%) (8.3-16.3)
Standard culture at time of recruitment (%) (95% CI)	4/268 (1.5%) (0.5-4.0)
Standard culture throughout pregnancy – any GBS detected (%) (95% CI)	10/273 (3.7%) (1.9-6.8)
Swabs throughout pregnancy – any GBS detected (%) (95% CI)	27/273 (9.9%) (6.7-14.2)

This discrepancy is potentially important, as detection of GBS carriage at any point in pregnancy is a risk factor for neonatal GBS septicaemia. There is some evidence that this is a risk factor even with lower levels of GBS, as described previously (Anderson, Simhan et al. 2007).

In the 4/268 (1.5%, 95% CI 0.5-4.0) standard cultures that detected GBS, enhanced culture also detected GBS. Enhanced cultures detected GBS in an additional 28/268 (10.5%, 95% CI 7.2-14.9) samples.

We cannot determine whether the additional GBS detected by enhanced culture is because of vaginal flora, or bladder microbiome, from these data alone. It is of note that GBS was also detected in a CSU sample, however. What is important is that GBS detection was higher in enhanced culture samples than simultaneously-voided standard culture samples, or opportunistic urine or vaginal cultures throughout pregnancy. This study was not powered to detect differences in outcomes related to GBS, however the role of urine in GBS carriage screening in relation to relevant outcomes is something that warrants further research.

There were no cases of neonatal GBS septicaemia or meningitis. One patient had a late miscarriage with chorioamnionitis and placental GBS. At recruitment, standard culture had shown non-significant growth, a vaginal swab had shown GBS, but unfortunately the enhanced cultures could not be analysed.

The study was not designed to explore the implications of sub-threshold levels of GBS bacteriuria. This is something that would warrant future study.

I compared outcomes for those with GBS present on enhanced culture, and no GBS (Table 10-17). Significance tests were not performed to avoid errors of multiplicity.

Table 10-17: Comparison of outcomes in GBS-positive enhanced culture versus GBS-negative

	GBS present on enhanced culture (n=32)	GBS absent on enhanced culture (n=241)
Preterm birth (n=268) (%) (95% CI)	2/32 (6.3%) (1.1-22.2)	21/236 (8.9%) (5.7-13.5)
Chorioamnionitis by placenta (n=23) (%) (95% CI)	1/2 (50%) (2.7-97.3)	10/21 (47.6%) (26.4-69.7)
Maternal blood cultures GBS (n=18) (%) (95% CI)	0/2 (0%) (0-80.2)	0/16 (0%) (0-24.1)
Possible intrapartum sepsis (n=263) (%) (95% CI)	3/32 (9.4%) (3.2-24.2)	11/231 (4.8%) (2.7-8.3)
Possible neonatal sepsis (n=257) (%) (95% CI)	5/31 (16.1%) (7.1-32.6)	17/226 (7.5%) (4.8-11.7)
Admission to NNU (n=263) (%) (95% CI)	5/32 (15.6%) (6.9-31.8)	26/231 (11.3%) (7.8-16.0)

There was a suggestion of higher rates of intrapartum sepsis, neonatal sepsis and admission to NNU among those with GBS on sediment culture.

To examine the effect of colony counts, I compared GBS colony counts in term and preterm births. Of those in whom GBS was detected, median GBS bacteriuria was 380889 CFU/ml (2 cases of 177 and 761,600) for preterm births, and was 231 CFU/ml (95% CI 64-3676, range 2-913,869) for those with term births. Taking into account all cases, median counts were 0 CFU/ml (95% CI 0-0) for both groups, with no difference between them ($p=0.660$, Mann Whitney U). Whilst almost uninterpretable here owing to small numbers, these data may contribute to future meta-analysis.

Enhanced culture provides additional information about GBS compared with standard culture. Earlier I described other bacteria isolated from enhanced culture where the standard culture had shown a single uropathogen. It is not clear of the relevance of those additional organisms. Given the burgeoning understanding of urinary microbiome, it is likely that those organisms found by enhanced culture tell a story of some kind. The degree to which any of these are pathogenic or otherwise cannot be interpreted from quantitative microbiology alone. Understanding the integration of host response, through measurable factors in the urine, symptoms, clinical outcomes, or other elements, is needed to start to understand the implications. I believe it is critical to question the dominant role of the standard culture in diagnosing UTI in pregnancy, given these findings.

Having compared standard and alternative tests with each other, I next compared alternative tests with each other to understand more about their relationships.

10.4 Relationships between different tests

Next, I explored relationships between different alternative tests, using graphical methods and correlation where appropriate. I have included only voided samples rather than CSU.

10.4.1 Correlations between different alternative tests

First, I compared symptom scores and quality of life (shown in Table 10-18). There were moderate correlations between pain symptoms and voiding symptoms ($r_s = 0.5$, $p < 0.01$), overactive bladder symptoms and voiding symptoms ($r_s = 0.4$, $p < 0.01$), and between 'your health today' and EQ-5D-5L index value ($r_s = 0.5$, $p < 0.01$). There was a weak negative correlation between EQ-5D-5L index value and total symptoms ($r_s = -0.3$, $p < 0.01$), and a weak positive correlation between overactive bladder and stress incontinence symptoms ($r_s = 0.3$, $p < 0.01$), and between pain symptoms and overactive bladder symptoms ($r_s = 0.4$, $p < 0.01$). I did not include correlations between total symptoms and symptom groups as these are not independent.

Table 10-18: Correlations between symptom scores and quality of life

* $p < 0.05$ ** $p < 0.01$

r_s	1	2	3	4	5	6	7	8
1. Gestation								
2. Age	-0.15 *							
3. Stress incontinence symptoms	0.06	0.004						
4. Overactive bladder symptoms	-0.004	-0.002	0.34 **					
5. Voiding symptoms	-0.08	-0.01	0.17 **	0.43 **				
6. Pain symptoms	-0.10	-0.13 *	0.02	0.36 **	0.45 **			
7. Total symptoms	-0.05	-0.04						
8. Your health today	0.04	0.16 *	-0.05	-0.18 *	-0.27 **	-0.22 **	-0.26 **	
9. EQ-5D-5L index value	-0.12	0.06	-0.08	-0.22 **	-0.30 **	-0.32 **	-0.34 **	0.49 **

This demonstrated a weak-moderate correlation between the different symptom types, and a moderate correlation between the two measures of quality of life. There were weak negative correlations between the symptom groups and measures of quality of life. There was no relationship with gestation or age.

Next, I compared fresh unspun microscopy cell counts, ATP and colony counts along with total symptoms and quality of life (Table 10-19). There was a moderate correlation between epithelial cell count and white cell count ($r_s=0.6$, $p<0.01$, Figure 10-9) and between red cell count and white cell count ($r_s=0.4$, $p<0.010$). There were weak positive correlations between: white cell count and gestation ($r_s=0.3$, $p<0.01$, Figure 10-10); epithelial cell count and gestation ($r_s=0.2$, $p<0.01$); total colony counts and white cell count ($r_s=0.3$, $p<0.01$); epithelial cell count and red cell count ($r_s=0.3$, $p<0.01$); total colony counts and epithelial cell count ($r_s=0.3$, $p<0.01$). ATP did not correlate with any of these measures.

Table 10-19: Correlations between fresh microscopy cell counts, ATP, colony counts

* $p<0.05$ ** $p<0.01$

r_s	1	2	3	4	5	6	7	8
1. Gestation								
2. Age	-0.15 *							
3. White cell count	0.33 **	-0.16 **						
4. Red cell count	0.03	-0.02	0.44 **					
5. Epithelial cell count	0.22 **	-0.09	0.56 **	0.29 **				
6. ATP	-0.07	-0.12 *	-0.05	-0.04	0.09			
7. Total symptoms	-0.05	-0.04	-0.05	-0.05	-0.09	0.12		
8. Your health today	0.04	0.16 *	-0.01	0.04	-0.09	-0.07	-0.26 **	
9. Total colony counts	0.07	-0.09	0.30 **	0.08	0.28 **	0.07	0.03	-0.15 *

Figure 10-9: Scatter plot comparing fresh microscopy white cell counts and epithelial cell counts (logarithmic scales)

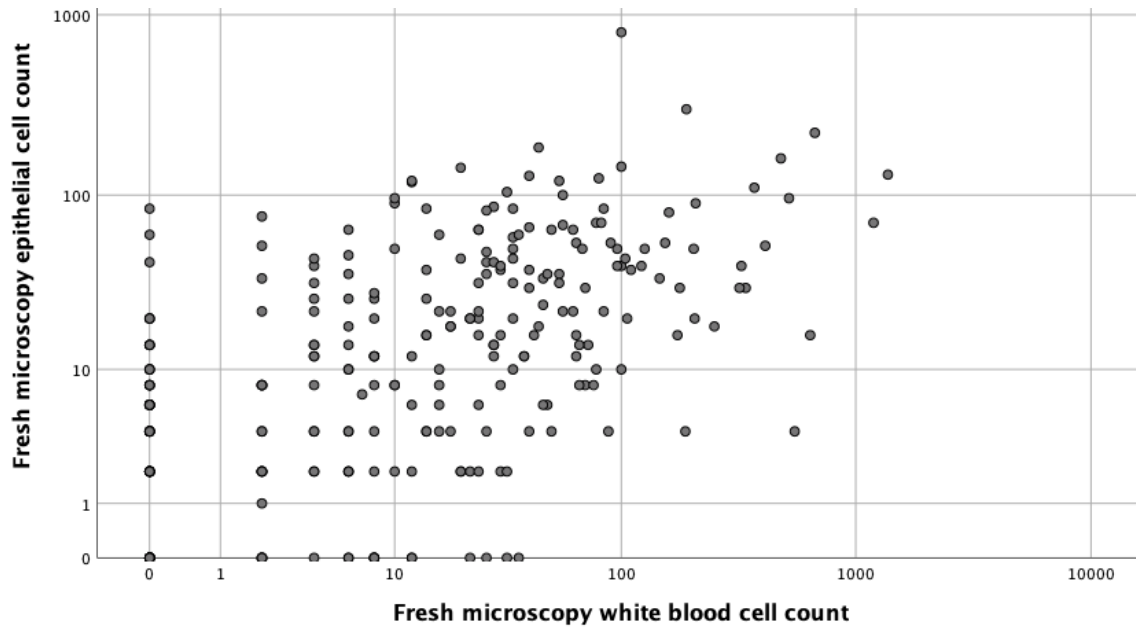
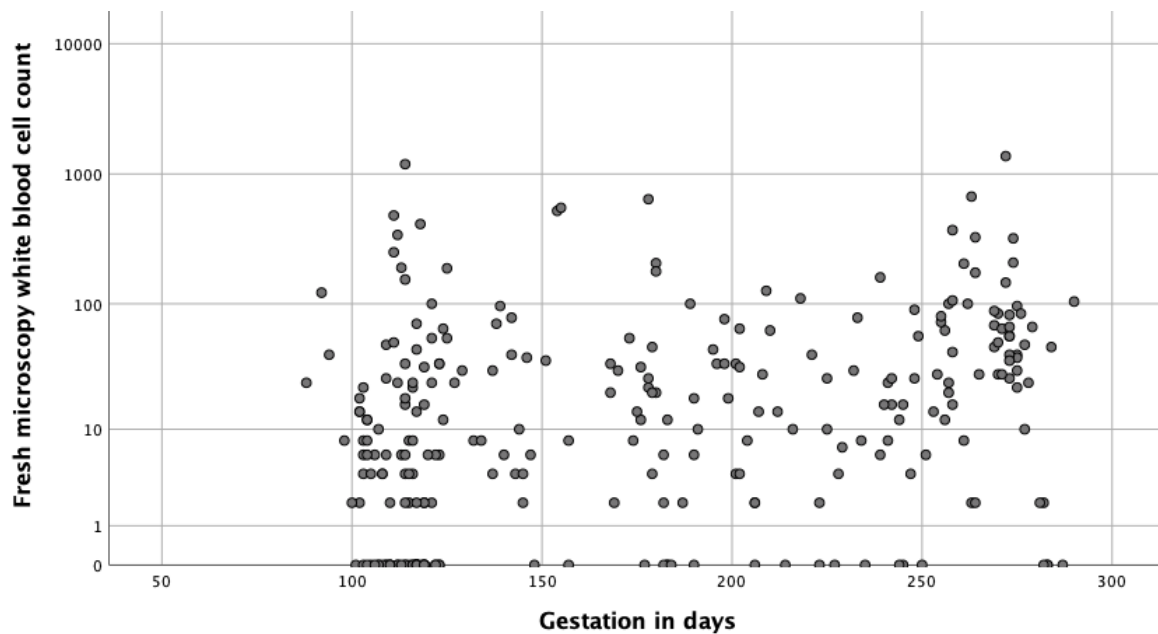


Figure 10-10: Scatter plot comparing gestation (days) with fresh microscopy white cell counts (logarithmic scale)



The correlation between cell counts could have several explanations: (1) cell counts increase due to the same stimulus (2) cell counts increase in parallel due to different factors. If epithelial cells were due to contamination, they might not be expected to correlate with white cells, which are taken as a sign of host response to infection, unless white cells also represent contamination.

The relationship between gestation and white and epithelial cells was weak, and a review of the scatter plot shows that this has little meaningful clinical application, given the spread of cell counts at all gestations.

It is surprising that there was no relationship between cell counts and total symptom scores. In the chronic LUTS context, symptom scores are seen to oscillate in a way that mirrors the cell counts (Swamy, Barcella et al. 2018). However, these are single values and what might be a baseline of normal for one participant might represent UTI for another; the change in values over time might be more illustrative.

Next, I compared urinary cytokines with gestation, age and each other (see Table 10-20). I excluded non-independent comparisons. There was a strong correlation between IL-6 and total protein ($r_s=0.7$, $p<0.01$, Figure 10-11), between lactoferrin and IL-8 ($r_s=0.7$, $p<0.01$, Figure 10-12) and between lactoferrin and IL-8 normalised ($r_s=0.7$, $p<0.01$). There was a moderate correlation between IL-8 and IL-6 ($r_s=0.5$, $p<0.01$), total protein and IL-8 ($r_s=0.5$, $p<0.01$), and between lactoferrin normalised and IL-8 normalised ($r_s=0.5$, $p<0.01$).

Table 10-20: Comparison of urinary cytokines.* $p < 0.05$ ** $p < 0.01$

r_s	1	2	3	4	5	6	7
1 Gestation							
2 Age	-0.15 *						
3 IL-6	0.02	-0.09					
4 IL-6 normalised	-0.06	-0.07					
5 IL-8	0.06	-0.28 **	0.50 **	0.36 **			
6 IL-8 normalised	-0.01	-0.29 **	0.29 **	0.25 **			
7 Lactoferrin	0.08	-0.18 **	0.21 **	0.15 *	0.69 **	0.69 **	
8 Lactoferrin normalised	-0.03	-0.12	-0.19 **	-0.07	0.30 **	0.51 **	
Total protein	0.16 **	-0.10	0.69 **		0.55 **		0.24 **

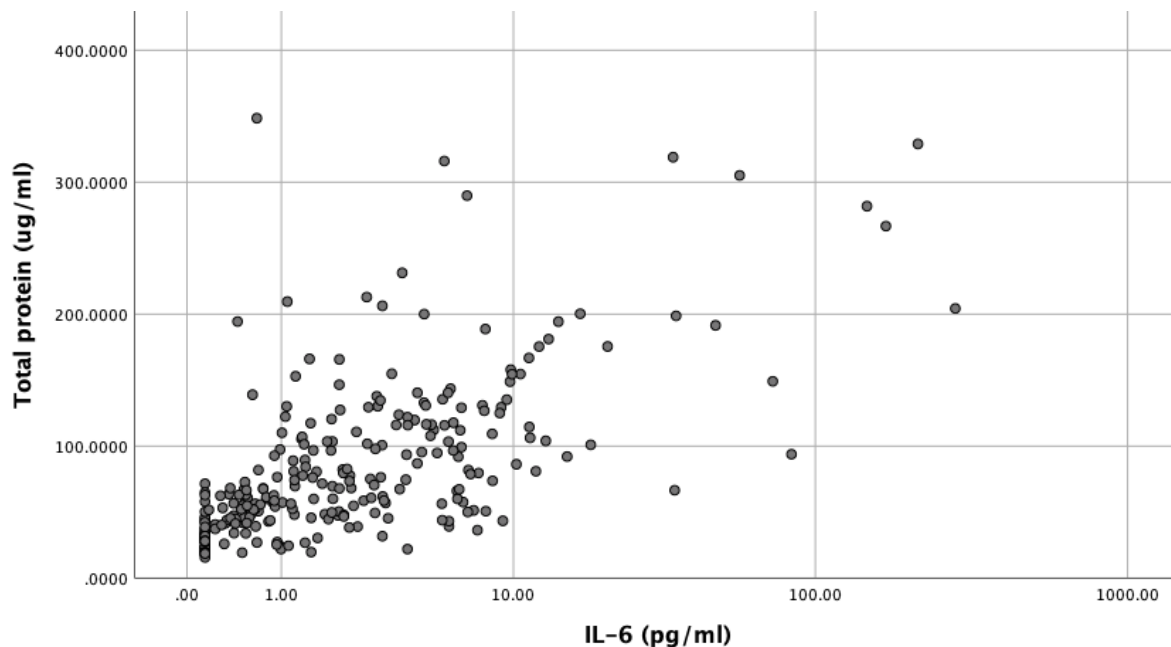
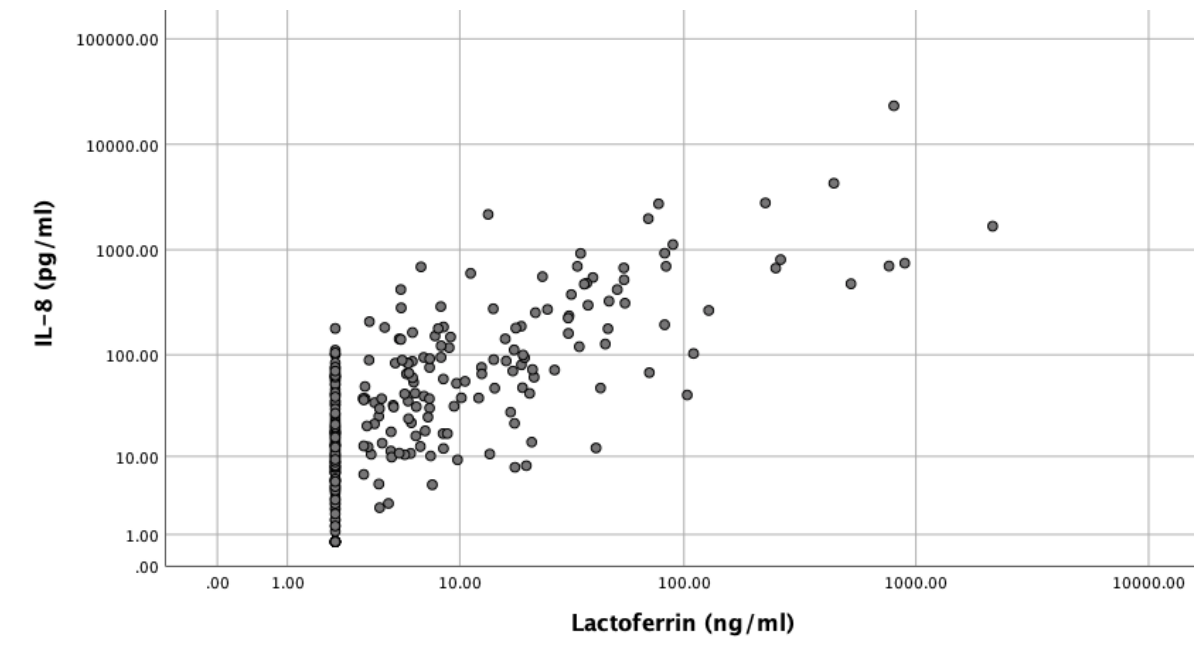
Figure 10-11: Scatter plot comparing IL-6 (logarithmic scale) with total protein

Figure 10-12: Scatter plot comparing lactoferrin with IL-8 (logarithmic scales)



Correlations between the urinary cytokines are moderate to strong, suggesting they are demonstrating something similar to each other. The relationship of each to protein is unexpected; if protein is to be used to normalise, I would expect it to have a more random pattern reflecting something else about each patient's renal excretion. If it has a positive correlation with the urinary cytokines that suggests it may not be independent of the cytokines. Is there a relationship between proteinuria and infection? A systematic review found no association between asymptomatic bacteriuria and proteinuria (Carter, Tomson et al. 2006). There was no relationship between cytokines and gestation.

Next, I compared the urinary cytokines against fresh microscopy cell counts, enhanced culture colony counts, ATP and symptoms (Table 10-21). I included only the non-normalised values for reasons given above. This showed that there was a strong correlation between epithelial cell count and IL-8 ($r_s=0.6$, $p<0.01$, Figure 10-13), and moderate correlation between white cell count and IL-8 ($r_s=0.5$, $p<0.01$), and between epithelial cell count and lactoferrin ($r_s=0.4$, $p<0.01$). There was no correlation between symptoms score and urinary cytokines. There was a weak correlation between ATP and IL-6 ($r_s=0.2$,

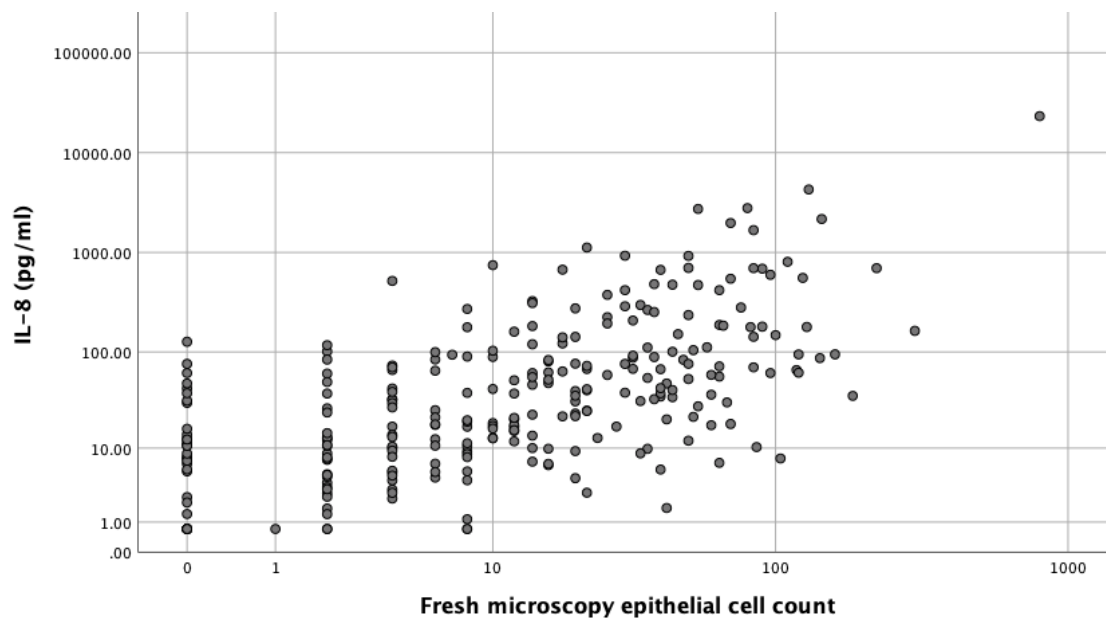
$p < 0.01$) and IL-8 ($r_s = 0.2$, $p < 0.01$), and between total colony count and IL-8 ($r_s = 0.2$, $p < 0.01$).

Table 10-21: Comparison of urinary cytokines with other measures

* $p < 0.05$ ** $p < 0.01$

r_s	1	2	3	4	5	6	7
1 IL-6							
2 IL-8	0.50 **						
3 Lactoferrin	0.21 **	0.68 **					
4 ATP	0.21 **	0.25 **	0.18 **				
5 White cell count	0.30 **	0.52 **	0.38 **	-0.05			
6 Epithelial cell count	0.33 **	0.60 **	0.40 **	0.09	0.56 **		
7 Total symptoms	0.01	0.04	0.01	0.12	-0.05	-0.09	
8 Total colony count	0.08	0.24 **	0.18 **	0.07	0.30 **	0.28 **	0.03

Figure 10-13: Scatter plot comparing epithelial cell count with IL-8



The positive relationship between IL-8 and epithelial cells is interesting. This is consistent with earlier research that suggested urothelial cells release IL-8 and will be discussed further later.

The lack of relationship between symptoms and urinary cytokines is also interesting. There was a large range in symptom scores among the participants, however these do not correlate with anything except a weak negative correlation with quality of life scores. This may be because urine symptoms in pregnancy are not pathological, or it may be because the tests have not explored pathology, or it may be because the symptom scoring system is not effective as a one-off symptoms assessment in pregnancy.

Having compared alternative tests to each other, next I compared standard and alternative tests against clinical outcomes.

10.5 Comparison of standard and alternative tests against standard culture and clinical outcomes

I have argued previously that outcome-based analysis is essential to move forward with UTI in pregnancy research. Because standard culture is the primary clinical indicator, and considered the gold standard, I first compared the standard culture against the other tests. I then compared all of the tests against preterm birth as a clinical outcome of interest. I had planned to compare against pyelonephritis, but no cases occurred.

10.5.1 Comparison of standard and alternative tests with standard culture

10.5.1.1 Methods

I compared standard culture positive versus negative (which included 'screening culture negative', 'non-significant growth', and 'mixed growth') (Table 10-22). I compared risk factors, tests and outcomes and independent variables against standard culture as the dependent in a secondary analysis (Table 10-23).

10.5.1.2 Results

There were 22 positive and 244 negative cultures. Table 10-22 describes the relationship of these results to the consilience variables.

There was a correlation with fresh urine microscopy pyuria, epithelial cell count, the quality of life VAS for “Your health today” and the enhanced culture total colony counts. Using a Bonferroni correction (37 analyses, significance threshold 0.0014), no correlations remained significant. Whilst multiplicity should be considered when evaluating such findings, the convergence is plausible.

Table 10-22: Comparison of risk factors, tests and outcomes against standard culture (positive versus non-positive) using pooled data.

	Standard culture non-positive (n=244)	Standard culture positive (n=22)	Significance
Age (median, years) (95% CI)	34.3 (33.7-35.0)	34.3 (31.3-38.0)	MWU=2865 p=0.600
Parity (nulliparous) (%) (95% CI)	115/244 47.1% (41.0-53.4)	8/22 36.4% (19.7-57.0)	χ^2 p=0.332
BMI (median, kg/m ²) (95% CI)	23.8 (23.4-24.6)	22.5 (20.8-28.0)	MWU p=0.312
Ethnicity (non-white) (%) (95% CI)	155/244 63.5% (57.3-69.3)	11/22 50% (30.7-69.3)	χ^2 p=0.252
Social deprivation (lower 5 deciles) (%) (95% CI)	173/240 72.1% (66.1-77.4)	11/22 50% (30.7-69.3)	χ^2 p=0.048
Smoking (current smoker) (%) (95% CI)	16/242 6.6% (4.1-10.5)	0/22 0% (0-14.9)	χ^2 p=0.213
Dipstick (any of leu/pro/nit positive) (%) (95% CI)	165/244 (67.6%) (61.3-73.4)	16/22 (72.7%) (49.6-88.4)	χ^2 p=0.647
Fresh microscopy white cell count (median, μl^{-1}) (95% CI)	12 (8-18)	49 (8-104)	MWU=3632 p=0.006
Fresh microscopy red cell count (median, μl^{-1}) (95% CI)	2 (0-2)	1 (0-12)	MWU=2783 p=0.761
Fresh microscopy epithelial cell count (median, μl^{-1}) (95% CI)	12 (8-14)	41 (10-50)	MWU=3519 p=0.016
ATP (RLU) (95% CI)	5847 (5154-6857)	6800 (3733-14388)	MWU=3018 p=0.334
Total LUTS score /40 (median) (95% CI)	5 (5-6)	5 (3-13)	MWU=2768 p=0.928

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	Standard culture non-positive (n=244)	Standard culture positive (n=22)	Significance
EQ-5D-5L 'Your health today' (%) (95% CI)	80 (75-80)	65 (50-85)	MWU=1179 p=0.039
EQ-5D-5L index value Median (95% CI)	0.84 (0.84-0.85)	0.84 (0.66-1.00)	MWU=1639 p=0.951
Any bacteriuria present by sediment culture (%) (95% CI)	242/244 (99.2%) (96.8-99.9)	22/22 (100%) (81.5-100)	FE p=1.000
Sediment culture total colony counts (CFU/ml) (95% CI)	1583 (1010-2670)	51464 (884-220480)	MWU=3755 p=0.002
Number of isolates (95% CI)	3 (3-3)	3 (1-4)	MWU=2278 p=0.223
Total protein (median, µg/ml) (95% CI)	64.8 (58.7-71.6)	92.0 (36.3-120.5)	MWU=2807 p=0.723
IL-6 (median, pg/ml) (95% CI)	1.5 (1.2-2.1)	2.4 (0.5-5.6)	MWU=2789 p=0.761
IL-6 normalised for total protein (median, ng/g) (95% CI)	21 (17-27)	24 (9-56)	MWU=2775 p=0.792
IL-8 (median, pg/ml) (95% CI)	32.2 (20.4-41.0)	38.3 (20.4-140.2)	MWU=3117 p=0.210
IL-8 normalised for total protein (median, ng/g) (95% CI)	0.43 (0.33-0.53)	0.57 (0.28-1.61)	MWU=3117 p=0.210
Lactoferrin (median, ng/ml) (95% CI)	3.4 (2.2-4.7)	6.9 (2.2-16.4)	MWU=3192 p=0.113
Lactoferrin normalised for total protein (median, g/g) (95% CI)	71 (59-81)	95 (46-194)	MWU=3048 p=0.276
Presence of clue cells (%) (95% CI)	192/242 (79.3%) (73.6-84.2)	19/22 (86.4%) (64.0-96.4)	FE p=0.583

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	Standard culture non-positive (n=244)	Standard culture positive (n=22)	Significance
Gestation at delivery (days) (95% CI)	275 (273-277)	273 (264-276)	MWU=2010 p=0.130
Preterm birth (%) (95% CI)	21/240 (8.8%) (5.6-13.3)	1/21 (4.8%) (0.3-25.9)	FE p=1.000
Pyelonephritis in pregnancy (%) (95% CI)	0/244 (0%) (0-1.9)	0/22 (0%) (0-18.5)	n/a
Sepsis or possible sepsis intrapartum (%) (95% CI)	11/236 (4.7%) (2.5-8.4)	3/20 (15%) (4.0-38.9)	χ^2 p=0.051
Sepsis or possible sepsis postpartum (%) (95% CI)	18/236 (7.6%) (4.7-12.0)	2/20 (10%) (1.8-33.1)	χ^2 p=0.70
Sepsis or possible sepsis neonatal (%) (95% CI)	18/231 (7.8%) (4.8-12.2)	3/19 (15.8%) (4.2-40.5)	χ^2 p=0.23
Urinary symptoms at triage (%) (95% CI)	6/95 (6.3%) (2.6-13.8)	0/5 (0%) (0-53.7)	n/a
Clinician diagnosis included UTI (%) (95% CI)	19/88 (21.6%) (13.8-31.9)	1/4 (25%) (1.3-78.1)	χ^2 p=0.87
Shortest measured cervix (median, mm) (95% CI)	(n=65) 29 (28-30)	(n=2) 24.5 (15-34)	MWU=54.5 p=0.71
Any positive MSU in this pregnancy (%) (95% CI)	51/244 20.9% (16.3-26.4)	22/22 100% (85.1-100)	χ^2 p<0.001
Any mixed growth MSU in this pregnancy (%) (95% CI)	108/244 44.3% (38.2-50.5)	11/22 50% (30.7-69.3)	χ^2 p=0.382
Booking MSU shows mixed growth (%) (95% CI)	43/240 17.9% (13.6-23.3)	3/22 13.6% (4.8-33.3)	FE=3.13 p=0.381

A binomial logistic regression (Table 10-23) was performed to determine the effects of white cell count, epithelial cell count, enhanced culture colony counts and social deprivation score on the likelihood that participants would have a positive standard culture result. The quality of life VAS of 'your health today' was omitted as it was only applied to a subset of participants. The logistic regression model was statistically significant ($\chi^2=22.6$, $df = 4$, $p<0.001$). The model explained 18.8% (Nagelkerke R^2) of the variance in culture. $\log_{10}$ total CFU added significantly to the prediction ($p=0.005$) as did social deprivation score ($p=0.027$) however $\log_{10}$ white cell count ($p=0.196$) and $\log_{10}$ epithelial cell count ($p=0.618$) did not. Greater abundance colony counts on enhanced culture correlated with a positive culture. This is to be expected but supports a view that culture abundance was an important factor in Kass' pregnancy outcome predictions, as opposed to a simple positive/negative dichotomy.

Table 10-23: Logistic regression for standard culture positive versus non-positive

	B	SE	Wald	df	Significance	Odds ratio	95% CI for OR
$\log_{10}$ white cell count	0.510	0.39	1.68	1	0.196	1.67	0.77-3.61
$\log_{10}$ epithelial cell count	0.240	0.48	0.25	1	0.618	1.27	0.50-3.26
$\log_{10}$ total colony count	0.567	0.20	7.91	1	0.005	1.76	1.19-2.62
Index of multiple deprivation (0=lower half, 1=upper half)	1.059	0.48	4.88	1	0.027	2.88	1.13-7.38
Constant	-5.869	0.96	37.60	1	0.000	0.003	

So far in this analysis I have defined standard culture negative as including the three categories of screening culture negative, non-significant growth and mixed growth. This is a clinical interpretation, where a standard culture result is either positive or negative. To further explore the correlations with standard

culture, I repeated the analysis with all four categories of standard culture result.

There were 22 positive cultures, 33 mixed growth, 49 non-significant growth, and 162 screening culture negative. Table 10-24 shows the relationship of these results to the consilience variables.

There was a correlation with BMI, fresh microscopy white cell and epithelial cell counts, the quality of life VAS for "Your health today", sediment culture total colony counts, number of isolates, gestation at delivery, any mixed growth in the pregnancy, and mixed growth at booking. Using a Bonferroni correction (37 analyses, significance threshold 0.0014), BMI, total colony counts and number of isolates remained significant.

I have plotted the consilience variables total colony counts from the enhanced culture, fresh microscopy white cell count and epithelial cell count, and BMI, against the four categories of the standard culture results (Figure 10-14).

There is a correlation, and we shall return to this association with microbial abundance in the discussion.

Table 10-24: Comparison of risk factors, tests and outcomes against standard culture (positive versus mixed versus non-significant growth versus negative) using pooled data

	Negative (n=162)	Non- significant growth (n=49)	Mixed growth (n=33)	Positive (n=22)	Sig.
Age (median, years) (95% CI)	34.5 (33.6-35.1)	34.2 (33.0-36.0)	33.0 (31.2-36.3)	34.3 (31.3-38.0)	KW p=0.894
Parity (nulliparous) (%) (95% CI)	75/162 (46.3%) (38.5-54.3)	24/49 (49.0%) (34.6-63.5)	16/33 (48.5%) (31.2-66.1)	8/22 36.4% (19.7-57.0)	χ^2 p=0.782
BMI (median, kg/m ²) (95% CI)	23.1 (22.3-23.7)	25.0 (23.6-27.5)	27.1 (25.1-29.4)	22.5 (20.8-28.0)	KW p<0.001
Ethnicity (non- white) (%) (95% CI)	100/162 (61.7%) (53.7-69.2)	31/49 (63.3%) (48.3-76.2)	24/33 (72.7%) (54.2-86.1)	11/22 50% (30.7-69.3)	χ^2 p=0.393
Social deprivation (lower 5 deciles) (%) (95% CI)	117/161 (72.7%) (65.0-79.2)	33/47 (70.2%) (54.9-82.2)	23/32 (71.9%) (53.0-85.6)	11/22 50% (30.7-69.3)	χ^2 p=0.187
Current smoker (%) (95% CI)	9/160 (5.6%) (2.8-10.7)	5/49 (10.2%) (3.8-23.0)	2/33 (6.1%) (1.1-21.6)	0/22 0% (0-18.5)	FE p=0.464
Dipstick (any leu/pro/nit positive) (%) (95% CI)	106/162 (65.4%) (57.5-72.6)	37/49 (75.5%) (60.8-86.2)	22/33 (66.7%) (48.1-81.5)	16/22 (72.7%) (49.6-88.4)	χ^2 p=0.569
Fresh microscopy white cells (median, μl^{-1}) (95% CI)	10 (6-14)	20 (8-34)	24 (6-36)	49 (8-104)	KW p=0.003
Fresh microscopy red cells (median, μl^{-1}) (95% CI)	0 (0-2)	4 (0-8)	0 (0-8)	1 (0-12)	KW p=0.238
Fresh microscopy epithelial cells (median, μl^{-1}) (95% CI)	10 (8-14)	20 (10-36)	18 (6-60)	41 (10-50)	KW p=0.010
ATP (RLU) (95% CI)	5847 (5192-6921)	5131 (3530-8168)	6302 (4211-8475)	6800 (3733-14388)	KW p=0.779
Total LUTS score /40 (median) (95% CI)	5 (4-6)	6 (4-8)	5 (4-9)	5 (3-13)	KW p=0.857

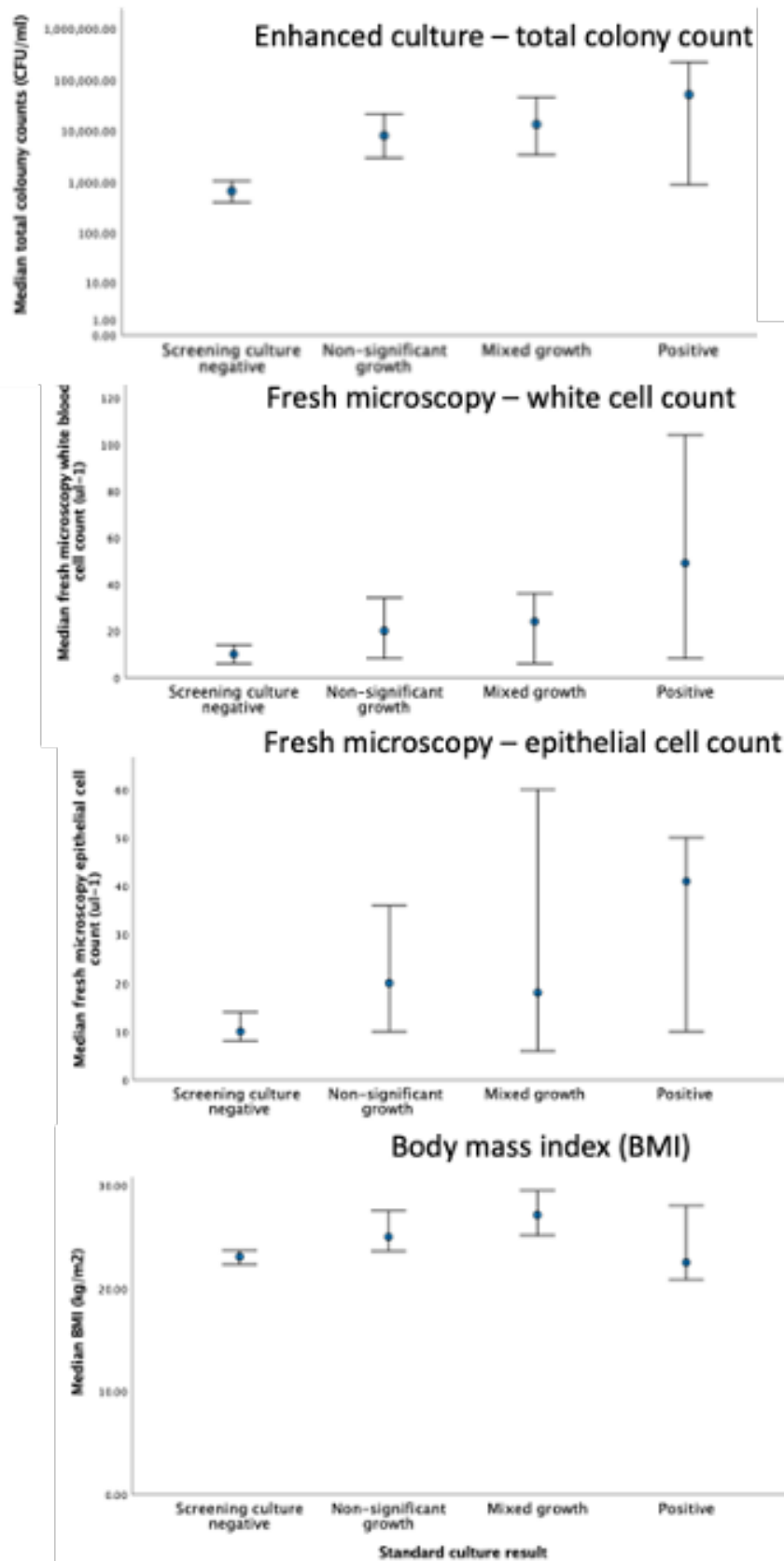
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	Negative (n=162)	Non- significant growth (n=49)	Mixed growth (n=33)	Positive (n=22)	Sig.
EQ-5D-5L 'Your health today' (%) (95% CI)	80 (80-85)	80 (70-84)	72.5 (70-85)	65 (50-85)	KW p=0.041
EQ-5D-5L index value Median (95% CI)	0.84 (0.83-0.88)	0.74 (0.70-0.88)	0.88 (0.84-1.00)	0.84 (0.66-1.00)	KW p=0.288
Any bacteriuria present by sediment culture (%) (95% CI)	160/162 (98.8%) (95.2-99.8)	49/49 (100%) (90.9-100)	33/33 (100%) (87.0-100)	22/22 (100%) (81.5-100)	FE p=0.630
Sediment culture total colony counts (CFU/ml) (95% CI)	666 (401-1051)	8123 (2987-21628)	13470 (3407-45882)	51464 (884-220480)	KW p<0.001
Number of isolates (95% CI)	3 (3-3)	3 (3-4)	3 (3-4)	3 (1-4)	KW p<0.001
Total protein (median, µg/ml) (95% CI)	63.0 (55.8-71.6)	60.0 (56.3-79.6)	84.2 (55.2-110.1)	92.0 (36.3-120.5)	KW p=0.427
IL-6 (median, pg/ml) (95% CI)	1.5 (1.1-2.1)	2.1 (0.9-3.6)	1.2 (0.6-3.0)	2.4 (0.5-5.6)	KW p=0.956
IL-6 normalised for total protein (median, ng/g) (95% CI)	24 (17-32)	22 (13-41)	13 (9-36)	24 (9-56)	KW p=0.689
IL-8 (median, pg/ml) (95% CI)	24.7 (16.9-46.4)	35.8 (17.0-49.5)	35.7 (18.0-83.4)	38.3 (20.4-140.2)	KW p=0.399
IL-8 normalised for total protein (median, ng/g) (95% CI)	0.36 (0.29-0.50)	0.58 (0.32-0.86)	0.44 (0.29-0.81)	0.57 (0.28-1.61)	KW p=0.427
Lactoferrin (median, ng/ml) (95% CI)	2.2 (2.2-4.2)	4.1 (2.2-6.0)	5.6 (2.2-9.4)	6.9 (2.2-16.4)	KW p=0.190
Lactoferrin normalised for total protein (median, g/g) (95% CI)	74 (57-85)	69 (49-104)	66 (45-108)	95 (46-194)	KW p=0.660
Presence of clue cells (%) (95% CI)	120/155 (77.4%) (69.9-83.6)	39/46 (84.8%) (70.5-93.2)	23/32 (71.9%) (53.0-85.6)	15/19 (79.0%) (53.9-93.0)	FE p=0.429

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	Negative (n=162)	Non- significant growth (n=49)	Mixed growth (n=33)	Positive (n=22)	Sig.
Gestation at delivery (days) (95% CI)	276 (274-279)	271 (265-274)	278 (273-283)	273 (264-276)	KW p=0.004
Preterm birth (%) (95% CI)	11/159 (6.9%) (3.7-12.4)	8/48 (16.7%) (8.0-30.8)	2/33 (6.1%) (1.1-21.6)	1/21 (4.8%) (0.3-25.9)	FE p=0.190
Spontaneous preterm birth (%) (95% CI)	5/158 (3.2%) (1.2-7.6)	5/48 (10.4%) (3.9-23.5)	0/33 (0%) (0-13.0)	0/21 (0%) (0-19.2)	FE p=0.092
Pyelonephritis in pregnancy (%) (95% CI)	0/157 (0%) (0-3.0)	0/46 (0%) (0-9.6)	0/33 (0%) (0-13.0)	0/20 (0%) (0-18.5)	n/a
Sepsis or possible sepsis intrapartum (%) (95% CI)	6/157 (3.8%) (1.6-8.5)	3/46 (6.5%) (1.7-18.9)	2/33 (6.1%) (1.1-21.6)	3/20 (15%) (4.0-38.9)	FE p=0.324
Sepsis or possible sepsis postpartum (%) (95% CI)	12/157 (7.6%) (4.2-12.3)	3/46 (6.5%) (1.7-18.9)	3/33 (9.1%) (2.4-25.5)	2/20 (10%) (1.8-33.1)	FE p=0.875
Sepsis or possible sepsis neonatal (%) (95% CI)	11/156 (7.1%) (3.8-12.6)	5/44 (11.4%) (4.3-25.4)	2/31 (6.5%) (1.1-22.8)	3/19 (15.8%) (4.2-40.5)	FE p=0.427
Urinary symptoms at triage (%) (95% CI)	3/66 (4.6%) (1.2-13.6)	3/18 (16.7%) (4.4-42.3)	0/11 (0%) (0-32.1)	0/5 (0%) (0-53.7)	FE p=0.179
Clinician diagnosis included UTI (%) (95% CI)	11/59 (18.6%) (10.1-31.3)	6/18 (33.3%) (14.4-58.8)	2/11 (18.2%) (3.2-52.2)	1/4 (25%) (1.3-78.1)	FE p=0.557
Shortest measured cervix (median, mm) (95% CI)	(n=46) 30 (28-31)	(n=14) 28 (26-33)	(n=5) 29 (25-32)	(n=2) 24.5 (15-34)	KW p=0.838
Any positive MSU in this pregnancy (%) (95% CI)	28/162 (17.3%) (12.0-24.2)	13/49 (26.5%) (15.4-41.3)	10/33 (30.3%) (16.2-48.9)	22/22 100% (85.1-100)	χ^2 p<0.001
Any mixed growth MSU in this pregnancy (%) (95% CI)	46/162 (28.4%) (21.7-36.1)	29/49 (59.2%) (44.3-72.7)	33/33 (100%) (87.0-100)	11/22 50% (30.7-69.3)	χ^2 p<0.001
Booking MSU shows mixed growth (%) (95% CI)	20/159 (12.6%) (8.0-19.0)	15/48 (31.3%) (19.1-46.4)	8/33 (24.2%) (11.7-42.6)	3/22 13.6% (4.8-33.3)	FE= p=0.018

Figure 10-14: Standard culture compared against enhanced culture, cell counts, and quality of life (median $\pm$ 95% confidence intervals)



I compared individual LUTS scores against standard culture (Figure 10-15). There were differences only in post-micturition dribbling ($p=0.013$), bladder pain unrelieved by voids ($p=0.045$) and pain radiating to legs ($p=0.043$). After applying a Bonferroni correction for multiplicity (threshold $p<0.0013$) none remained significant.

Figure 10-15: Individual LUTS compared with standard culture result

10.5.1.3 Discussion

There was very little to distinguish standard culture positive from non-positive, particularly in terms of urinary symptoms, ATP, urinary cytokines, presence of clue cells, and obstetric and neonatal outcomes. The only risk factors which differed were index of multiple deprivation, with more deprived postcodes associated with negative cultures. This is probably a spurious finding from the multiplicity of the data. The laboratory tests which correlated were fresh microscopy white cell and epithelial cell counts, and enhanced culture total colony counts, which were higher in samples that were standard culture positive. Women with positive culture were more likely to report a lower 'Your health today' VAS on quality of life assessment. Only higher socioeconomic status by postcode, and higher enhanced culture colony counts, remained predictive of positive standard culture in a logistic regression model.

There was no difference in total LUTS scores, however the difference in quality of life score may hint at a 'bothersome-ness' of urinary symptoms in UTI that might be present. Interestingly there was no difference between most individual LUTS. While studies in non-pregnant women have reported symptoms that are associated with UTI (Dune, Price et al. 2017), in this study there were only three symptoms that were different, one more common in MSU negative women. Dysuria was rare and was not associated with positive culture; this is an important finding since this may be treated as diagnostic in clinical practice (MacDermott 1994).

It is perhaps surprising that positive and negative standard cultures differed little in terms of symptomatology before treatment, with symptoms being common in both groups. It is not clear how this relates to what is often assumed to be *asymptomatic* bacteriuria. The role of women's symptoms in UTI in pregnancy warrants further research.

The lack of difference in outcomes is not surprising. The impact of a positive culture on outcome is likely to be minimal, even if pathophysiologically there is a link between UTI by standard culture and negative outcomes. This is because a positive culture is likely to be treated with antibiotics according to standard guidelines, as discussed previously.

The number of participants in the positive culture group was small at 22. It is possible that no differences were found owing to type II errors, particularly if effect sizes are small and variability is large. To power a study to compare positive cultures against negative cultures one would need to plan a controlled study where untreated positives were compared against negatives, ideally randomised if clinical equipoise permitted this trial to run.

The Kazemier study (Kazemier, Koningstein et al. 2015) had this design, and was powered based on what was thought to be the risk of pyelonephritis and preterm birth from untreated asymptomatic bacteriuria. At the outset of the trial, it was thought that the primary outcome would be detected in 10% of the treatment group and 25% in the placebo group. This gave a requirement of 110 in each group for 80% power and 5% type 1 error probability. To achieve that, it was calculated that 4400 women would need to be screened for asymptomatic bacteriuria.

Interim analysis after recruitment of a third of participants showed that the primary outcome was far rarer, occurring in 1.7%, because the rate of pyelonephritis was lower than predicted. It was calculated that 1900 women would need to be recruited to the study. Recruitment was thus stopped on safety grounds.

Currently UK practice is still to perform screening for asymptomatic bacteriuria, as described previously (2.5.2); the UK is not currently in clinical equipoise on this topic. An alternative approach would be an observational study of women with untreated mixed growth at booking compared with negative cultures at booking. If we hypothesised that untreated mixed growth carries the same risk of pyelonephritis as untreated positive cultures, then we could apply the Kazemier data (Kazemier, Koningstein et al. 2015) to predict the numbers with pyelonephritis (in that study 24/4035 (0.6%) bacteriuria-negative women had pyelonephritis compared with 5/208 (2.4%) untreated bacteriuria-positive). With an alpha of 0.05 and power of 80%, we would need 441 women in the mixed growth group and 1233 in the negative cultures group, with an enrolment ratio of 1:3 (mixed growth: negative cultures), which approximately reflects the proportions in this study (18.5% mixed growth versus 46.9% negative at booking). Such a study would thus need to recruit 1644 women. A

similar calculation could be used to compare results of urine cultures taken later in the pregnancy.

If the intention of comparing positive standard cultures with negative cultures was to compare the alternative tests, rather than clinical outcomes, then the sample size calculations would mirror those used for other parts of the study comparing colony counts, symptoms or white cell counts, which gave sample sizes of 20, 34 and 40 respectively (see section 0). We have seen that colony counts and white cell counts were higher in the positive culture group; a larger sample size of at least 34 in the positive culture group would be needed to demonstrate a difference in symptoms.

Given the predominant belief that positive cultures are associated with harm in pregnancy, it remains even less feasible to envisage an ethical study design that would confirm the maternal and fetal consequences of not treating a positive culture in a symptomatic or high risk woman in pregnancy.

It has been noted that the negative group included non-significant growth and mixed growth. This was a deliberate decision, as clinicians treat these results as equivalent to negative, in general. However, if there was a gradation in urinary pathology between negative, non-significant and mixed growth, then this inclusion could mask a difference with positive cultures, requiring an even larger sample size to disentangle.

The analysis with standard culture split into four categories was included to explore the ideas that (1) differences between non-positive and positive might be masked by including non-significant and mixed growth in non-positive and (2) there may be a spectrum of pathology from negative culture through non-significant growth and mixed growth. Interestingly, the correlations were almost identical, with a positive relationship with total colony counts, and fresh microscopy white cell and epithelial cell counts through the ordinal categories of culture result, a triangulation which is supportive of the concept of a spectrum of pathology.

There was a difference in distribution according to BMI, with an appearance that a higher BMI was associated with non-significant and mixed growth. This is interesting and requires further investigation. Raised BMI is associated with

urinary tract infection in pregnancy (Nohr, Timpson et al. 2009, Magann, Doherty et al. 2013). I was unable to find a study comparing mixed growth cultures with BMI. In this study there was no association seen with BMI and positive versus non-positive culture.

The correlation between standard culture and enhanced culture colony counts suggests that they are measuring similar things, with enhanced culture demonstrating the spectrum of microbial growth rather than forcing a dichotomy. Future work could extend the approach taken here, and explore in more detail the total bacterial burden irrespective of species as a marker of the need for treatment. This assumes a method to standardise the culture process; in this study care was taken to ensure minimal delay in culture, which would affect bacterial burden, but real-life laboratory transfer processes are more haphazard.

The replicating pattern of correlation between standard culture categories and white cell counts, epithelial cell counts, and total colony counts, with a reverse pattern of quality of life measurement, suggests that standard culture categories represent a continuum. If mixed growth and non-significant growth were solely markers of contamination, one would expect less consistent patterns of cell shedding and bacterial growth.

Positive standard culture is the gold standard against which all other tests are compared. When it was developed as a screening test in the era of Kass, the risk of pyelonephritis and morbidity was very high; in the modern era this risk is considerably lower. It is essential to re-evaluate this gold standard in the face of modern evidence. This will be considered in more detail in the discussion.

It should be noted, as with all the secondary analyses I have performed, that this is hypothesis-generating only and would need further research to test it. There are multiple comparisons, and so the risk of a chance association is high.

10.5.2 Comparison of standard and alternative tests compared with preterm birth as a secondary outcome

10.5.2.1 Methods

I compared risk factors, tests and outcomes and independent variables against preterm birth as the dependent in a secondary analysis (Table 10-25). I used preterm birth by any cause. Note that 46 women were term when recruited; these have been excluded from this analysis.

10.5.2.2 Results

There were several differences between those who delivered at 37 weeks or more (term) versus those who delivered before 37 weeks (pre-term), in addition to gestation at delivery ($p < 0.001$) and suspected neonatal sepsis ($p = 0.014$). These were: total colony count of enhanced culture ($p = 0.001$); EQ-5D-5L index value ($p = 0.008$); booking MSU showing mixed growth ($p = 0.008$); cervical cerclage ($p = 0.002$); any mixed growth during pregnancy ($p = 0.046$); and number of MSUs sent during pregnancy ($p = 0.015$).

Using a Bonferroni correction (46 analyses, significance threshold 0.0011), the only remaining difference was total colony count of enhanced culture (see Figure 10-16).

Table 10-25: Comparison of risk factors, tests and outcomes for term birth versus preterm birth using pooled data

	Term birth (n=199)	Preterm birth (n=23)	Significance
Age (median, years) (95% CI)	34.0 (33.2-35.1)	35.1 (33.7-38.3)	MWU p=0.272
BMI (median, kg/m ²) (95% CI)	23.5 (22.9-24.4)	24.8 (22.8-28.4)	MWU p=0.093
Multiparous (% multiparous) (95% CI)	101/199 (50.8%) (43.6-57.9)	13/23 (56.5%) (34.9-76.1)	χ^2 p=0.663
Current smoker (%) (95% CI)	13/198 (6.6%) (3.7-11.2)	2/22 (9.1%) (1.6-30.6)	FE p=0.651
Non-white ethnicity (%) (95% CI)	72/199 (36.2%) (29.6-43.3)	6/23 (26.1%) (11.1-48.7)	χ^2 p=0.369
Social deprivation (lower 5 deciles) (%) (95% CI)	136/196 (69.4%) (62.4-75.7)	19/22 (86.4%) (64.0-96.4)	χ^2 p=0.136
Previous UTI (%) (95% CI)	26/193 (13.5%) (9.1-19.3)	5/23 (21.7%) (8.3-44.2)	FE p=0.341
Dipstick (any of leu/pro/nit positive) (%) (95% CI)	129/199 (64.8%) (57.7-71.4)	17/23 (73.9%) (51.3-88.9)	χ^2 p=0.489
Fresh microscopy white cell count (median, μL^{-1}) (95% CI)	10 (8-16)	4 (0-12)	MWU p=0.425
Fresh microscopy red cell count (median, μL^{-1}) (95% CI)	0 (0-2)	2 (0-10)	MWU p=0.152
Fresh microscopy epithelial cell count (median, μL^{-1}) (95% CI)	12 (8-16)	10 (4-14)	MWU p=0.321
Standard lab culture positive (%) (95% CI)	11/196 (5.6%) (3.0-10.1)	1/22 (4.6%) (0.2-24.9)	FE p=1.000
ATP (median, RLU) 95% CI	6302 (5302-6998)	6967 (3825-9527)	MWU p=0.583

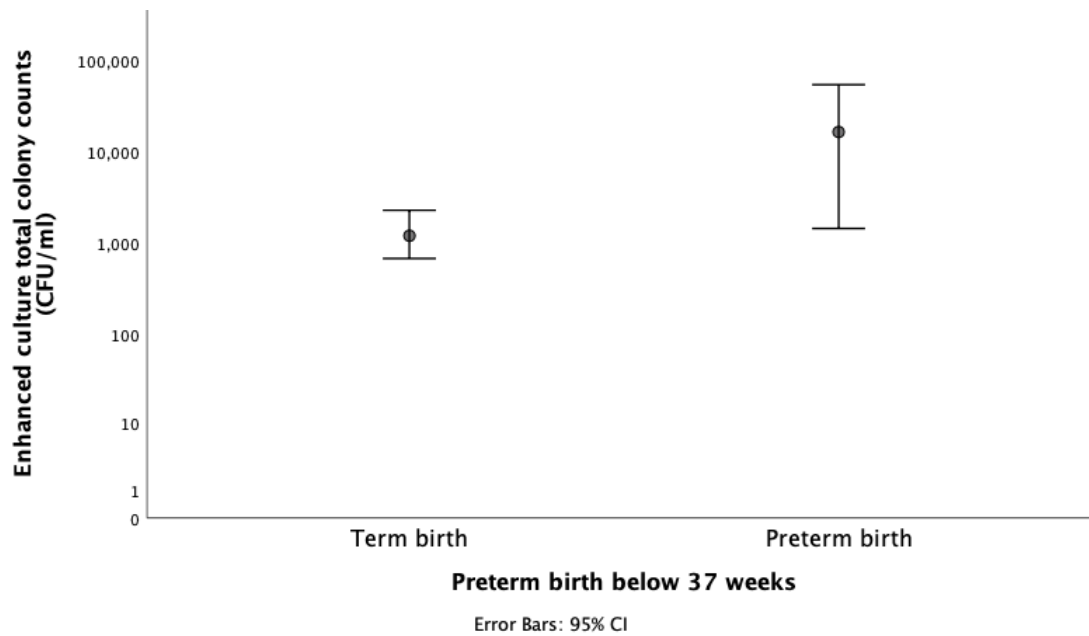
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	Term birth (n=199)	Preterm birth (n=23)	Significance
Any bacteriuria present by sediment culture (%) (95% CI)	197/199 (99.0%) (96.0-99.8)	23/23 (100%) (82.2-100)	F E p=1.000
Sediment culture total colony counts (CFU/ml) (95% CI)	1220 (688-2317)	16681 (1467-54812)	MWU p=0.001
Number of isolates (95% CI)	3 (3-3)	3 (3-3)	MWU p=0.955
Total LUTS score /40 (median) (95% CI)	6 (5-6)	5 (3-10)	MWU p=0.966
EQ-5D-5L 'Your health today' (%) (95% CI)	80 (75-80)	75 (50-87)	MWU p=0.342
EQ-5D-5L index value Median (95% CI)	0.837 (0.837-0.879)	0.725 (0.635-0.837)	MWU p=0.008
Total protein (median, µg/ml) (95% CI)	67.4 (58.7-78.8)	56.3 (40.2-68.2)	MWU p=0.172
IL-6 (median, pg/ml) (95% CI)	1.5 (1.2-1.9)	2.3 (0.5-7.2)	MWU p=0.560
IL-6 normalised for total protein (median, ng/g) (95% CI)	22 (16-27)	34 (10-77)	MWU p=0.256
IL-8 (median, pg/ml) (95% CI)	36.7 (23.4-51.5)	17.1 (12.9-55.6)	MWU p=0.605
IL-8 normalised for total protein (median, ng/g) (95% CI)	0.46 (0.33-0.62)	0.42 (0.28-0.70)	MWU p=0.900
Lactoferrin (median, ng/ml) (95% CI)	3.3 (2.2-4.8)	4.7 (2.2-10.6)	MWU p=0.448
Lactoferrin normalised for total protein (median, g/g) (95% CI)	69 (56-82)	85 (53-169)	MWU p=0.209
Presence of clue cells (%) (95% CI)	155/197 (78.7%) (72.2-84.1)	17/23 (73.9%) (51.3-88.9)	χ^2 p=0.791
Gestation at delivery (days) (95% CI)	276 (274-278)	239 (223-257)	MWU p<0.001
Sepsis or possible sepsis intrapartum (%) (95% CI)	9/196 (4.6%) (2.3-8.8)	3/21 (14.3%) (3.8-37.4)	χ^2 p=0.065

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	Term birth (n=199)	Preterm birth (n=23)	Significance
Sepsis or possible sepsis postpartum (%) (95% CI)	16/196 (8.2%) (4.9-13.2)	1/21 (4.8%) (0.3-25.9)	χ^2 p=0.581
Sepsis or possible sepsis neonatal (%) (95% CI)	12/193 (6.2%) (3.4-10.9)	4/18 (22.2%) (7.4-48.1)	χ^2 p=0.014
Urinary symptoms at triage (%) (95% CI)	4/81 (4.9%) (1.6-12.8)	2/7 (28.6%) (5.1-69.7)	FE p=0.070
Clinician diagnosis included UTI (%) (95% CI)	17/76 (22.4%) (13.9-33.7)	2/6 (33.3%) (6-75.9)	FE p=0.619
Shortest measured cervix (median, mm) (95% CI)	(n=59) 29 (28-30)	(n=10) 29 (27-35)	MWU p=0.871
Any positive MSU in this pregnancy (%) (95% CI)	54/199 (27.1%) (21.2-34.0)	6/23 (26.1%) (11.1-48.7)	$\chi^2=0.011$ p=1.000
Any mixed growth MSU in this pregnancy (%) (95% CI)	91/199 (45.7%) (38.7-52.9)	16/23 (69.6%) (47.0-86.0)	$\chi^2=4.692$ p=0.046
Booking MSU shows mixed growth (%) (95% CI)	32/197 (16.2%) (11.5-22.3)	9/23 (39.1%) (20.5-61.2)	FE p=0.019
Cerclage this pregnancy (%) (95% CI)	13/199 (6.5%) (3.7-11.2)	7/23 (30.4%) (14.1-53)	FE p=0.002
Progesterone this pregnancy (%) (95% CI)	15/199 (7.5%) (4.4-12.4)	3/23 (13.0%) (3.4-34.7)	FE p=0.420
Number of MSUs sent during pregnancy (95% CI)	3 (3-4)	4 (3-7)	MWU p=0.015
Any GBS positive swab throughout the pregnancy (%) (95% CI)	19/125 (15.2%) (9.6-23.0)	3/19 (15.8%) (4.2-40.5)	FE p=1.000

Figure 10-16: Preterm birth compared against enhanced culture total colony counts ($\log_{10}$ median $\pm$ 95% confidence intervals)



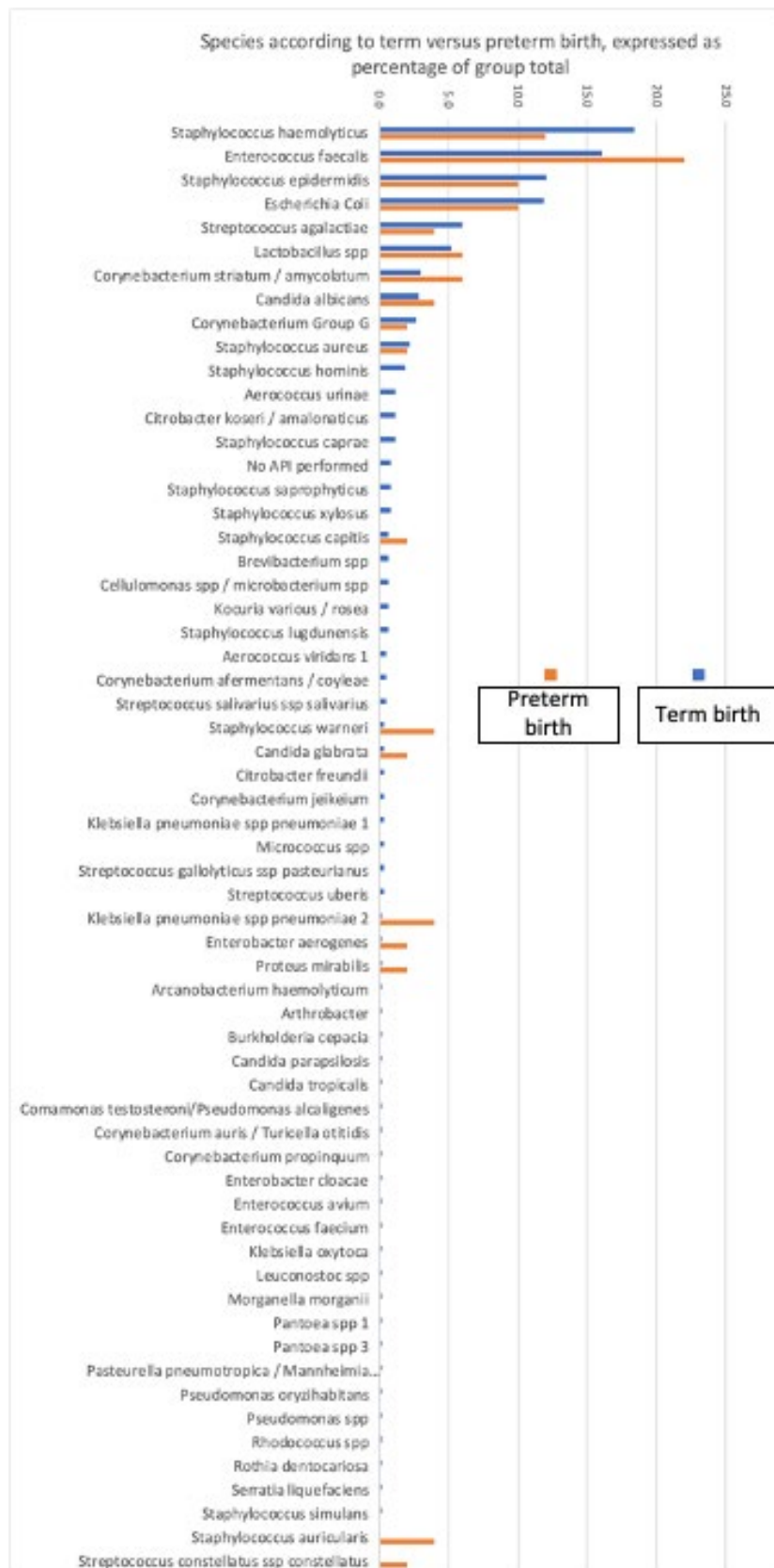
A binomial logistic regression (Table 10-26) was performed to determine the effects of enhanced culture colony counts, number of MSUs in pregnancy, any mixed growth culture in pregnancy, and booking culture showing mixed growth, on the likelihood that participants would have a preterm birth. The quality of life index value was omitted as it was only applied to a subset of participants. Having a cerclage was omitted as this was not an independent variable at the time of recruitment. The logistic regression model was statistically significant ($\chi^2=20.2$, $df = 4$, $p<0.001$). The model explained 18% (Nagelkerke R^2) of the variance in culture result. $\log_{10}$ total CFU added significantly to the prediction ($p=0.016$) as did number of MSUs in pregnancy ($p=0.015$) however the others did not. Higher $\log_{10}$ total CFU, and higher number of MSUs in pregnancy, were associated with an increased likelihood of a preterm birth. It should be noted that positive cultures were not more likely, so a higher number of MSU samples may reflect greater burden of pre-pregnancy urinary pathology, symptoms, or attendance at the preterm birth clinic where there is a high index of suspicion for urinary pathology.

Table 10-26: Logistic regression for term birth versus pre-term birth

	B	SE	Wald	df	Significance	Odds ratio	95% CI for OR
Any mixed growth culture in pregnancy	-0.133	0.62	0.05	1	0.829	0.88	0.26-2.92
Mixed growth at booking	1.079	0.59	3.34	1	0.068	2.94	0.93-9.36
Number of MSUs overall	0.215	0.09	5.93	1	0.015	1.24	1.04-1.47
log10 total colony count	0.493	0.20	5.86	1	0.016	1.64	1.10-2.44
Constant	-5.869	0.91	31.5	1	0.000	0.006	

I compared species identified at enhanced culture in women who had preterm birth, compared with women who had term births (Figure 10-17). Given the small numbers it is not possible to say if there is any meaningful difference in species between the two groups. Importantly, uropathogens and non-uropathogens were noted in both groups.

Figure 10-17: Comparison of enhanced culture isolates in preterm birth compared with term birth (pooled data)



The need for cervical cerclage could be considered a marker for very high risk of preterm birth. I therefore performed a secondary analysis, comparing women with cervical cerclage with those without. Of 20 women with cervical cerclage, 14 had already had it inserted at the time of recruitment, and 6 went on to have it inserted. I explored the relationship between cervical cerclage, timing of cervical cerclage in relation to recruitment to the study, and test results (Table 10-27). This was intended for hypothesis generation only.

This showed no differences in test results other than gestation at delivery being lower for those with cerclage, which might be expected in such a high-risk cohort. However, given the small numbers a lack of differences is not surprising. There was a trend that compared with those who were recruited after cerclage insertion, those who were recruited before cerclage insertion had higher enhanced culture cell counts, higher white cell and epithelial cell counts, and higher urinary cytokines. This is consistent with a recent study in which women with a treated positive culture prior to cerclage placement were more likely to deliver preterm than those with negative culture pre-cerclage, whereas there was no difference in women with positive versus negative cultures following cerclage placement (Kunpalin, Burul et al. 2020). The relationship between urinary pathology and cerclage warrants further assessment in future.

Table 10-27: Comparison of outcomes and test results according to cervical cerclage status

	Cerclage prior to recruitment (n=14)	Cerclage following recruitment (n=6)	No cervical cerclage (n=207)	Significance
PTB (%) (95% CI)	6/14 42.9% (21.4-67.4)	1/6 16.7% (3.0-56.4)	16/202 7.9% (4.9-12.5)	FE=12.79 p=0.001
Spontaneous PTB (%) (95% CI)	2/14 14.3% (4.0-40.0)	1/6 16.7% (3.0-56.4)	8/201 4.0% (2.0-7.7)	FE=5.37 p=0.081
Gestation at delivery in days (95% CI)	262 (184-288)	268 (237-291)	275 (273-277)	KW p=0.006
Standard culture positive (%) (95% CI)	0/12 0% (0-24.3)	0/6 0% (0-39.0)	13/192 6.8% (4.0-11.2)	FE p=1.000
Dipstick positive (%) (95% CI)	8/14 57.1% (32.6-78.6)	4/6 66.7% 30.0-90.3	138/207 66.7% (60-72.7)	FE p=0.796
Total colony counts (median CFU/ml) (95% CI)	4057 (306-44042)	7223 (9.6-1099395)	1371 (845-2670)	KW p=0.293
Total LUTS (median /40) (95% CI)	6.5 (1-14)	5 (2-32)	6 (5-6)	KW p=0.736
ATP (RLU) (95% CI)	8328 (3037-13709)	7444 (1041-13162)	6302 (5215-6967)	KW p=0.563
White cell count (median, μL^{-1}) (95% CI)	2 (0-22)	15 (0-100)	10 (8-16)	KW p=0.079
Red cell count (median, μL^{-1}) (95% CI)	2 (0-10)	2 (0-660)	2 (0-2)	KW p=0.929
Epithelial cell count (median, μL^{-1}) (95% CI)	5 (2-20)	18 (0-800)	12 (8-16)	KW p=0.302
IL-6 (median, pg/ml) (95% CI)	2.1 (0.4-11.3)	7.7 (0.1-56.9)	1.5 (1.2-2.0)	KW 0.283
IL-8 (median, pg/ml) (95% CI)	26.2 (5.4-102.8)	61.4 (1.4-23160)	35.2 (22.0-48.3)	KW 0.826
Lactoferrin (median, ng/ml) (95% CI)	4.2 (2.2-45.4)	5.3 (2.2-803.9)	3.5 (2.2-5.0)	KW 0.928
Clue cells (%) (95% CI)	13/14 (92.9%) (64.2-99.6)	6/6 (100%) (51.7-100)	158/204 (77.5%) (71.0-82.9)	FE 0.260

10.5.2.3 Comparison of term births with spontaneous preterm births

As discussed previously, preterm birth is a syndrome with multiple phenotypes. One distinction is between medically-indicated preterm birth and spontaneous preterm birth, which includes spontaneous preterm labour and preterm prelabour rupture of membranes (PPROM). Although I initially planned to compare all preterm births against all term births, I have separated spontaneous from medically-indicated and performed a repeat analysis of that described above (10.5.2.2).

I have compared risk factors, tests and outcomes for term births against spontaneous preterm birth (Table 10-28). Of the 23 preterm births, 11 were known to be spontaneous.

Findings are similar to those described for all types of preterm birth in Table 10-25. There were significant differences in enhanced culture total colony counts ($p=0.021$), and mixed growth at booking ($p=0.028$). There were significant differences in gestation at delivery ($p<0.001$), which would occur by definition, and significant differences in possible intrapartum sepsis ($p<0.001$) and possible neonatal sepsis ($p<0.001$), again consistent with spontaneous preterm birth. Cerclage in the current pregnancy was commoner in the spontaneous preterm birth group ($p=0.041$).

Using a Bonferroni correction (46 analyses, significance threshold 0.0011), the only remained differences were gestation at delivery and possible intrapartum and neonatal sepsis.

Table 10-28 Comparison of risk factors, tests and outcomes for term birth versus *spontaneous* preterm birth using pooled data

	Term birth (n=199)	<i>Spontaneous</i> preterm birth (n=11)	Significance
Age (median, years) (95% CI)	34.0 (33.2-35.1)	35.1 (29.9-38.3)	MWU p=0.449
BMI (median, kg/m ²) (95% CI)	23.5 (22.9-24.4)	24.1 (21.1-30.4)	MWU p=0.548
Multiparous (% multiparous) (95% CI)	101/199 (50.8%) (43.6-57.9)	7/11 (63.6%) (31.6-87.6)	χ^2 p=0.540
Current smoker (%) (95% CI)	13/198 (6.6%) (3.7-11.2)	0/11 (0%) (0.0-32.1)	FE p=1.000
Non-white ethnicity (%) (95% CI)	72/199 (36.2%) (29.6-43.3)	3/11 (27.3%) (7.3-60.7)	FE p=0.750
Social deprivation (lower 5 deciles) (%) (95% CI)	136/196 (69.4%) (62.4-75.7)	9/11 (81.8%) (47.8-96.8)	FE p=0.511
Previous UTI (%) (95% CI)	26/193 (13.5%) (9.1-19.3)	2/11 (18.2%) (3.2-52.2)	FE p=0.650
Dipstick (any of leu/pro/nit positive) (%) (95% CI)	129/199 (64.8%) (57.7-71.4)	6/11 (54.6%) (24.6-81.9)	FE p=0.527
Fresh microscopy white cell count (median, μl^{-1}) (95% CI)	10 (8-16)	4 (0-40)	MWU p=0.111
Fresh microscopy red cell count (median, μl^{-1}) (95% CI)	0 (0-2)	0 (0-16)	MWU p=0.929
Fresh microscopy epithelial cell count (median, μl^{-1}) (95% CI)	12 (8-16)	4 (0-20)	MWU p=0.090
Standard lab culture positive (%) (95% CI)	11/196 (5.6%) (3.0-10.1)	0/10 (0%) (0.0-32.5)	FE p=1.000
ATP (median, RLU) 95% CI	6302 (5302-6998)	8016 (3649-9527)	MWU p=0.468

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	Term birth (n=199)	Spontaneous preterm birth (n=11)	Significance
Any bacteriuria present by sediment culture (%) (95% CI)	197/199 (99.0%) (96.0-99.8)	11/11 (100%) (67.9-100)	FE p=1.000
Sediment culture total colony counts (CFU/ml) (95% CI)	1220 (688-2317)	16681 (994.3-103776.8)	MWU p=0.021
Number of isolates (95% CI)	3 (3-3)	3 (2-3)	MWU p=0.341
Total LUTS score /40 (median) (95% CI)	6 (5-6)	5 (2-12)	MWU p=0.967
EQ-5D-5L 'Your health today' (%) (95% CI)	80 (75-80)	80 (50-95)	MWU p=0.996
EQ-5D-5L index value Median (95% CI)	0.837 (0.837-0.879)	0.725 (0.500-1.000)	MWU p=0.160
Total protein (median, µg/ml) (95% CI)	67.4 (58.7-78.8)	41.3 (28.3-106.3)	MWU p=0.163
IL-6 (median, pg/ml) (95% CI)	1.5 (1.2-1.9)	2.3 (0.3-11.4)	MWU p=0.486
IL-6 normalised for total protein (median, ng/g) (95% CI)	22 (16-27)	60 (6-182)	MWU p=0.183
IL-8 (median, pg/ml) (95% CI)	36.7 (23.4-51.5)	16.2 (10.8-55.6)	MWU p=0.195
IL-8 normalised for total protein (median, ng/g) (95% CI)	0.46 (0.33-0.62)	0.40 (0.13-0.82)	MWU p=0.440
Lactoferrin (median, ng/ml) (95% CI)	3.3 (2.2-4.8)	2.2 (2.2-8.3)	MWU p=0.392
Lactoferrin normalised for total protein (median, g/g) (95% CI)	69 (56-82)	78 (32-152)	MWU p=0.943
Presence of clue cells (%) (95% CI)	155/197 (78.7%) (72.2-84.1)	9/10 (90%) (54.1-99.5)	FE p=0.691
Gestation at delivery (days) (95% CI)	276 (274-278)	231 (138-251)	MWU p<0.001
Sepsis or possible sepsis intrapartum(%) (95% CI)	9/196 (4.6%) (2.3-8.8)	3/9 (33.3%) (9.0-69.1)	χ^2 p<0.001

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	Term birth (n=199)	<i>Spontaneous</i> preterm birth (n=11)	Significance
Sepsis or possible sepsis postpartum (%) (95% CI)	16/196 (8.2%) (4.9-13.2)	1/9 (11.1%) (0.6-49.3)	χ^2 p=0.754
Sepsis or possible sepsis neonatal (%) (95% CI)	12/193 (6.2%) (3.4-10.9)	4/9 (44.4%) (15.3-77.3)	χ^2 p<0.001
Urinary symptoms at triage (%) (95% CI)	4/81 (4.9%) (1.6-12.8)	1/4 (25%) (1.3-78.1)	FE p=0.219
Clinician diagnosis included UTI (%) (95% CI)	17/76 (22.4%) (13.9-33.7)	0/4 (0%) (0-60.4)	FE p=0.573
Shortest measured cervix (median, mm) (95% CI)	(n=59) 29 (28-30)	(n=5) 29 (18-40)	MWU p=1.000
Any positive MSU in this pregnancy (%) (95% CI)	54/199 (27.1%) (21.2-34.0)	2/11 (18.2%) (3.2-52.2)	FE p=0.731
Any mixed growth MSU in this pregnancy (%) (95% CI)	91/199 (45.7%) (38.7-52.9)	8/11 (72.7%) (39.3-92.7)	$\chi^2=4.692$ p=0.120
Booking MSU shows mixed growth (%) (95% CI)	32/197 (16.2%) (11.5-22.3)	5/11 (45.5%) (18.1-75.4)	FE p=0.028
Cerclage this pregnancy (%) (95% CI)	13/199 (6.5%) (3.7-11.2)	3/11 (27.3%) (7.3-60.7)	FE p=0.041
Progesterone this pregnancy (%) (95% CI)	15/199 (7.5%) (4.4-12.4)	1/11 (9.1%) (0.5-42.9)	FE p=0.850
Number of MSUs sent during pregnancy (95% CI)	3 (3-4)	4 (2-9)	MWU p=0.148
Any GBS positive swab throughout the pregnancy (%) (95% CI)	19/125 (15.2%) (9.6-23.0)	2/10 (20%) (3.5-55.8)	FE p=0.654

10.5.2.4 Discussion

In all of these comparisons, the low rate of preterm birth restricted sampling. This is a common problem in studies of preterm birth. If an enriched sample is used which selects women particularly at risk the findings may not be generalizable to the population. The rate of preterm birth amongst primiparous women without apparent risk factors is around 6% (Langhoff-Roos, Kesmodel et al. 2006). Being able to predict preterm birth in low risk women would be a great advance in preterm birth prevention.

The higher median total colony count on enhanced culture is an intriguing finding, particularly since there was no difference in the rate of positive standard cultures. This warrants further exploration. It fits with the other difference that booking culture showing mixed growth was around twice as common among those who delivered preterm, although this was not significant once a Bonferroni correction was applied. Mixed growth is a confusing finding with inconsistent management and has largely been excluded from UTI studies as it has been assumed to be contamination.

The mixed growth being linked to preterm birth but a significant single species culture not being associated may be explained by the enhanced culture data. These showed microbial abundance to be associated with preterm birth and mixed growth would be a manifestation of that situation. However, there is another matter, positive cultures would have been labelled as UTI and treated, whereas mixed growth would have been rejected as contaminated. If that is the case, the data contradict the assumption that mixed growth is a benign event. Unfortunately, we did not collect the data necessary to analyse the occurrence of antibiotic interventions. This should be incorporated in future studies.

A previous study of subjects attending a clinic for those with increased risk of preterm birth found that mixed growth at booking was associated with lower gestation at delivery (Nwokoro 2013). The current study includes low risk women, but the findings are similar.

The additional analysis of spontaneous preterm birth versus term birth was limited by the low numbers with spontaneous preterm birth (n=11). It showed

similar findings as the original analysis using preterm birth by all causes ($n=23$), including the rate of mixed growth at booking being higher in those with spontaneous preterm birth compared with term birth. However the application of the Bonferroni correction meant that no differences remained significant. Larger numbers would be needed to explore these findings with more certainty.

10.6 Discussion and conclusions

In this chapter I pooled the MSU data from the Abdominal pain, PTB and MSU-CSU studies, to: (1) describe the relationship of tests to each other; (2) compare alternative tests against standard culture; (3) compare tests against clinical outcomes. In addition, these pooled data would add to the literature about urinary tests in pregnancy, particularly for tests that had not been performed before in pregnancy, including enhanced sediment culture, urinary ATP, urinary lactoferrin, and urinary clue cells.

There was some clinical heterogeneity in the three pooled studies, whilst methodological heterogeneity was minimised by using parallel study designs. The clinical heterogeneity may be a positive feature, since urinary tests are applied to all pregnant women, and understanding their performance across a range of settings may aid in generalisability. If collecting additional data with further parallel-methods studies, a statistical assessment of heterogeneity could be performed.

There was a positive relationship between white cell counts by standard microscopy and fresh unspun microscopy, and categories of dipstick leucocyte esterase. There was a similar relationship between epithelial cell counts by standard microscopy and fresh microscopy. There was a positive relationship between dipstick protein and total protein.

While dipstick has been discredited as a predictor of UTI, either by standard culture or in chronic LUTS patients as discussed previously, this does suggest that a nuanced interpretation of dipstick might be helpful in the absence of fresh microscopy. Importantly, negative dipstick could be associated with pyuria, so could not be used for ruling-out UTI. The correlation with white cell count could help by measuring treatment response.

Enhanced culture showed bacteriuria in nearly all samples. There was a positive relationship between enhanced culture colony counts and quantitative categories of the standard culture report. Standard culture positive organisms were all identified in paired enhanced culture isolates, as well as additional organisms. Enhanced culture identified GBS in many participants in whom it was not detected by standard tests.

These findings support the hypothesis that urine of pregnant women is not sterile. Enhanced culture is able to detect live organisms in a way that standard culture does not. This calls into question the validity of standard culture reports. The ability to identify GBS in polymicrobial cultures where it has not been identified by other tests is worrying; the implications for neonates are not known.

LUTS were very common. There were correlations between LUTS categories, and a negative correlation between total symptom scores and quality of life scores, but total symptom scores did not correlate with other urinary markers.

ATP did not correlate with any other measures. Total colony counts had weak correlation with cell counts and cytokines.

Fresh microscopy white cell and epithelial cell counts correlated positively with urinary cytokines, particularly IL-8. This is consistent with previous studies relating cytokine release with white cells and urothelial cells (Hedges and Svanborg 1994). Lactoferrin and IL-8 were particularly strongly correlated with each other. While lactoferrin has previously been considered as a marker for UTI, it has not been studied in pregnancy, and the relationship with IL-8 has not been described before.

Correlation does not allow conclusions about causation. It is a non-nuanced way to explore relationships between markers. A machine-learning approach in a much bigger study may be more appropriate to understand the complex relationships between these markers.

Using standard culture as a gold standard for UTI, none of the alternative tests performed as well as a surrogate marker for UTI. The only differences between non-positive and positive culture groups were white cell and epithelial cell

counts, total colony counts at enhanced culture, and one marker of quality of life; none of these remained significant after a Bonferroni correction.

This suggests either that none of the alternative tests are able to replace standard culture, or that standard culture is not adequate as a gold standard for UTI diagnosis.

Using the outcome of preterm birth there were few differences between groups. Total colony count, a marker of quality of life, mixed growth at booking, cervical cerclage, mixed growth during pregnancy, and number of MSUs sent during pregnancy were all different. Only total colony counts remained significant after Bonferroni correction.

It is important to remember that comparison against the outcome of pyelonephritis was planned, but not possible as there were no cases in this study.

One interpretation of these results may be that preterm birth is associated with a degree of bacteriuria, insufficient to trigger antibiotic treatment using current guidelines. It may be that those with positive standard cultures have an increased risk of preterm birth, however are being given antibiotics which mitigates that risk. Women with bacteriuria that does not meet treatment threshold may be more vulnerable to preterm birth for some reason. Number of MSUs sent in pregnancy may be a reflection on recurrent mixed growth, or a woman who is reporting urine symptoms, or reflect the practice in the preterm birth clinic of sending more MSUs in women at risk of preterm birth. In this study, insufficient data was collected about use of antibiotics throughout the pregnancy so an analysis based on treatment could not be performed. A larger study would be needed to explore these findings in more depth. There is sufficient evidence here to drive a large study looking at the implications of 'mixed growth', including parallel analyses using enhanced culture and sequencing methods to understand the contents of the mixed growth, as well as analysis of antibiotic use.

These are all secondary analyses, and while a comparison against standard culture and preterm birth were planned, the specifics of this analysis were not detailed at the outset. A Bonferroni correction was applied to reduce the risk of

false positive findings. The use of Bonferroni is controversial, as it is particularly conservative and increases the false negative rates (Perneger 1998).

There is a risk of inappropriately identifying findings on the basis of this, given there were fewer than 300 participants. Initially I planned to analyse using the outcomes of urosepsis, neonatal outcomes, and antibiotic treatment, however numbers were too small to warrant this. The data have been collected and would be useful for future systematic review and meta-analysis taking into account outcomes determined to be important by a core outcome set approach.

Having analysed the three studies individually, and pooled data from the three studies, I was able to address my research questions. My final aim was to work towards an alternative understanding of urinary pathology in pregnancy using a different perspective to the classical microbiologically-centred view. To do this I revisited my literature review, focusing on assumptions about UTI in pregnancy, and reconsidered them in light of the data from these studies. In the next chapter I will describe this analysis, and critically appraise the alternative tests applied.

11 Discussion

11.1 Introduction

The final aim for this thesis was to work towards an alternative understanding of urinary pathology in pregnancy different to the classical culture-centred perspective, taking inspiration from Bayesian approaches, the damage-response framework, and outcomes-based approaches. This results from the modern recognition of flaws in urine culture. I shall first summarise what has been published on the importance of UTI in pregnancy since this study began. I will then revisit the assumptions underlying UTI diagnosis in pregnancy, and discuss how my results shed more light on these. Finally, I shall critically appraise, and discuss the utility of, the alternative tests that I used in this study.

11.2 Importance of research into urinary pathology in pregnancy

11.2.1.1 Introduction

Recent research continues to show that urinary pathology in pregnancy is a significant problem in terms of maternal and neonatal risks. There are also legitimate concerns about antibiotic use.

11.2.2 Urinary pathology in pregnancy and maternal conditions

11.2.2.1 Pyelonephritis and sepsis

A single-centre cohort study of 110 cases of pyelonephritis in pregnancy over 6 years found that morbidity could not be predicted by fever at the time of presentation to hospital (DeYoung, Whittington et al. 2019). In particular, there was no difference in admission to intensive care, or occurrence of adult respiratory distress syndrome in those with fever compared with those without fever. Only 46% had positive urine cultures. The commonest risk factor was prior UTI in the pregnancy. The preterm birth rate in this study was 13/88 (14.8%). These data emphasise that pyelonephritis is potentially dangerous, incompletely understood, and not necessarily correlated with positive cultures.

A multi-centre study of 123 cases of pyelonephritis in pregnancy over 2 years found that one fifth of cases required hospital admission of at least five days, with 6/123 (4.9%) requiring intensive care (Valent, Peticca et al. 2017). 14% had negative cultures and 19% had mixed growth which was assumed to imply contamination. This study also demonstrated the seriousness of pyelonephritis in pregnancy, and dissociation from standard cultures.

11.2.2.2 Pre-eclampsia

UTI was found to be associated with pre-eclampsia in a US cohort study (Easter, Cantonwine et al. 2016). After controlling for confounders, UTI in pregnancy was associated with an odds ratio for pre-eclampsia of 3.2 (95% CI 2.0-5.1). A 2018 meta-analysis showed that UTI during pregnancy was a risk factor for the development of pre-eclampsia (odds ratio 1.3, 95% CI 1.2-1.4) (Yan, Jin et al. 2018). This is an outcome that has not been explored in the current study but should be included in future research and considered as a

core outcome. Pre-eclampsia is important not only as a serious obstetric condition with significant maternal and neonatal morbidity, but also because it may lead to medically-indicated preterm birth, which would confound the relationship between UTI and spontaneous preterm birth.

11.2.3 Urinary pathology in pregnancy and fetal/neonatal conditions

11.2.3.1 Preterm birth and low birthweight

Recent studies continue to sow confusion over the relationship between UTI and preterm birth.

A database study of >2,000,000 pregnant women in the USA between 2007-2012 found that UTIs were associated with an increased risk of preterm birth, particularly spontaneous preterm birth, from 6.7% without any UTI to 12.3% with UTI (aRR 1.4, 95% confidence interval 1.3-1.5) (Baer 2019). This study used emergency presentations for UTI, and this implies that the index cases had symptomatic UTI.

A single-centre cohort study of women undergoing cervical cerclage found that UTI in the first trimester before cervical cerclage was associated with increased chance of preterm birth after cerclage, even when the UTI was treated (Kunpalin, Burul et al. 2020). This did not apply for UTI diagnosed following cerclage.

A cluster-randomised controlled trial in Bangladesh, found no benefit of introducing a screening and treatment programme for vaginal and urinary tract infections between 13-19 weeks on preterm birth rates (Lee, Mullany et al. 2019). This study analysed nearly 8000 pregnancy outcomes. The rate of UTI was 8.6% overall. UTI was defined by culture of a single uropathogen of either high-burden (10^5 CFU/ml) or intermediate growth (10^3 - 10^5 CFU/ml). This study was analysing on culture result and not symptoms.

In the Bangladeshi study preterm birth incidences were 21.8% in the intervention group compared with 20.6% in the control group (relative risk 1.07, 95% CI 0.9-1.2). Around 15% of urine samples were excluded because of contamination. The treatment for UTI was initially 3 days of cefixime, although this was switched to 7 days of nitrofurantoin owing to high levels of antibiotic

resistance locally. There was a clearance of the culture in 71% of those found positive. There were no differences in rates of clinical UTI after 20 weeks, pyelonephritis, or babies that were small for gestational age.

It is suggested that the inability of an intervention trial of this size to show a benefit to screening for and treating UTI in the first half of pregnancy, demonstrates that there is no causative link between significant bacteriuria and preterm birth (McClure and Goldenberg 2019). However, in this study the rate of preterm birth is far higher than in the UK, and the structure of care is dramatically different. Compliance with treatment was poor. It is surprising that there was no difference in pyelonephritis rates given that this association has been consistently found in many other studies.

A systematic review and meta-analysis has shown that GBS bacteriuria is associated with preterm birth (Bianchi-Jassir, Seale et al. 2017).

11.2.3.2 Neonatal or childhood impact of UTI in pregnancy

Periconceptual UTIs (in the month prior to pregnancy and to end of first trimester) were associated with birth defects in a recent large birth defect registry (Howley, Feldkamp et al. 2018). It is not clear if this association was related to the infection, the treatment, or associated risk factors. However, this demonstrates the importance of including outcomes in all pregnancy-related UTI studies. UTI was associated with congenital cataracts, cleft lip, duodenal atresia, small intestinal atresia, colonic atresia, encephalocele, renal agenesis / hypoplasia, and some cardiac defects.

UTI in pregnancy was also associated with increased risk of hospitalisation for infectious pathology in childhood in a single-centre cohort study including nearly 250,000 deliveries (Cohen, Gutvirth et al. 2019). Using a similar dataset, UTI in pregnancy was associated with long-term neurological morbidity in offspring, after adjusting for gestation at birth (Mazurksy 2020).

11.2.4 Issues with antibiotics

Globally, antibiotic resistance is a concern. In 2017 the World Health Organisation (WHO) published a list of twelve priority pathogens to drive research into alternative treatments. The critical priority includes carbapenem-

resistant and third generation cephalosporin-resistant *Enterobacteriaceae*, a group which includes *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus* spp., which are all known uropathogens (WHO 2017). WHO's recommendation to improve antimicrobial stewardship aims to avoid inappropriate antibiotic prescribing, which is dependent on accurate diagnostic tests.

Along the theme of reducing antimicrobial resistance, the Longitude Prize is a £10million reward for development of "point-of-care diagnostic test that will conserve antibiotics for future generations" (Longitude-Prize 2020). This is a noble concept, however it risks being driven by the current gold standard of standard culture, rather than reconsidering the accepted methods of diagnosing UTI.

There is recent evidence of birth defects associated with macrolide antibiotic use in the first trimester of pregnancy (Fan, Gilbert et al. 2020). While this would be an unusual choice of antibiotics for UTI, it demonstrates the caution required with prescribing in the first trimester, and the need to ensure benefits outweigh the risks.

There is evidence that antibiotics during pregnancy are associated with increased asthma susceptibility in children (Zhao, Su et al. 2015). Antibiotics in third trimester were associated with asthma by age 5 in a study using sibling controls (Mulder, Pouwels et al. 2016). A study of >200,000 mother-child pairs found antenatal antibiotics were associated with increased risk of asthma in children (Loewen, Monchka et al. 2018). Another study of >130,000 mother-child pairs found antenatal UTI was associated with increased risk of childhood asthma, with a dose-response relationship; the authors attributed this effect to maternal antibiotic use (Wu, Feldman et al. 2016). This relationship has been causally demonstrated in a mouse model (Alhasan, Cait et al. 2020).

There is also evidence emerging of the effect of antibiotics in pregnancy on development of the neonatal microbiome (Nogacka, Salazar et al. 2017). The long-term implications of this are not yet known.

11.2.5 Conclusion

A recent systematic review of the benefits and harms of screening for and treating asymptomatic bacteriuria in pregnancy concluded that there was insufficient data to examine the risk of harms (Angelescu, Nussbaumer-Streit et al. 2016). In a different systematic review of asymptomatic bacteriuria in pregnancy screening and treatment including patient preferences, authors found that patient opinions varied widely and identified their understanding of risks and benefits as an area of inadequate research (Wingert, Pillay et al. 2019).

The spectrum of urinary pathology in pregnancy carries significant morbidity; treating it is not without risk. It is essential that the diagnostic approach to UTI is fit for purpose to maximise benefit while minimising harm.

11.3 Returning to the assumptions underlying diagnosis of UTI

11.3.1 Introduction

In chapter 3.2 I hypothesised that assumptions are made when diagnosing UTI in pregnancy, and I challenged them. Here I will revisit each in turn, using results from this study and from more recently published studies.

11.3.2 Assumption 1: Altered urinary symptoms in pregnancy can nearly always be attributed to the pregnancy

11.3.2.1 Introduction

My original literature search found a gap in the role of LUTS in diagnosing UTI in pregnancy, and whether any specific symptoms are more likely to be associated with UTI.

11.3.2.2 Data from this study

There was no difference in the presence of LUTS between women with a positive standard culture (21/24, 87.5%) versus those with a negative standard culture (245/273, 89.7%, $p=1.0$). There was no difference in total LUTS between women with a positive culture (median 5, 95% CI 3-11) versus a negative culture (median 5, 95% CI 4-6) ($p=0.664$). There were only 24 participants with positive cultures, so the risk of a type 2 error is substantial. As described above (10.5.1.3), at least 34 participants in each group would be required to demonstrate a difference in LUTS.

There was no clinically meaningful correlation between symptoms and other markers studied, whether microbiological or immunological markers.

It was striking that a wide range of LUTS was prominent across all studies. While the range was from 0-32 (maximum 40), only 23/273 (8.4%) reported no LUTS at all. In the normal controls (apparently asymptomatic at recruitment), only 4/51 (7.8%) reported no LUTS.

This high prevalence of LUTS in apparently asymptomatic women is problematic for the definition of asymptomatic bacteriuria. Without detailed symptoms assessment, research on asymptomatic bacteriuria in pregnancy

may not be accurate. Urinary pathology may be more prevalent than previously thought, but whether this is pathology or physiology is not clear.

One suggestion was to redefine UTI according to relevant clinical outcomes. LUTS distributions did not vary when compared to preterm birth.

One potential criticism is that the Artemis LUTS questionnaire did not assess how bothersome symptoms were. Perhaps non-bothersome symptoms are physiological rather than pathological. The questionnaire specifically did not include a bothersome rating, to avoid adjectival scaling and maintain objectivity (Khasriya, Barcella et al. 2018).

I analysed quality of life alongside LUTS to explore the impact of symptoms. There was a weak negative correlation overall between total LUTS and quality of life scores, but no way to know if this is a consequence of the symptoms or other confounding factors.

No individual LUTS correlated with standard culture positivity. The study was not powered to look at individual LUTS, so their predictive role cannot be commented on. This is an argument for all UTI in pregnancy studies to include symptom questionnaires so that this can be combined in meta-analysis. But there is a more pressing concern in that we do not seem to have identified a symptomatic phenotype that presages dangerous UTI.

11.3.2.3 Newer contributions from the literature

The study that validated the Artemis LUTS inventory found that symptoms in chronic LUTS patients, particularly voiding symptoms, correlated with microscopic pyuria and measures of quality of life (Khasriya, Barcella et al. 2018).

A study that attempted to define UTI in broader ways than standard culture with Kass criteria (self-report by patient, standard culture with 10 CFU/ml or greater, and enhanced culture with 10 CFU/ml or greater, using transurethral catheter specimens), found that dysuria was better than frequency or urgency for predicting UTI (Dune, Price et al. 2017). This was in urogynaecology patients. We remain unaware of a study in pregnancy that has tried to assess the utility of LUTS to predict UTI since 1994 (MacDermott 1994).

11.3.2.4 Conclusions

Urinary symptoms, detected using the Artemis LUTS inventory, are common in pregnancy, but to what extent this is pathological is a mystery. The high prevalence of LUTS calls into question studies of asymptomatic bacteriuria in pregnancy. In this study, LUTS did not differentiate positive and negative standard culture, questioning the validity of the standard culture definition of UTI.

It may be that symptoms in pregnancy *are* more common than outside pregnancy, and that this 'normal' increased symptomatology masks pathological symptoms. This study used a snapshot approach; a longitudinal view may be more helpful.

Future studies should include detailed symptom analysis, whether intending to study asymptomatic or symptomatic UTI. Such data could then be pooled in meta-analysis with potential to analyse at the individual symptom level.

11.3.3 Assumption 2: Normal urine is sterile

11.3.3.1 Introduction

In chapter 3.2.2 I presented evidence that normal urine is not sterile and introduced the concept of a bladder microbiome. Here I present data from this study, which supports the hypothesis that urine of healthy pregnant women is not sterile. I also discuss more recent data examining the bladder microbiome in pregnancy.

11.3.3.2 Data from this study

All three studies included enhanced culture, performed using spun urinary sediment. Micro-organisms were detected in 271/273 (99.3%, 95% CI 97.1-99.9) of voided samples using this method. This compares with standard culture, which was positive in 22/266 (8.3%, 95% CI 5.4-12.4) and not negative (i.e. non-significant growth, mixed growth and positive) in 104/266 (39.1%, 95% CI 33.3-45.3).

It could be argued that these results are due to contamination. However, the same sample was used for both standard and enhanced cultures. Participants

were asked to do a clean-catch mid-stream specimen, to minimise the risk of contamination with perineal organisms. I have presented evidence that the epithelial cells are usually urothelial cells.

Using catheter specimens, 10/34 (29.4%, 95% CI 15.7-47.7) participants had growth by enhanced cultures compared to standard cultures with 2/33 (6.1%, 95% CI 1.1-21.6) positive and 3/33 (9.1%, 95% CI 2.4-25.5) non-negative. The standard culture misses bacteriuria.

11.3.3.3 Newer contributions from the literature

Many studies have focussed on symptomatic patients or those at high risk of UTI. A recent study of 101 healthy controls, using enhanced culture methods, found bacteria in most participants, when most routine cultures were negative, and was able to characterise normal flora (Coorevits, Heytens et al. 2017).

In the literature review, I introduced the concept of the urinary microbiome.

This reframing may lead to a new way of thinking about culture results.

Brubaker and Wolfe, in a 2017 review of the (non-pregnant) female urinary microbiome, describe a paradigm change in which clinicians treating a urine infection should no longer aim to kill all the bacteria, and in which negative urine microbiological results should be reported as 'below detection limits' rather than sterile (Brubaker and Wolfe 2017).

A 2017 study was the first to demonstrate that urine of pregnant women is also not sterile (Jacobs, Thomas-White et al. 2017). In this study, 51 pregnant patients, undergoing catheterisation for a medically-indicated reason, were studied by enhanced culture and 16s RNA sequencing. Patients who had received antibiotics in the past four weeks were excluded. Routine culture was negative for all participants. Organisms were detected in 35/51 (68.6%) using sequencing and 34/51 (66.7%) using enhanced culture. The combination of both tests detected bacteria in 41/51 (80%) participants. The same conclusion about urotype (a cluster of samples with similar community structures, named on the basis of the predominant organism) was reached in 33/51 (64%) of participants. By using catheterisation, the authors felt they were able to avoid doubts about contamination. This suggests that there is a urinary microbiome

in pregnancy, about which we need to understand more. Research in this area has begun.

The urinary microbiome may be affected by caesarean delivery, according to a study which reported altered urinary microbiota 24 hours following caesarean, compared with pre-delivery (Liu, Lv et al. 2018). This study did not control for vaginal delivery or the effect of catheterisation. The effect of urinary microbiome on preterm birth is also of interest. One study compared 49 cases (delivery below 37 weeks) and 48 controls (delivery at 37 weeks or more) and examined associations between urinary microbiota and preterm birth in the second trimester (Ollberding, Volgyi et al. 2016). They did not account for symptoms or use of antibiotics. There were no differences between cases and controls in taxa richness, or community composition. They reported a microbiota clustering partition dominated by *S. marcescens* associated with preterm birth. A small study comparing samples in first and second trimester found stability in the microbiome urotype across trimesters (Sung 2020).

11.3.3.4 Conclusions

In this study, bacteriuria was detected in nearly all voided samples using enhanced culture. Even in catheter specimens, the detection rate of enhanced culture was higher than standard culture. This is consistent with the hypothesis that urine in pregnancy is not sterile. Recent developments in urinary microbiome studies show that there is a urinary microbiome in pregnancy that is yet to be well studied and understood.

The danger of rejecting apparently contaminated samples is that contamination is impossible to define, leading to the rejection of all mixed growth and low count results, as happens currently. This may lead to missing potential pathology.

11.3.4 Assumption 3: Urine dipsticks help to diagnose UTI in pregnancy

11.3.4.1 Introduction

In chapter 3.2.3 I appraised evidence for dipsticks and found that while they may support a diagnosis of asymptomatic bacteriuria, they are a poor

screening test for it. Not enough was known about dipsticks in symptomatic patients in pregnancy, with minimal evidence relating dipsticks to outcomes.

11.3.4.2 Data from this study

In this study I found that dipstick was unable to differentiate standard positive culture from negative, and compared against preterm birth as an outcome there was also no difference. There was a positive relationship between several of its components and other tests, including: leucocyte esterase and fresh microscopy white cell count; blood and fresh microscopy red cell count; and protein and total protein concentration. Only 1/273 (0.4%) were nitrite positive.

Overall, 185/273 (67.8%) of voided samples had a positive dipstick, suggesting its role for triage is limited. Importantly, a proportion of women with dipstick negative for leucocyte esterase had pyuria on fresh microscopy.

11.3.4.3 Newer contributions from the literature

In 2016 a prospectively registered systematic review and meta-analysis examined onsite tests to detect asymptomatic bacteriuria in pregnancy (Rogozinska, Formina et al. 2016). The gold standard was 10^5 CFU/ml of a single organism. Any studies using a different definition were excluded, as were case-control studies.

Twenty-one studies (involving 9491 women) reported accuracy data for the detection of nitrites using dipstick, and eight (5940 women) for the combination of positive nitrites or leucocytes. Pooled sensitivity for nitrites was 0.55 (CI 0.42-0.67) with specificity 0.99 (CI 0.98-0.99). Pooled sensitivity for nitrites or leucocytes was 0.73 (CI 0.59-0.83) with specificity 0.89 (CI 0.79-0.94).

Including nitrites or leucocytes increased the sensitivity at the cost of decreasing specificity. It was concluded that the test was inadequate for diagnosing asymptomatic bacteriuria, although may have value as a screening test in resource-poor settings with limited access to culture. They highlighted poor reporting in individual studies and paucity of data.

This did not address the utility of dipsticks in a symptomatic population, or take into account other criteria for UTI in pregnancy. Importantly, it did not report on clinical outcomes other than comparison with gold standard.

A questionnaire study of antenatal units in England about screening for asymptomatic bacteriuria included questions about dipsticks (Kirby, Simpson et al. 2016). Of the 18 replies (100 total contacted) one used dipstick to screen for asymptomatic bacteriuria, which they would confirm by culture. In response to the question what they would consider a positive result on dipstick for asymptomatic bacteriuria, the responses were as follows: blood 6 (35%), leucocyte 4 (24%), nitrite 6 (35%), protein 6 (35%), showing that there is little consistency in interpretation. Only one unit had a guideline about how to interpret dipstick for asymptomatic bacteriuria.

11.3.4.4 Conclusions

Urine dipsticks with positive features may help support a clinical diagnosis of UTI in pregnancy, but negative dipsticks do not rule it out. There is a link between leucocyte esterase on dipstick, and white cells on microscopy. Positive nitrite, although rare, predicts positive standard culture. Protein and blood are fairly accurate. Voided samples and catheter samples do not match, particularly for protein, which may be clinically important in detecting pre-eclampsia.

Future UTI studies involving dipsticks should include clinical outcomes, to allow better evaluation of dipsticks for predicting morbidity in UTI in pregnancy.

11.3.5 Assumption 4: Mixed growth of a urine culture indicates contamination

11.3.5.1 Introduction

In chapter 3.2.4 I considered evidence that some mixed or polymicrobial growth may be associated with worse outcomes, and that the relevance of this in pregnancy needs to be better understood.

11.3.5.2 Data from this study

Enhanced culture showed mixed growth (two or more isolates) in 252/307 (82.1%, 95% CI 77.2-86.1) samples. The median number of isolates was 3 (95% CI 3-3) with a range of 0 to 7. In voided samples, 247/273 (90.5%, 95% CI 86.2-93.6) had mixed growth.

Standard culture showed mixed growth in 33/299 (11.0%, 95% CI 7.8-15.3). Of these, 33/33 (100%, 95% CI 87.0-100) had mixed growth by enhanced culture. 17/24 (70.8%, 95% CI 48.8-86.6) positive standard cultures had mixed growth by enhanced culture, and 147/192 (76.6%, 69.8-82.2) of negative standard cultures had mixed growth by enhanced culture. Non-contaminated urine is usually defined as negative or positive standard culture; the high proportion of these that have mixed growth on enhanced culture suggests this definition is flawed. The concern is that 'contaminated' results may be ignored clinically, without understanding their significance. As an example, GBS was found by enhanced culture in 6/33 (18.2%) mixed growth and 6/49 (12.2%) non-significant growth; these would be ignored if assumed to be contamination.

Secondary analysis showed that mixed growth at booking, and on standard culture throughout pregnancy, were more common in preterm birth.

Mixed growth was less common in MSUs conducted for this study, compared with other MSUs during the index pregnancies. This may be related to the emphasis and instructions around the clean-catch, mid-stream voiding method.

11.3.5.3 Newer contributions from the literature

Outside of pregnancy, a study (Armbruster, Smith et al. 2017) that looked at polymicrobial UTI using a murine model of catheter-associated UTI found that multiple microbes increased the pathogenicity of *proteus mirabilis*, perhaps mediated via urease production.

A recent review notes that polymicrobial interactions are common, often with increased pathogenicity, suggested mechanisms being: "metabolite exploitation, immune modulation, niche optimization, and virulence induction" (Tay, Chong et al. 2016).

Polymicrobial infections are being studied as a way to understand pathogenicity (Kline and Lewis 2016), for example the interaction between *Group B streptococcus* and *E. coli* (Kline, Schwartz et al. 2012), or between *E. coli* and *P. mirabilis* (Alteri, Himpel et al. 2015).

Considering pregnancy, a recent review highlighted that GBS often coexists with other pathogens, and so risks not being reported (Kline and Lewis 2016).

Some studies on UTI in pregnancy exclude mixed growth samples as contamination. For example, in the Dutch asymptomatic bacteriuria RCT mentioned previously (Kazemier, Koningstein et al. 2015) they excluded 686/5621 (13.4%) for contaminated dipslides (more than two isolates or two with none above diagnostic threshold), essentially mixed growth, for whom outcomes were not collected. This was more than double the positive cultures (255/5132, 5.0%).

Since this study started, a new UK guideline has acknowledged some UTIs may be due to mixed infection and recommends a "re-test if symptomatic" ((PHE) 2018).

It has been suggested that boric acid tubes should have been used for analysis of urine in this study. At the time of doing the study, boric acid tubes were not in routine use in this hospital. Boric acid is used to preserve urine for culture when there is a risk of delay in transfer to the laboratory (Porter and Brodie 1969). A systematic review published in 2016 identified 9 studies between 1969 and 1999 studying urine preservation using boric acid, as well as refrigeration (LaRocco, Franek et al. 2016). Both boric acid and refrigeration were found to adequately preserve urine specimens prior to their processing for 24 hours. Urine kept at room temperature for more than four hours had overgrowth of both clinically significant and contaminating micro-organisms, using standard Kass criteria. A more recent study of the effect of boric acid preservation on fresh microscopy cell counts found that both boric acid and refrigeration delayed loss of white blood cells, but that there was 40% loss by 4 hours regardless (Kupelian, Horsley et al. 2013). A full discussion of the risks and benefits of boric acid transportation is outwith the remit of this thesis, however it should be noted that boric acid containers may also lead to false

negatives owing to over-suppression of bacteria when underfilled with urine (Nickander, Shanholtzer et al. 1982).

It is important to note that urines for enhanced culture in this study were all processed within four hours of being produced, reducing the risk of overgrowth of contaminating organisms. All fresh microscopy was performed immediately after voiding, in the clinical environment. It may be that the standard culture yields, including positive cultures and mixed or non-significant growth, may have been adversely increased by the non-use of boric acid containers. The trust where this research was conducted is planning to implement these in the near future and it would be important to study the impact of this, taking into account clinical outcomes as well as reported growth rates.

11.3.5.4 Conclusions

Mixed growth has not been adequately studied in pregnancy, owing to the assumption that it represents contamination and elimination from research studies. This study suggests a higher rate of preterm birth in those with mixed growth on standard culture, and undetected GBS in apparently contaminated samples.

11.3.6 Assumption 5: Significant bacteriuria has a threshold of 10^5 CFU/ml

11.3.6.1 Introduction

Previously I described evidence challenging the threshold of 10^5 CFU/ml for significant bacteriuria in pregnancy. In acutely dysuric women outside pregnancy, 10^2 CFU/ml may be sufficient (Stamm, Counts et al. 1982), however standard culture is not routinely reported at this threshold.

11.3.6.2 Data from this study

Enhanced culture colony counts showed weak positive correlation with white cell counts ($r_s=0.303$, $p<0.01$), epithelial cell counts ($r_s=0.290$, $p<0.01$) and IL-8 ($r_s=0.242$, $p<0.01$). They did not correlate with symptoms or ATP.

When comparing positive standard cultures against negative standard cultures, white cell counts, epithelial cell counts and enhanced culture colony counts were higher for positive cultures, but not dichotomised. This suggests the standard culture dichotomises a spectrum and may represent the most severe end of that spectrum, without evidence that sub-threshold values are not harmful.

Enhanced culture colony counts were higher in preterm birth (median 16681CFU/ml, 95% CI 1467-54812) compared with term birth (1220CFU/ml, 95% CI 688-2317, $p=0.001$). This was despite no change in rates of positive standard culture.

When enhanced culture counts were compared with standard culture categories, there was a quantitative relationship. Non-significant growth includes single isolate growth below diagnostic threshold. Median non-significant growth colony counts (8123, 95% CI 2987-21628) appeared lower than positive (51464, 95% CI 884-220480). Secondary analysis showed that standard culture non-significant growth at recruitment was reported in 8/22 (36.4%, 95% CI 18.0-59.2) cases of preterm birth and in 40/239 (16.7%, 12.4-22.2, $p=0.023$) non-preterm birth cases. Although numbers were small and confidence intervals wide, it is not clear that growth below diagnostic threshold is not associated with a negative outcome.

There were no cases of pyelonephritis in this study, so it was not possible to analyse the contribution of colony counts to developing pyelonephritis.

11.3.6.3 Newer contributions from the literature

A recent study compared MSU culture with enhanced sediment culture in new and relapsed patients with chronic LUTS, and controls, using the same methods as here (Sathiananthamoorthy, Malone-Lee et al. 2019). The MSU culture grossly underestimated the amount of (polymicrobial) growth, from 36.4% of new patient MSUs, compared with 84.8% sediment cultures. No quantitative culture method, whether MSU or enhanced culture, was able to distinguish between patients and controls.

11.3.6.4 Conclusions

These data do not support the concept that significant bacteriuria has a threshold of 10^5 CFU/ml. Future studies should report bacteriuria below 10^5 CFU/ml and evaluate clinical outcomes to assess this relationship more fully.

11.3.7 Assumption 6: Epithelial cells in the urine are a marker of contamination

11.3.7.1 Introduction

In chapter 3.2.6 I outlined that epithelial cells are assumed to indicate perineal contamination. I have been unable to find the origin of this assertion. In non-pregnant studies, there is evidence that epithelial cells are often urothelial. There is evidence that urothelial cells are shed as an inflammatory response. There was a paucity of studies in pregnancy with which to debate this issue.

11.3.7.2 Data from this study

Epithelial cells had a moderate correlation with white cells ($r_s=0.56$, $p<0.01$) and IL-8 ($r_s=0.60$, $p<0.01$). There was a weak correlation with IL-6 ($r_s=0.33$, $p<0.01$), lactoferrin ($r_s=0.40$, $p<0.01$), and total colony count ($r_s=0.28$, $p<0.01$).

The correlation of epithelial cell counts with white cell counts and urinary IL-8 not only helps refute the idea that epithelial cells represent contamination, since contamination might vary randomly with white cell counts, but also supports the idea that shedding of urothelial cells is an inflammatory response.

Uroplakin-3 staining was used in both Abdominal pain (n=18) and MSU-CSU (n=66) studies to assess urothelial origin of epithelial cells in pregnancy. In both these, median proportion of uroplakin-positive epithelial cells was >90% with no difference between paired MSU and CSU samples (p=0.910). This is consistent with research in non-pregnant patients with chronic LUTS (Horsley, Malone-Lee et al. 2013).

Positive standard culture had higher median epithelial cell counts (41, 95% CI 10-50) than non-positive standard cultures (12, 95% CI 8-14, p=0.016). This seems inconsistent with them representing contamination. There was no association with preterm birth.

11.3.7.3 Newer contributions from the literature

More recent studies in chronic LUTS patients suggest epithelial cells as markers of host response. One study comparing patients with pyuria and overactive bladder symptoms with controls found that epithelial cell counts were one factor in predicting total colony counts by enhanced culture (Gill, Kang et al. 2018). Cross-over data from the Whittington chronic LUTS service, during a period when it temporarily closed, showed an increase in epithelial cell counts when patients stopped antibiotic treatment, and a subsequent decrease when they restarted treatment (Swamy, Kupelian et al. 2019).

One definition of contamination used has been epithelial cell counts greater than white cell counts, which occurred in 11/336 (3.3%) in one study in pregnancy and led to these samples being excluded from analysis (Schneeberger, van den Heuvel et al. 2013). In the current study, 125/307 (40.7%) of samples had epithelial cell count higher than the white cell count; to have excluded them would have risked missing pathology.

Since this study commenced, UK UTI guidelines have now changed, with the comment that epithelial cells do not necessarily represent contamination ((PHE) 2018).

A 2016 retrospective cross-sectional study (Mohr, Harland et al. 2016) of 19,328 patients presenting to a US medical centre aimed to determine the value of using epithelial cell counts to predict contamination. Contaminated cultures were defined as mixed bacterial flora, the presence of multiple skin flora, or the growth of *Gardnerella vaginalis*, *beta-hemolytic Streptococcus*, or *Candida spp*. The authors found that the epithelial cell count was a poor predictor of contamination, although could predict poor performance of urinalysis (with either ≥ 5 WBC/ml or nitrite positive) to predict bacteriuria.

11.3.7.4 Conclusions

This study provides new data about urinary epithelial cells in pregnancy, suggesting they are mostly urothelial in nature and reflect host response to infection. Standard lab microscopy epithelial cell categories correspond to fresh microscopy counts, which may clinically be useful. Epithelial cells in pregnancy cannot be assumed to represent contamination, and should be interpreted clinically.

11.3.8 Assumption 7: Micro-organisms can be categorised as uropathogens or contaminants

11.3.8.1 Introduction

In 3.2.7 I argued that dichotomising microbes into uropathogens and contaminants may be overly simplistic, with insufficient evidence in pregnancy to justify this.

11.3.8.2 Data from this study

In all three studies, enhanced culture species common to each study group included both traditional uropathogens (such as *E. coli* and *E. faecalis*) and contaminants (such as *S. haemolyticus*, *S. epidermidis*). In particular, typical contaminants, such as *Lactobacillus spp* and *Corynebacteria spp*, were identified by CSU as well as MSU.

When species associated with preterm birth or term birth were compared, the same pattern was seen.

11.3.8.3 Newer contributions from the literature

Guidelines for diagnosis of UTI, updated since the start of this study, still refer to uropathogens in diagnostic criteria. The PHE 2018 guideline, discussing lower colony counts and mixed growth, states that:

"If there are 2 isolates and each organism $>10^6$ CFU/L ($>10^3$ CFU/mL) including possible pathogen like *E. coli* or *S. saprophyticus* the patient might have a UTI."((PHE) 2018)

The 2019 European Urology Association Guidelines on Urological Infections also refers to the key pathogens:

"The most common causative agent of uncomplicated UTIs is *E. coli*, followed by *Staphylococcus saprophyticus*" (Bonkat 2019)

It states that in lower colony counts, for symptomatic patients, the presence of uropathogens confirms the diagnosis of UTI. However, it is not certain that uropathogens are always pathogenic, nor that non-pathogens are not pathogenic.

In an enhanced culture comparison of patients with overactive bladder symptoms and controls (Gill, Kang et al. 2018), while there was mostly division of uropathogens in patients and non-uropathogens in controls, *E. faecalis* was found more commonly in patients, and *Citrobacter* was found more commonly in controls.

Some microbes have pathogenic and non-pathogenic subtypes; standard culture may not distinguish these. Non-pathogenic *E. coli* have been researched as a way to prevent UTI in catheterised patients, as a non-pathogenic biofilm that prevents pathogenic biofilm formation (Zhu, Yu et al. 2017). Probiotic use of *Lactobacillus crispatus* to restore vaginal flora has shown promising in vitro results in protecting vaginal epithelial cells against uropathogenic *E. coli* (Butler, Silvestroni et al. 2016).

While *Corynebacterium spp* has been associated with contamination, certain species, isolated in hospital patients, have been linked with biofilm formation and antibiotic resistance (Chandran, Puthukkichal et al. 2016). A study of 762 coryneform isolates found that around one fifth were associated with infection,

of which 22% were from urine (Leal, Jones et al. 2016). In particular, novel findings of UTI were identified, caused by *Actinomyces neuui* (21%) and *Corynebacterium aurimucosum* (21%). It was not possible to determine whether the isolates were clinically significant without additional information.

In pregnancy, different species of vaginally isolated lactobacillus have been associated with different obstetric outcomes. *Lactobacillus iners* has been associated with preterm birth, while *Lactobacillus crispatus* has been associated with term delivery (Kindinger, Bennett et al. 2017). In a study of 51 asymptomatic pregnant women, using enhanced culture and sequencing, both *L. iners* (5/51) and *L. crispatus* (5/51) were identified in catheter specimens of urine (Jacobs, Thomas-White et al. 2017). A sequencing study comparing 49 women with preterm birth (<37 weeks) with 48 controls also found that second trimester urinary microbiota in the preterm birth group were enriched with *L. iners*, among other organisms (Ollberding, Volgyi et al. 2016).

Many of the studies so far have been cross-sectional. It is important to understand how urinary microbiome changes over time in pregnancy, or whether it is stable. A recent study compared clean catch MSUs in first and second trimesters and found them to be very similar, with lactobacillus the dominant organism at both time points for most women (Sung, Halverson et al. 2020). Further longitudinal studies using enhanced culture and sequencing methods would be required, to establish their reproducibility over time.

The rapidly growing field of microbiota analysis means that the understanding of pathogens versus contaminants or non-pathogens is likely to change over the next few years.

11.3.8.4 Conclusions

Enhanced culture in pregnancy in the current study, as well as other studies using enhanced culture and microbiome approaches, raise questions about the classification of uropathogens and non-uropathogens. Further research using outcomes will be needed to advance.

11.3.9 Assumption 8: Catheter specimens avoid the problem of contamination

This has been discussed in detail in Chapter 9.

11.3.10 Conclusion

The eight assumptions have all been at least partly challenged by the current study. These assumptions underpin clinical practice, and some make up the gold standard for UTI diagnosis.

Next, I discuss how to move forward from this.

11.4 Potential solutions to an imperfect reference standard

Having challenged assumptions underlying UTI diagnosis in pregnancy, we are left with a flawed gold standard. This creates at least two problems, explained in a recent analysis of the issues of assessing accuracy and economic value of a new test in the absence of a perfect reference test (Xie, Sinclair et al. 2017). Firstly, accuracy will always appear to be less even if the new test is better. Secondly, if the new test is more expensive, it will perform more poorly against the imperfect but cheaper reference test. One suggestion was a Bayesian approach, using latent-class meta-analysis to estimate the sensitivity and specificity of a new test, that takes into account the imperfect accuracy of an old test.

A second approach is to revisit Kass' methodology (Kass 1957), who determined a relevant clinical outcome (pyelonephritis) and determined the best test to discriminate this. This is also the approach taken by the UCL Chronic LUTS group, who used a clinical diagnosis of UTI flare (symptoms and urinary white cells) in patients with chronic UTI and compare other tests against this (Sathiananthamoorthy, Malone-Lee et al. 2018).

Clinical outcomes of interest are thankfully rare, as demonstrated by the Dutch asymptomatic bacteriuria RCT which closed early owing to the rarity of the primary outcome of either preterm birth or pyelonephritis (Kazemier, Koningstein et al. 2015). However, this makes design of appropriate studies challenging, to include enough participants for adequate power. There is a role here for systematic review and meta-analysis of studies that include data about outcomes. I have established through literature review that outcome-focussed studies of UTI in pregnancy are rare. A solution to reduce research waste, and facilitate useful meta-analysis, has been core outcome sets in research, which has been supported by the initiative Core Outcome Measures in Effectiveness Trials (COMET) (Williamson, Altman et al. 2017).

Since planning this research, a set of core outcomes for research into effectiveness of interventions in preterm birth has been developed (van 't Hooft, Duffy et al. 2016). This followed a systematic review of outcomes reported in intervention studies for preterm birth (Meher and Alfirevic 2014)

which noted an ongoing wide variation in reported outcomes, making it difficult to incorporate findings into systematic reviews, thought to be the highest level of evidence (Howick 2011). Screening for UTI in pregnancy could be considered an intervention to prevent preterm birth and so future UTI studies should incorporate these. Core outcome sets are also being developed in urogynaecology research (Pergialiotis, Durnea et al. 2018). I argue that a core outcome set for research in UTI in pregnancy is urgently required, to ensure that future studies can lead to meaningful advancement in UTI testing.

It is important to note that since this study began, UK guidelines for UTI diagnosis have adapted to include more nuance, including different culture thresholds ((PHE) 2018). This does not preclude the need to revisit UTI diagnosis in pregnancy from an outcomes-perspective, however.

11.5 Critical appraisal of alternative methods for diagnosis of UTI

I have already discussed many of the specific findings from each of these alternative methods. In this section I critically appraise each method in turn as a research tool or potential diagnostic instrument.

11.5.1 Standardised assessment of lower urinary tract symptoms (LUTS)

11.5.1.1 Introduction

I justified the use of the Artemis LUTS inventory in chapter 4.2, and summarised results in chapter 10. Here I appraise it as an instrument in this context.

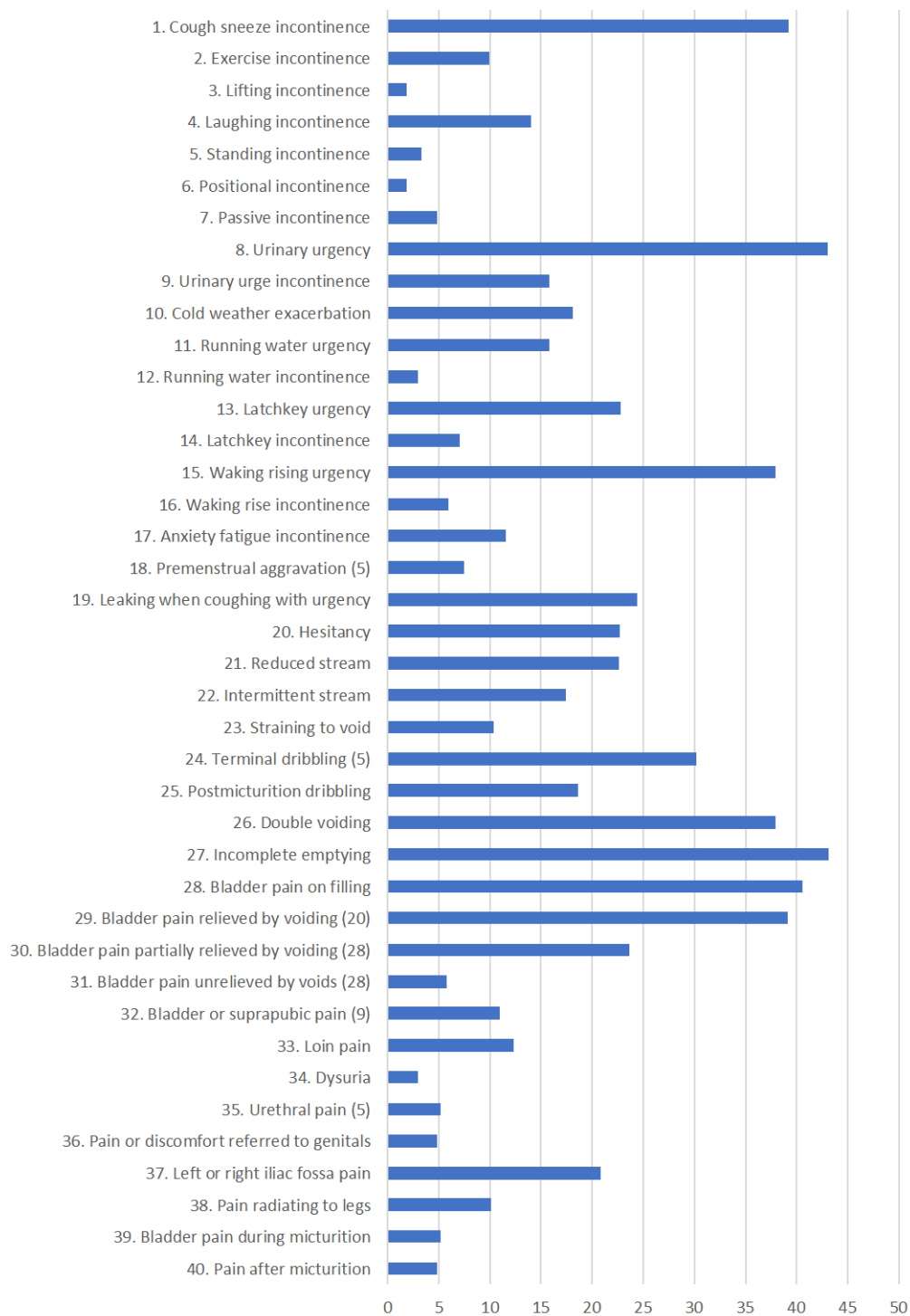
11.5.1.2 Data from this study

No patient feedback was collected about the acceptability of the questionnaire in this context. All patients attempted it, mostly completely although there were some missing responses. Figure 11-1 shows responses from the questionnaire across all three studies. Those with 5 or more missing responses are marked with the number of missing responses shown in brackets.

Most questions were answered, with only 4 missed by more than 5 respondents. The commonest symptoms were incomplete emptying, urinary urgency, and bladder pain on filling, experienced by more than 40% of respondents.

The range of total LUTS was 0-32/40. This suggests that the questionnaire sampled a broad spectrum of LUTS.

Close analysis of responses showed inconsistencies. Question 28, about pain on bladder filling, was answered positively by 110/271 (40.6%) respondents. The following three questions were sub-questions of this, referring to the effect of voiding on the pain. However, in total 171 respondents gave a positive answer to one of these questions, rather than 110. Similarly, one of the questions refers to premenstrual aggravation. This was positive in 20/268 (7.5%) of respondents, which either suggests chronic symptoms, or inappropriate responses for pregnancy.

Figure 11-1: Individual LUTS scores (percent reporting symptom, n=273)

** Number in brackets refers to number of missing responses if more than 4 missing*

There was a trend towards a higher LUTS score when the patient thought they had a UTI (Table 11-1), suggesting an element of face validity and when the clinician diagnosed UTI (Table 11-2).

Table 11-1: Relationship between patient's perception of UTI versus total LUTS score

Does patient think it is a UTI?	Number of cases	Median LUTS score	95% CI of median	Range
Patient does not think it is a UTI	72	5.5	4-7	0-22
Patient thinks it might be a UTI	10	8.5	5-11	4-12
Patient thinks it is a UTI	4	9.5	1-13	1-13

Table 11-2: Relationship between clinician's diagnosis of UTI and total LUTS score

Clinician diagnosis of UTI	Number of cases	Median LUTS score	95% CI of median	Range
No UTI	74	5.5	4-8	0-22
UTI	20	8	6-11	0-21

In the Chronic LUTS setting, symptoms were found to be predictive of pyuria, and voiding symptoms correlated particularly well (Khasriya, Barcella et al. 2018). In contrast, Table 11-3 showed no difference between the same three categories of pyuria in terms of total LUTS (Kruskal-Wallis, $p=0.225$).

Table 11-3: Comparison of total LUTS score with degree of pyuria

	Number of cases	Median total LUTS score	95% CI of median	Range
No pyuria	58	6	5-8	0-18
Pyuria 1-9	64	5	4-8	0-22
Pyuria >9	183	5	4-6	0-32

As demonstrated previously, there was no relationship between total LUTS and preterm birth.

Other methods of assessment of utility and validity of a questionnaire assessment tool, such as interobserver variability, have not been assessed in this study. As the questionnaire was validated outside pregnancy, formal validation of its use in pregnancy is something that could be considered in future.

11.5.1.3 Newer contributions from the literature

Since this study, two further papers have been published that describe in more detail the use of the Artemis LUTS inventory.

It allows monitoring of symptoms over time, and can detect treatment success. In a case series of 624 new female patients with chronic LUTS and pyuria ($\geq 1\text{WBC}/\mu\text{l}$) (Swamy, Barcella et al. 2018), median total LUTS score of these patients at first clinic attendance was 11.0. This broke down into urgency (4.0), pain (3.0), voiding (3.0) and stress incontinence (0.0). Symptom scores decreased over time with treatment and at the point of discharge average symptom scores were below 4 ($p < 0.001$).

In 2015, the same Chronic LUTS clinic unexpectedly closed for four weeks; patient outcomes were monitored (Swamy, Kupelian et al. 2019). 221 of 1035 active patients in the clinic were affected. Of these, 199/221 (90%) reported deterioration in their symptoms when treatment was stopped. Symptom scores increased following cessation of treatment. Once antibiotic regimens were recommenced, symptom scores fell again to levels similar to those prior to the suspension ($p < 0.001$).

In both these studies, white cell counts and epithelial cell counts mirrored the symptom scores.

The Artemis LUTS inventory has also contributed to pathophysiological understanding of chronic LUTS, through the demonstration of a phenomenon of damped oscillations (Swamy, Barcella et al. 2018). There was a pattern of oscillations in symptom scores over time, which correlated with pyuria and was used as a mechanism of monitoring response to treatment. In 73% of patients these were damped oscillations, that is a series of oscillations of decreasing amplitude. This may have treatment implications.

The current study was a one-off snapshot with no longitudinal data. It would be important to assess the validity of Artemis as a longitudinal tool in pregnancy,

11.5.1.4 Conclusions

The Artemis LUTS inventory appeared to be useable by participating patients, with few incomplete responses. It did not predict pyuria in the same way as non-pregnant chronic LUTS patients, which should be explored further. Recent data from chronic LUTS patients have demonstrated the utility of the questionnaire in that setting to assess response to treatment and stopping treatment, and have contributed to pathophysiological understanding of chronic UTI. The longitudinal role of the questionnaire in pregnancy needs to be better understood.

11.5.2 Quality of life assessment

11.5.2.1 Introduction

I discussed the findings about quality of life, and how they relate to other tests and outcomes, in chapter 10. Here I appraise the EuroQol EQ-5D-5L questionnaire tool in this research setting.

11.5.2.2 Data from this study

Table 11-4 summarises quality of life data from all three studies. There was missing data from 10/207 (4.8%) questionnaires.

The least common problem was self-care, and most common was pain/discomfort. Median 'your health today' was 80% (95% CI 75-80, range 20-100). There was a difference between groups in the Abdominal pain study, but not in the other studies.

Table 11-4: Summary of quality-of-life index data combined from all three studies

	No problems	Slight problems	Moderate problems	Severe problems	Unable to do / extreme	Missing response	Totals
Mobility	151 (72.9%)	32 (15.5%)	10 (4.8%)	4 (1.9%)	1 (0.5%)	9 (4.3%)	207 (100%)
Self-care	175 (84.5%)	19 (9.2%)	4 (1.9%)	1 (0.5%)	0 (0%)	8 (3.9%)	207 (100%)
Usual activities	130 (62.8%)	49 (23.7%)	13 (6.3%)	3 (1.4%)	4 (1.9%)	8 (3.9%)	207 (100%)
Pain / discomfort	95 (45.9%)	62 (29.7%)	32 (15.5%)	8 (3.9%)	1 (0.5%)	9 (4.3%)	207 (100%)
Anxiety / depression	130 (62.8%)	53 (25.6%)	10 (4.8%)	3 (1.4%)	1 (0.5%)	10 (4.8%)	207 (100%)

When comparing against outcomes, quality of life by VAS 'your health today' differed between positive and negative standard culture ($p=0.008$), however index value did not. For preterm birth, index value was lower for preterm birth ($p=0.008$) however there was no difference by VAS. This inconsistency between the two measures needs further investigation.

The wide range of results shows the tool covers the spectrum of experience.

11.5.2.3 Newer contributions from the literature

The earlier version of the EuroQol tool (EQ-5D-3L) was used in a longitudinal cohort study of pregnant women (Morin, Claris et al. 2019). It showed that quality of life dropped between 4th and 8th months of pregnancy, with a greater decrease in pathological pregnancies, with a drop of around 0.1 index points per month. Index values were more discriminating than VAS scores.

11.5.2.4 Conclusions

The tool worked well in this context, with few patients unable to complete it. There was a difference in scores between cases and controls in the Abdominal pain study, suggesting there is validity when used in this context. It would be appropriate to continue to encourage the use of generic QoL assessment tools in future UTI in pregnancy research.

11.5.3 Fresh unspun microscopy

11.5.3.1 Introduction

The performance of fresh microscopy for white cell counts and epithelial cell counts has been discussed previously. Here I will briefly summarise these conclusions, evaluate the advantages and disadvantages of this method, and consider newer findings from the literature.

11.5.3.2 Data from this study

White cell counts differed more between studies using the pooled data, than between groups within a study (Table 11-5, Table 11-6). Median white cell counts for each study were markedly different; there was a similar trend for red cell and epithelial cell counts. Thus, there would appear to be an unexpected sampling effect.

The study groups differed by gestation at recruitment. There was a weak positive correlation between gestation and white cell counts ($r_s=0.333$, $p<0.01$), and red cell counts ($r_s=0.219$, $p<0.01$), which may partly account for this. Figure 11-2 and Figure 11-3 show the inter-relationships between gestation, study, and white cell or epithelial cell counts respectively. The similarity between study groups suggests there may be a study effect. It is of note that although most of the MSU-CSU samples were late gestation, four (representing the two cervical cerclages) were at an early gestation, and also had relatively high cell counts.

Table 11-5: Fresh unspun microscopy cell counts compared across studies

Study	Number of cases (n)	Median WCC (95% CI) / μL^{-1}	Median RBC (95% CI) / μL^{-1}	Median ECC (95% CI) / μL^{-1}	Median gestation in days (95% CI)
Abdominal pain	159	14 (10-24)	0 (0-2)	14 (8-18)	190 (180-206)
PTB	80	2 (0-4)	0 (0-2)	8 (4-12)	114 (111-117)
MSU-CSU	68 (34 patients)	52 (42-66)	6 (2-10)	28 (22-40)	272 (269-273)

Table 11-6: Fresh unspun microscopy cell counts compared across study groups

Study group	Number of cases (n)	Median WCC (95% CI) / μL^{-1}	Median RBC (95% CI) / μL^{-1}	Median ECC (95% CI) / μL^{-1}
Pain cases	50	13 (6-24)	1.5 (0-4)	14 (8-26)
Acute controls	58	10 (6-28)	0 (0-2)	14 (6-30)
Normal controls	51	20 (14-32)	0 (0-6)	8 (4-16)
Low risk	13	0 (0-34)	0 (0-14)	10 (2-32)
High risk – PTB	37	2 (0-4)	0 (0-2)	8 (4-14)
High risk – non-PTB	30	3 (0-12)	0 (0-6)	8 (4-12)
CS MSU	32	67 (46-96)	4 (2-12)	41 (22-64)
CS CSU	32	46 (34-64)	7 (2-24)	22 (12-30)
CC MSU	2	32 (24-40)	2 (2-2)	44 (22-66)
CC CSU	2	40 (30-50)	8 (0-16)	35 (18-52)

Figure 11-2: Scatter plot of white cell count versus gestation, separated by study

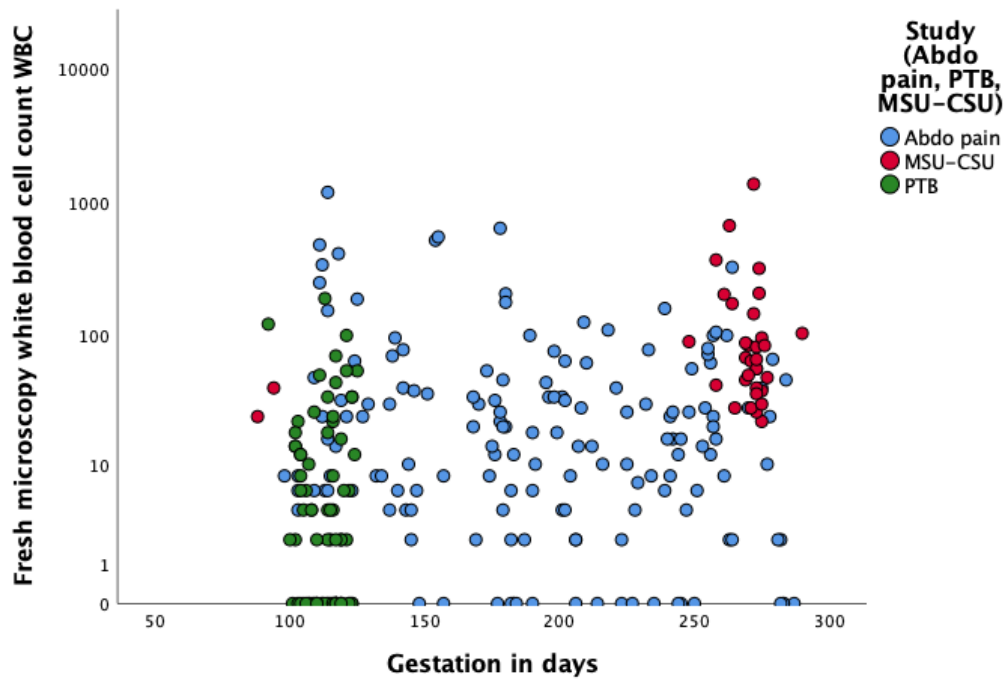
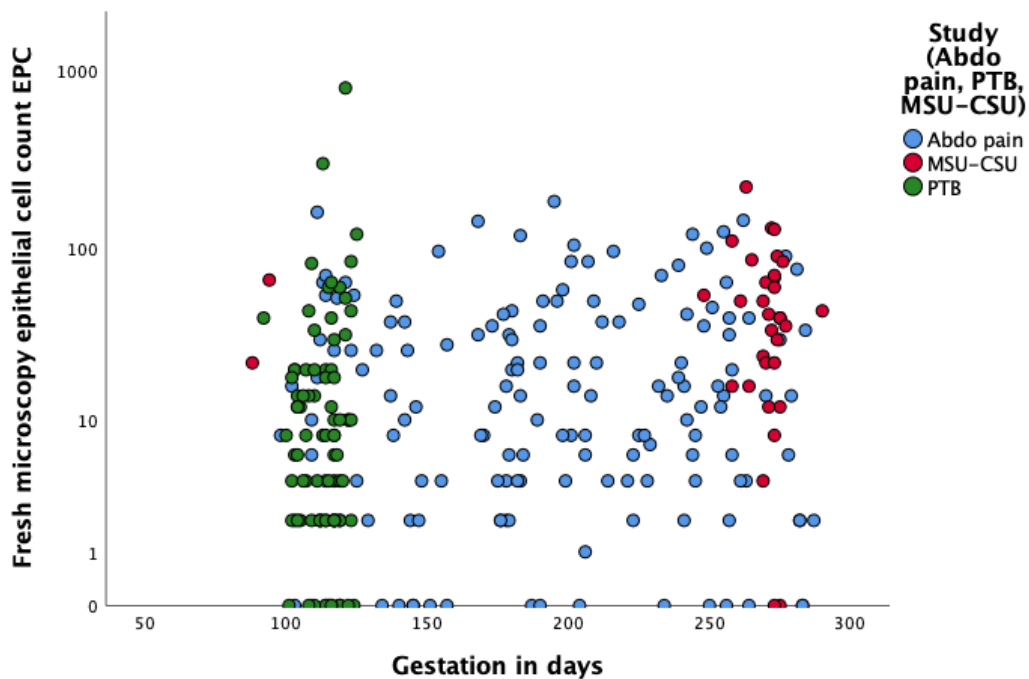


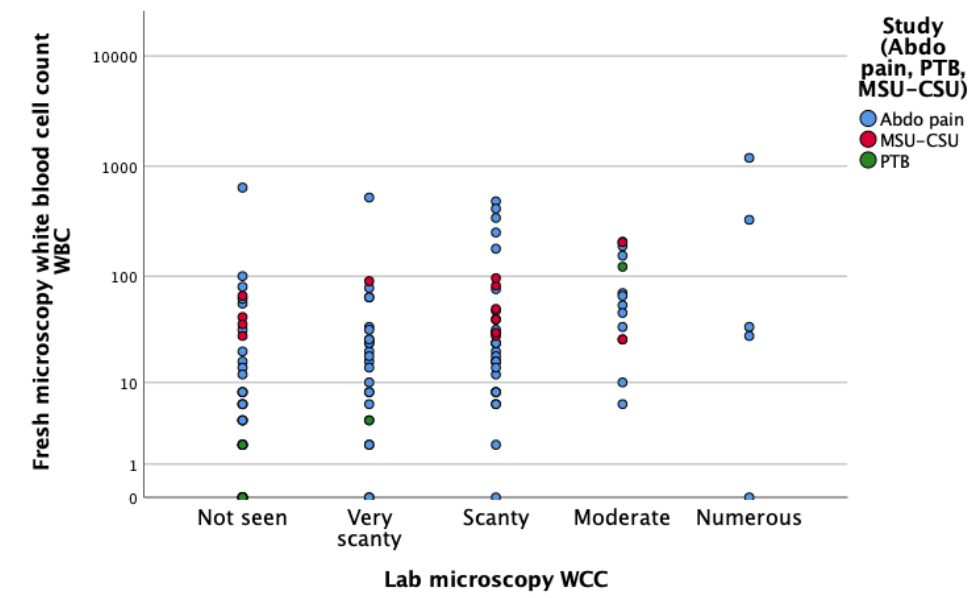
Figure 11-3: Scatter plot of epithelial cell count versus gestation, separated by study



To assess for a study effect, cell counts were compared with other methods of measuring those components, according to study group. First fresh

microscopy was compared with standard microscopy (Figure 11-4). There were only 115 cases of this including 5 in the PTB study, 13 in the MSU-CSU study and 97 in the Abdominal pain study. Although there was a discrepancy in MSU-CSU cases of no white cells seen on standard microscopy having relatively high fresh microscopy readings, there were samples from the Abdominal pain study with a similar discrepancy. Next, fresh microscopy was compared against dipstick, showing a similar pattern (Figure 11-5). Although there is much variation, there is a possibility of systematic over-reading of white cell counts in the MSU-CSU study. There were also two instances of fresh microscopy count of zero with dipstick of 2+, from the PTB study, which may hint at systematic under-reading in that study.

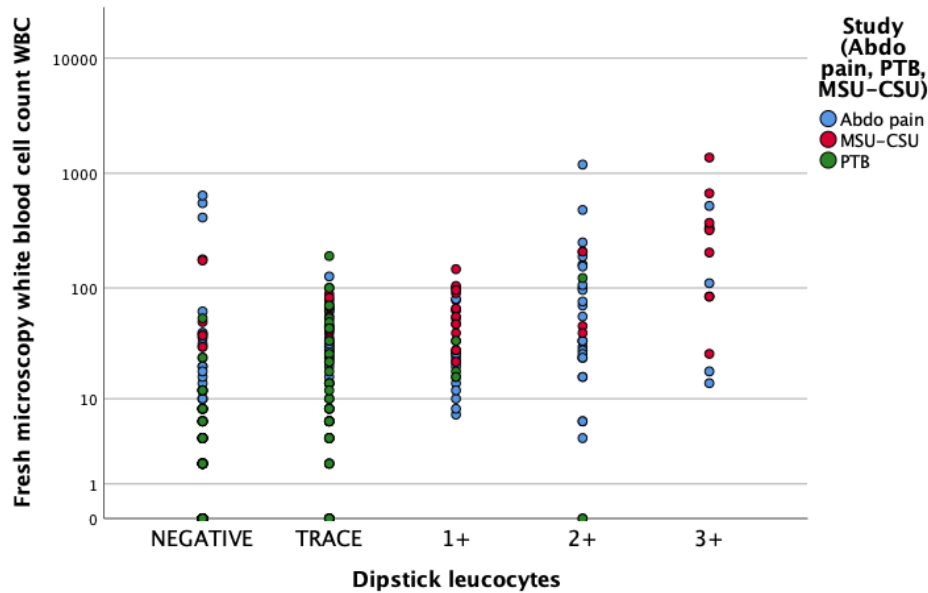
Figure 11-4: Comparison of standard microscopy white count with fresh unspun microscopy



A different operator conducted the MSU-CSU microscopy compared with Abdominal pain and PTB microscopy, which also involved a third person. Although all were trained by the same person, including double counting the same slides, it is possible that their technique was subsequently different. To assess this would require an analysis of inter-observer variability, by double-counting the same samples. Although this was planned, the nature of

recruitment was that it was not possible to double-count the fresh microscopy samples as only one researcher was available at a time.

Figure 11-5: Comparison of dipstick leucocyte esterase with fresh microscopy white cell counts



When fresh microscopy red cell count was compared against standard microscopy (Figure 11-6) there was a discrepancy, particularly with red cells being seen on fresh microscopy but not on standard microscopy, with no obvious effect of study. This may be an effect of the delay between analysis for standard microscopy. On dipstick, however, there was a similar effect, with some high fresh microscopy red cell counts for negative dipstick (Figure 11-7). Dipstick was performed prior to fresh microscopy so delay was not a factor.

Figure 11-6: Comparison of fresh microscopy red cell counts with standard microscopy red cell counts

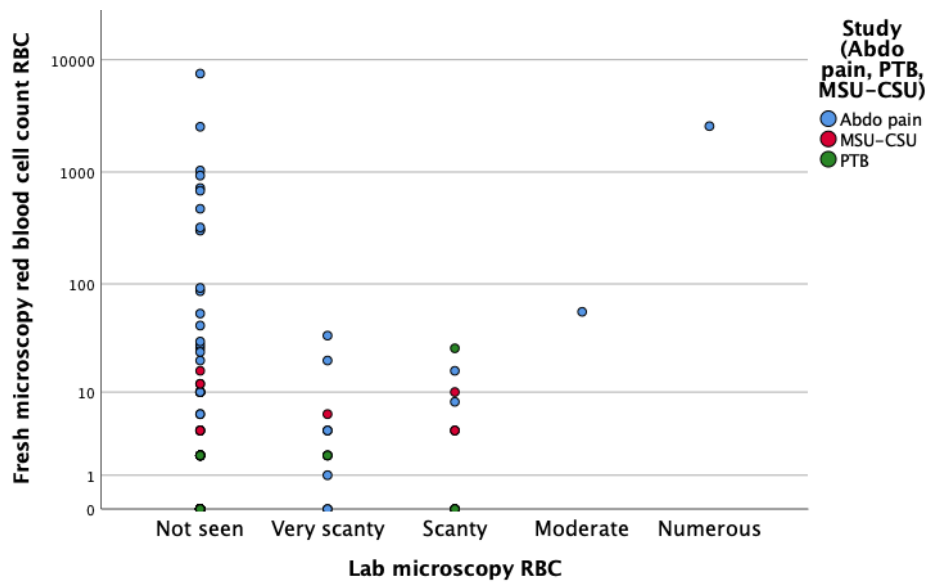
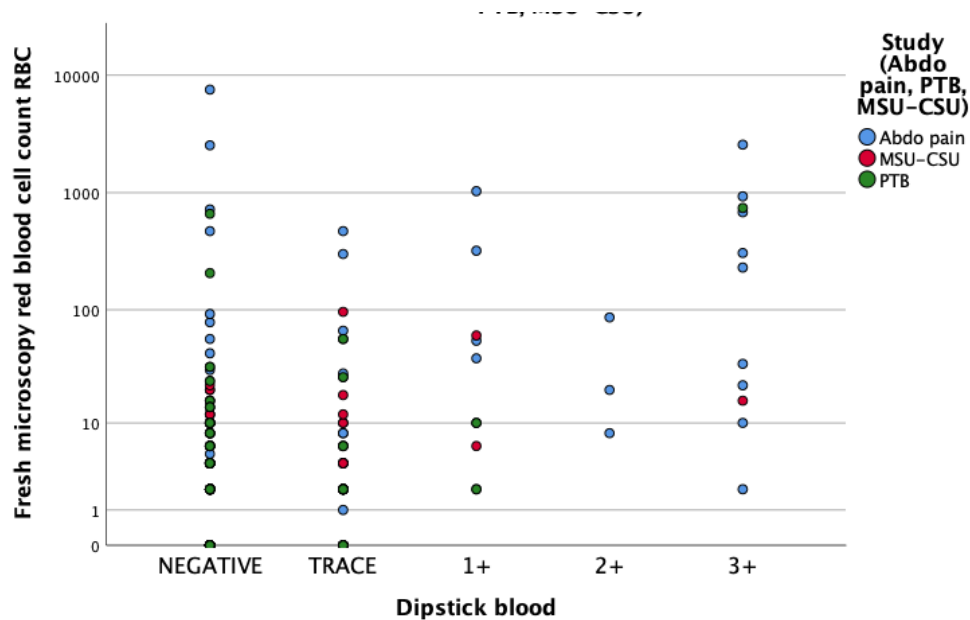


Figure 11-7: Comparison of fresh microscopy red cell counts with dipstick red cell counts



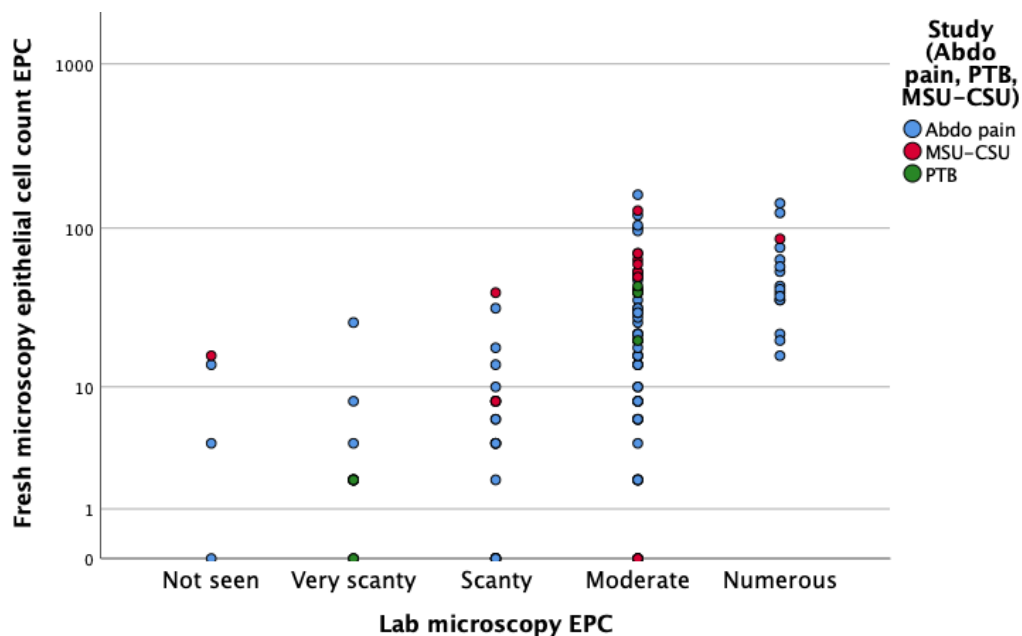
When fresh microscopy epithelial cell counts were compared against standard microscopy (Figure 11-8), there was a positive relationship, with median counts for the 'numerous' category higher than those in the 'not seen', 'very scanty' and 'scanty' categories, demonstrated by non-overlapping confidence

intervals. This suggests that attention to standard microscopy reports for epithelial cell counts could potentially be useful, if there were no facility for fresh microscopy.

Table 11-7: Comparison of fresh microscopy epithelial cell counts against standard microscopy

Standard microscopy epithelial cells	n	Median epithelial cell count / μ l-1	95% CI / μ l-1	Range / μ l-1
Not seen	6	11	0-28	0-28
Very scanty	11	2	0-8	0-26
Scanty	22	5	0-8	0-40
Moderate	62	22	16-36	0-160
Numerous	16	43	36-76	16-142
<i>Not done</i>	<i>190</i>	<i>12</i>	<i>10-18</i>	<i>0-800</i>

Figure 11-8: Comparison of fresh microscopy epithelial cell counts with standard microscopy epithelial cell counts



11.5.3.3 Newer contributions from the literature

The presence of any pyuria on fresh unspun microscopy continues to be used by the Chronic LUTS clinic to define infection (Swamy, Barcella et al. 2018).

A recent study in Taiwan, where there is antenatal screening for asymptomatic pyuria instead of bacteriuria, showed that asymptomatic pyuria (defined as $\geq 15 \text{ WBC}\mu\text{l}^{-1}$ in the absence of urinary symptoms) was present in 253/1187 (21.3%) patients and was associated with preterm birth, preterm pre-labour rupture of membranes and low birth weight (Lai, Hsu et al. 2017). The patients with asymptomatic pyuria had higher rates of preterm birth (7.1% versus 3.3%, $p=0.007$). Patients with more than $15\mu\text{l}^{-1}$ squamous epithelial cells were excluded, as well as patients who delivered preterm for a medical indication or who were symptomatic.

It is plausible that given changes in the immune system in pregnancy, and in response to hormonal influence (Terlizzi, Gribaudo et al. 2017), urothelial cells may behave differently in pregnancy.

11.5.3.4 Conclusions

Fresh unspun microscopy in pregnancy needs further evaluation. The relationship with gestation needs to be understood, and more stringent assessment with double counting to establish acceptable intra-observer and inter-observer variability. There appears to be a relationship with quantitative standard microscopy and dipstick, which may facilitate use of the longitudinal approach adopted for chronic UTI management.

In the next section I appraise the value of ATP as a test.

11.5.4 Urinary ATP measurement

11.5.4.1 Introduction

In this section I briefly summarise ATP findings, critically appraise the test, and reconsider how this fits within the wider recent literature.

11.5.4.2 Data from this study

ATP differentiated between study groups in the Abdominal pain study – it was higher in the Acute Controls than Normal controls. It did not differentiate between any study groups in the PTB study. It was higher for CSU than MSU samples in the MSU-CSU study.

The most dramatic finding overall was higher ATP in the catheter specimens compared with any voided specimens. When paired MSU-CSU samples were compared, CSU was higher ($p < 0.001$). It is hypothesised that either the trauma of catheterisation, or the sudden void caused by the release of urine, contributed to this.

No data were collected regarding interobserver variability, or repeatability of measurements. As a swab test, read by machine, interobserver variability should not be an issue, however there may be differences in procedure, such as time to swab, and physical handling of the urine. It has since been reported that urinary ATP degrades rapidly over time unless frozen (Gill, Horsley et al. 2015), and in future time to test would be important to record.

Interestingly, there was no correlation between colony counts and ATP ($r_s = 0.065$, $p > 0.05$). This contrasts with independent findings that ATP swab RLU correlates with colony counts when directly inoculated (Omidbakhsh, Ahmadpour et al. 2014). Maybe there is no relationship, or it may be that the swab in neat urine does not accurately assess bacterial load in the urine, or performs differently to an ATP assay performed in the lab.

11.5.4.3 Newer contributions from the literature

Since this study, ATP research has continued in other areas. ATP swabs continue to be tested in hospital environments to assess bacterial load and cleanliness on surfaces (Nante, Ceriale et al. 2017).

The field of immunohistochemistry in relation to the urothelium has advanced considerably in recent years (Zupancic and Romih 2021). There is thought to be a uroepithelial-associated sensory web (Apodaca, Balestreire et al. 2007), where channels and receptors in the uroepithelium receive sensory inputs such as hydrostatic pressure and binding of mediators including ATP. These inputs may lead to responses such as turnover of umbrella cells and release of neurotransmitters and other mediators which communicate to underlying tissues. This would lead to the coordinated function of the bladder during cycles of filling and voiding.

There are different purinergic receptors which respond to ATP. Purinergic type 2 receptors are labelled P2, and are grouped into P2Y G-protein coupled receptors and P2X ligand-gated ion channels (Savio, de Andrade Mello et al. 2018).

P2X7 is involved with both innate and adaptive immune responses and can have a variety of hypothesised effects in infection. Animal studies suggest that pressure-induced extracellular ATP release in urothelial cells is amplified by P2X7 receptor activation and ATP-induced-APT release; this amplified ATP signal then brings about the caspase-1 inflammatory response, which initiates an inflammatory response via activation of pro-inflammatory cytokines (Dunton, Purves et al. 2018).

In a recent mouse model of UTI, bacterial infection led to activation of spinal neurons through the bladder efferent pathway, leading to an enhanced micturition reflex; it was found that ATP signalling, and P2X2 and P2X3 receptors were critical to this pathway. (Ueda, Kondo et al. 2020)

Bladder stretch appears to cause release of ATP, which interacts with purinergic receptors (P2X3) on subepithelial afferent nerves which transmit sensation to the central nervous system (Vlaskovska, Kasakov et al. 2001).

The complexity of the urinary tract and the interplay between fluid dynamics and pathology, and how research models may start to approach this, is described in Murray et al 2021 "recurrent urinary tract infection: a mystery in search of better model systems". Computational fluid dynamic simulations have demonstrated that shear stress experienced by cells is considerably higher in the urethra than in the bladder (Jin, Zhang et al. 2010) (Atesci, Aydogdu et al. 2014). Bladder umbrella cells during normal voiding may experience shear stress below the level required to activate shear-induced ATP release, whereas catheterisation may alter this flow pattern.

Studies in human subjects are also required to aid translational understanding. A urological study found that basal ATP release from the urothelium was higher in patients with overactive bladder with pyuria than those without pyuria and asymptomatic controls (Contreras-Sanz, Krska et al. 2016). Urinary ATP has been found to vary with time since last void (McLatchie 2018). Urinary ATP concentration was found to be higher when voiding more frequently, not as a simple function of urine dilution. Time since last void was not collected in this study.

Explanations for the high ATP in CSUs compared with MSUs could include trauma to the urothelium, or be related to the voiding pattern that occurs following catheterisation. If there were an inflammatory reaction following catheterisation, perhaps involving P2X7 or other inflammatory purinergic receptors, it might follow that cytokines would also rise. However, it was seen that IL-8 and lactoferrin were lower in CSUs, and undetectable IL-6 was more common in CSUs. This was a cross-sectional study with urine collected at a single time-point; there would need to be a series of experiments measuring ATP and cytokines at time points following catheterisation to determine if there was a cytokine response to catheterisation with a different time-course. It is interesting that there was no clinical correlation between urinary ATP and urinary cytokines, or any other markers of urinary pathology, in these studies, despite strong basic science and translational evidence about the role ATP plays in urothelial signalling.

The focus of this thesis is about the relationship between bacteria, inflammation and pregnancy outcomes. While the story of MSU-CSU ATP and

what this tells us about the uroepithelial response to catheterisation in pregnancy is an interesting one, it is a slight aside to this goal. However, this work may be the largest series of urinary ATP measurements in pregnancy.

11.5.4.4 Conclusions

This is, to our knowledge, the first study of urinary ATP, measured by rapid swab test, in pregnancy. There was no correlation with colony counts or other microbiological outcomes, or with clinical measures or outcomes. There was however a significant difference between voided and catheter specimens which suggests ATP is released by catheterisation, although the mechanism remains unclear.

The role of ATP as a clinical biomarker for infectious urinary tract pathology is not supported by this data.

11.5.5 Enhanced culture

11.5.5.1 Introduction

I have discussed sediment culture findings in detail previously. In this section I will critically appraise the use of the enhanced culture, and consider recent findings from the literature.

11.5.5.2 Data from this study

Enhanced culture detected bacteriuria in 271/273 (99.3%, 95% CI 97.1-99.9) voided samples of urine, compared with 104/266 (39.1%, 95% CI 33.3-45.3) by standard culture (including mixed growth and non-significant growth) and 22/266 (8.3%, 95% CI 5.4-12.4) positive by standard culture.

In CSU samples, sediment culture detected bacteriuria in 15/34 (44.1%, 95% CI 27.6-61.9) samples, compared with 3/33 (9.1%, 95% CI 2.4-25.5) by standard culture and 2/33 (6.1%, 95% CI 1.1-21.6) standard culture positive.

In neither MSU nor CSU samples was there a positive standard culture with negative bacteriuria by sediment culture.

There was a quantitative relationship between categories of standard culture and total colony counts at sediment culture, supporting the idea that the tests measured something similar. There was no relationship between colony counts and study groups, or with symptoms. Total colony counts were higher in those who delivered preterm ($p=0.001$), by an order of magnitude around 10. There was no relationship between colony counts and cell counts or cytokines.

The number of different isolates was higher than in previous studies in non-pregnant patients (Khasriya, Sathiananthamoorthy et al. 2013), with a median of 3 (95% CI 3-3) for voided samples, or mean 3.1 (95% CI 2.9-3.2) versus a mean of 1.36 (95% CI 1.2-2.1) in non-pregnant patients. There were no differences in number of isolates between study groups, except CSU which was median of 0 (95% CI 0-1).

Species were a combination of uropathogens and non-uropathogens without significant between-group differences, except for women at high risk of

preterm birth where there was a suggestion of a greater range of isolates identified compared to the other two groups.

The method used to identify organisms was laborious and for larger-scale identification of polymicrobial organisms for a larger study would need to be replaced by a faster and more accurate technology such as MALDI-TOF MS, or supplemented by sequencing data.

It should be noted that API testing for lactobacillus may be flawed (Boyd, Antonio et al. 2005). At the start of this study the significance of different lactobacilli species (identified using next generation sequencing) for preterm birth had not been widely published and so methods to optimise yield of lactobacillus were not researched.

As discussed above, the MSU-CSU data casts doubt on the hypothesis that voided samples are susceptible to contamination and CSU samples are not, rather they may be sampling different portions of the urinary sediment (Collins, Sathiananthamoorthy et al. 2020).

11.5.5.3 Newer contributions from the literature

Chromogenic agar has been shown to have benefits over conventional agar in terms of facilitating identification of mixed growth, as well as at a laboratory scale for reducing time required for identification of species, with similar results (Perry 2017).

The chromogenic agar used in this study, ChromID® media, has been reported to inhibit the growth of normal peri-urethral flora. Whilst concerns have been raised about detection of *Group B Streptococcus* (GBS) with chromogenic agar (Yarbrough, Wallace et al. 2016), a study using CPS3 (used for the primary cultures in this study) found that detection of gram positive bacteria, including GBS, was as good as with CBA (Ciragil, Gul et al. 2006). From 2016 our laboratory switched to the newer CPS Elite agar (CPSE), which was reported to have increased sensitivity for detection of uropathogens compared with its predecessors (Rigaill, Verhoeven et al. 2015).

Sediment culture to genus level was also used as part of the study comparing different urine sampling methods in LUTS patients and controls (Collins,

Sathiananthamoorthy et al. 2020). Growth using sediment cultures was greater using MSU or Peezy sampling methods compared with CSU. Microbial distributions for the three methods were similar, with dominant isolates being *Enterococcus* spp and *E. coli*. It was noted that there was genus overlap between patients and controls.

In a non-pregnancy comparison of standard culture and next-generation sequencing (NGS) (McDonald, Kameh et al. 2017), 44 symptomatic patients were randomised into treatment using standard culture (n=22), and treatment using NGS (n=22), with 22 asymptomatic controls. There were positive standard cultures in 13/44 symptomatic patients compared with 44/44 positive NGS; in controls there were 5/22 positive standard cultures and 21/22 positive NGS. Comparing symptom scores following treatment with a single targeted antibiotic for 7 days, the NGS group had greater improvement in symptom scores. This suggests a potential role for microbiome approaches in UTI management.

In another non-pregnant study of 33 new patients with LUTS and 29 asymptomatic controls (Sathiananthamoorthy, Malone-Lee et al. 2019), a comparison of enhanced culture (using the same method as this study) with sequencing showed that sequencing was superior to culture for bacterial detection and that culture failed to detect bacteria, including uropathogens.

Maternal bladder microbiome has been studied using both an enhanced culture method and 16S sequencing in 51 pregnant women requiring catheters for medical indications (Jacobs, Thomas-White et al. 2017). CSUs were used to avoid contamination. Standard culture did not identify bacteriuria, while enhanced culture identified bacteriuria in 34/51 (66.7%), and sequencing in 35/51 (68.6%). This was similar to the proportion in CSUs in our study, 15/34 (44.1%), as above. The combination of methods identified bacteriuria in 41/51 (80%) women. The same conclusion about urotype was reached in 33/51 (64%) of participants, or 23/28 (82%) of those with positive results from both methods. While 16S sequencing could identify non-culturable species, enhanced culture was able to identify microbiota to species level (creating 14 urotypes), compared with 16S sequencing which could identify only to a genus level (creating 6 urotypes).

Another maternal urinary microbiome study compared second-trimester samples in 49 women who delivered preterm (<37 weeks) and 48 socio-economically-matched controls (Ollberding, Volgyi et al. 2016). No difference was found in taxa richness, evenness, or community composition. Certain operational taxonomic units (OTUs) were enriched among cases, including *Prevotella*, *Sutterella*, *L. iners*, *Blautia*, *Kocuria*, Lachnospiraceae, and *S. marcescens*.

Urinary and vaginal microbiome outside pregnancy have been shown to be significantly associated, in particular *Lactobacillus spp.* was predominant in both (Komesu, Dinwiddie et al. 2020). In light of findings from vaginal microbiome studies about different *Lactobacillus spp* and preterm birth (Kindinger, Bennett et al. 2017), it is frustrating that we were unable to identify *Lactobacillus* to species level in this study.

Urinary microbiome in pregnancy may have clinical applications in future. It is reassuring that there is some relationship with enhanced culture methods. Understanding this relationship between enhanced culture and sequencing methods may help apply new understandings about urinary microbiome more cost-effectively and in low-resource settings.

11.5.5.4 Conclusions

Sediment culture, as a form of enhanced culture, is an accessible and affordable way to study polymicrobial growth in pregnancy. It supports findings that urine in pregnancy is not sterile, and that polymicrobial growth is common. This study showed greater microbial abundance in women experiencing preterm birth. Further work is required to compare sediment cultures with obstetric outcomes, particularly preterm birth, to understand how this is relevant. Understanding how it relates to the urinary microbiome, and in turn to the vaginal / placental / amniotic / gut microbiomes may also be important in future research. If a simple urine test can provide information about those other microbiome niches then this may function in the future as a screening test, if significant microbiome findings are present.

11.5.6 Urinary cytokines, chemokines and anti-microbial peptides

11.5.6.1 Introduction

Urinary cytokines were studied as they had shown potential as biomarkers for UTI, although with limited data in pregnancy. Here I summarise the data, critically appraise the tests, and consider recent literature.

11.5.6.2 Data from this study

Comparing study groups, the cytokines did not discriminate well. In the Abdominal pain study, the only between-group differences were for Lactoferrin ($p=0.006$). Median lactoferrin was 6.0ng/ml (95% CI 3.5-19.2) for pain cases compared with 2.2ng/ml (95% CI 2.2-3.3) for normal controls. LLOD was 2.2ng/ml, and the proportion below LLOD also differed between groups ($p=0.016$). There were no differences once lactoferrin was normalised. A striking finding was higher IL-8 and lactoferrin in women diagnosed with UTI compared with those presenting acutely but not diagnosed with UTI, despite no difference in the proportion with positive standard culture. This illustrates the importance of respecting the traditional clinical method. This applied also when normalised for protein. It should be noted that this was a clinical diagnosis based on symptoms, examination and dipstick, without benefit of microscopy and culture results.

There were no between-group differences for any cytokine in the PTB study.

In the MSU-CSU study, IL-8 and Lactoferrin were higher for MSU than CSU, whether normalised or not. Proportion with IL-6 below LLOD was lower for CSU although no other difference in distribution. Conversely, protein was higher in CSU samples. A comparison of voided and catheter specimen urinary cytokines is not previously documented in the literature. Higher IL-8 and lactoferrin in the MSU samples correlates with higher white cells and epithelial cells, and higher colony counts on enhanced culture. It suggests that cytokines are associated with the urinary sediment. Higher protein in CSU samples has potential implications for pre-eclampsia diagnosis and should be further investigated with paired protein: creatinine ratios in MSUs versus CSUs.

In the pooled data, there were no differences between any cytokines for either standard culture positive versus negative, or preterm birth versus term birth. This is a little surprising, given that total colony counts were higher for standard culture positive, and for preterm birth.

As the cytokines for the cerclage cases in MSU-CSU study appeared higher than caesarean cases, I compared cytokines across the three studies in those who had had cerclage already, those who went on to have cerclage, and those without cerclage. There was no difference between the three groups for any cytokine with or without normalisation.

The pooled values determined in this study (see Table 10-4 in chapter 10.3.2) were interesting to compare with previous studies in pregnancy (see Table 4-1 in chapter 4.7.10). Note that in none of those studies was the cytokine concentration normalised. The range of IL-6 was comparable with the study comparing cranberry versus placebo, although median values appeared higher in that study (Wing, Rumney et al. 2010). In the study comparing women with intra-amniotic infection with those without, a cut-off of IL-6 6pg/ml was selected to predict intra-amniotic infection (Dulay, Buhimschi et al. 2015). In our study, this would have applied to 55/273 (20.1%) of participants. This cut-off did not differentiate by standard culture, preterm birth or maternal sepsis. In one study, IL6 was undetectable in the urine although LLOD was not stated (Basso, Gimenez et al. 2005).

The two studies examining IL-8 compared pregnant women with asymptomatic bacteriuria with controls. The median values in our study were similar in magnitude to controls in one study (Basso, Gimenez et al. 2005), but without an effect for positive standard culture. The median values in the other study were far higher (Shelton, Boggess et al. 2001). It is not clear why this would be the case.

No previous studies examined lactoferrin in pregnancy, so the values here are the first description of urinary lactoferrin in pregnancy. In the 1999 study of UTI outside pregnancy, the mean lactoferrin in patients with UTI was 3300ng/ml +/- 646 (SEM) compared with 30ng/ml +/- 3 for healthy controls and 60ng/ml +/- 15 for patients without UTI (Arao, Matsuura et al. 1999). In a recent study in girls,

mean lactoferrin in controls was 670ng/ml +/- 320 compared with 1390 +/- 510 in those with UTI (Fatah 2018). In a recent study of patients with overactive bladder symptoms compared with controls, lactoferrin in patients was higher than controls with average around 30ng/ml (imprecise as value taken from graph) (Gill 2016).

The values found in this study could be used to contribute towards typical values in pregnancy. This is particularly relevant for lactoferrin, as not previously described in pregnancy.

It has been suggested that normal levels for cytokines are difficult to establish, given that they may vary in disparate body fluids, in different parts of the body, and research derives normal levels for a specific control group compared with an experimental group, rather than by examining healthy people representative of a wider population (Monastero and Pentyala 2017). In a study of 61 healthy non-pregnant women aged 18-30, median urinary IL-6 was 0.20pg/ml (IQR 0.10-0.41) and IL-8 was 7.49pg/ml (IQR 1.95-32.98) (Nobles, Bertone-Johnson et al. 2015). In a study of urinary cytokines and chemokines through the menstrual cycle, values were highest at menses but detectable through the cycle in most women. Median IL-6 at menses was 9pg/ml (IQR 5-19) and IL-8 was 64pg/ml (IQR 25-202) (Whitcomb, Mumford et al. 2014). The median results from our study lie between these two examples. This does not suggest a difference in urinary cytokines in pregnancy compared with outside pregnancy, however a longitudinal study would be needed to assess this question.

The relationship between biomarkers may help understand pathophysiology. Correlations have been described above. There was a moderate correlation between white cell counts, epithelial cell counts and urinary cytokines, most marked for IL-8. This is consistent with past studies in non-pregnant patients (Ko, Mukaida et al. 1993) (Oregioni, Delaunay et al. 2005).

Contrary to previous research (Hedges, Stenqvist et al. 1992), I found no relationship between urinary IL-6 and bacteriuria by standard culture, or by sediment culture. There was no relationship between urinary IL-6 and symptoms.

There was a wide variance in urinary cytokines which was not explained by study groups, standard culture, or preterm birth. The finding that the two highest values of IL-6 in the pooled data were in women in labour at term, and the third highest in a woman with ruptured membranes at term, suggests that urine samples around onset of labour may be contaminated with amniotic fluid or cervico-vaginal fluid, alternatively may represent a systemic or local inflammatory response. Either way, MSU IL-6 would not be a safe method to assess for urinary infectious pathology in this context.

There was a gap of up to two years between collecting samples and measuring cytokine levels in the supernatant. It is not known how this may have affected the result. However, it has been demonstrated that cytokines, including IL-6 and IL-8, are stable when stored at -80 degrees for two years, with degradation with multiple freeze-thaw cycles (de Jager, Bourcier et al. 2009).

A substantial proportion of lactoferrin samples, 147/306 (48.0%), were below the lower limit of detection (LLOD). Although this did not differ between study groups in any of the experiments, it may limit interpretation of results. The assay was validated for urine; however, a more sensitive assay may be preferable. At the time of performing the study, other more sensitive assays were not available, however there are now more assays on the market and some have a range as low as 20pg/ml (such as Abcam ab229392 Human Lactoferrin CatchPoint, released in 2018), compared to the assay used for this study which had a range starting from 3125pg/ml.

On the other hand, very few samples, 14/307 (4.6%), were below the LLOD for IL-8, using a sensitive assay. Previously it was reported that there is no IL-8 in normal healthy urine outside pregnancy (Agace, Hedges et al. 1993). These data suggest urinary IL-8 is almost ubiquitous in pregnancy.

I discussed the issue of normalisation previously. I had decided to analyse both non-normalised and normalised values, on the basis that one was not theoretically preferable, both had been used in the literature, and one might be more evidently useful when compared against outcomes. In these data, where there was a significant difference in urinary cytokines, this was consistent

whether normalised or not, apart from raised lactoferrin in pain cases in the Abdominal pain study.

11.5.6.3 Newer contributions from the literature

Cytokines in the urine may have arisen from several sources such as the blood in systemic inflammation (Nobles, Bertone-Johnson et al. 2015); from the kidney in ascending infection (Ko, Mukaida et al. 1993), or from kidney pathology such as nephrotic syndrome (Al-Eisa, Al Rushood et al. 2017). They may be released by white cells in the urine or by epithelial cells in the bladder (Hedges and Svanborg 1994). It is likely that bladder epithelial cells play a key role in defence against UTI (Hedges and Svanborg 1994) and so the source of cytokines from epithelial cells is of particular interest (Wu, Miao et al. 2017), (Abraham and Miao 2015).

Urinary IL-6 was studied in elderly patients comparing patients with asymptomatic bacteriuria (n=38) against negative cultures (n=57), symptomatic UTI (cystitis or pyelonephritis, n=49) and indwelling catheters (n=18, of whom 17/18 had significant bacteriuria and 10/18 had polymicrobial growth) (Kjolvmark, Tschernij et al. 2016). Asymptomatic bacteriuria was strictly defined by Kass criteria and excluded mixed growth. They found significantly higher median levels of urinary IL-6 in the UTI group (150pg/ml) compared with asymptomatic bacteriuria (4pg/ml) and negative controls (4pg/ml). IL-6 was significantly higher in patients with a permanent indwelling catheter compared to patients with asymptomatic bacteriuria without a catheter.

Urinary IL-6 related to bacteriuria but not symptoms in elderly nursing home residents (Sundvall, Elm et al. 2014). IL-6 was higher in those with positive urine cultures (median uIL-6 2.5ng/l, IQR 1.0-5.7) than those with negative cultures (1.3ng/l, IQR 0.6-2.8). This studied non-specific symptoms rather than LUTS.

A 2017 study of urinary microbiota in diabetic patients without a UTI diagnosis, found that detection of urinary IL-8 was associated with a different microbiota profile to those without detectable IL-8 (Ling, Liu et al. 2017).

In infants with UTI, IL-6 and IL-8 (normalised for creatinine) could not distinguish upper from lower UTI, or non-febrile UTI from asymptomatic bacteriuria, but could distinguish febrile UTI from asymptomatic bacteriuria (Krzemien, Szmigielska et al. 2016). It was hypothesised that the cytokines were responsible for the fever, and pyuria, by attracting neutrophils.

In patients with spinal cord lesions giving recurrent UTI/asymptomatic bacteriuria, urinary IL-6 was good at distinguishing UTI (defined as symptoms plus positive cultures) from asymptomatic bacteriuria, whereas IL-8 was not (Sunden, Butler et al. 2017). IL-6 above 25ng/l was associated with more severe symptoms.

It is interesting that in the current study, neither IL-6 nor IL-8 were associated with symptoms.

Inflammatory cytokines have continued to be studied in the context of preterm birth.

Plasma IL-6 was higher at 18 weeks in women with a short cervix at 16-24 weeks than in women with a long cervix (Venkatesh, Cantonwine et al. 2016).

Measurement of both IL-6 and IL-8 in cervico-vaginal fluid has been studied as a non-invasive approach, and has shown some diagnostic value in both PPRM and preterm labour (summarised in (Monastero and Penttala 2017)). However, cervico-vaginal IL-6 and IL-8 did not predict cervical shortening in women at risk of preterm birth (Chandiramani, Seed et al. 2012).

Serum IL-6 was higher in those with threatened preterm labour who went on to deliver preterm than those who did not (Herrera-Munoz, Fernandez-Alonso et al. 2017). The threatened preterm labour group were also more likely to have a history of urinary tract infection, although the absolute difference was small (21/64 versus 19/61).

Amniotic fluid IL-6 was associated with preterm birth in women with intact membranes (Chaemsaihong, Romero et al. 2016) as well as preterm prelabour rupture of membranes (Romero, Yoon et al. 1993).

A recent study examined the role of lactoferrin in uropathogenic *E. coli* in a mouse model and a cell culture model using human bladder epithelial cells

(Patras, Ha et al. 2019). Lactoferrin was found to be enriched in urinary exosomes of infected mice. Human bladder epithelial cells were confirmed as a source of lactoferrin following infection; this was a new finding. Exogenous lactoferrin reduced bladder bacterial burden and neutrophil infiltration in the mouse model.

Lactoferrin administration has been shown to reduce secretion of IL-6 and IL-8 in a cell culture model of *E. coli* infection (Haversen, Ohlsson et al. 2002).

Vaginal lactoferrin has been associated with the cervico-vaginal mucosal defence and is increased in dysbiosis (Valenti, Rosa et al. 2018). Vaginal lactoferrin has been studied as a potential treatment to prevent preterm birth in asymptomatic pregnant patients potentially at risk of preterm birth owing to a borderline short cervix of 25-29mm (Locci, Nazzaro et al. 2013). In a randomised study comparing lactoferrin with no treatment, IL-6 in vagino-cervical secretions and cervical length were measured, with a difference in the two groups following treatment, suggesting potential future therapeutic potential.

This study only examined three cytokines/chemokines. Other urinary cytokines may have potential as UTI biomarkers. For example, a murine model found IL-1 β , CXCL1, and CCL2 had strong predictive value for identifying mice that did not get infected, despite confounding variables (Armbruster, Smith et al. 2018). The same study also noted different cytokine response depending on the infecting organism. Another murine study found that both age and parity affected cytokine response to infection, specifically IL-1 β , IL-12(p40), and Monocyte Chemoattractant Protein-1 (MCP-1), and that *Streptococcus agalactiae* induced other cytokines, including IL-17, regardless of host factors (Sullivan, Carey et al. 2016).

11.5.6.4 Conclusions

Urinary cytokines in pregnancy in these studies were not related to standard culture, enhanced culture counts, organisms identified, urinary symptoms, or preterm birth.

They correlated with fresh unspun microscopy cell counts, which is important as these have been found to be useful in diagnosing flare of chronic UTI in

patients with chronic UTI. It also supports other pathophysiological studies linking cells with cytokine release.

MSU-CSU study showed that IL-8 and lactoferrin were higher in MSU than CSU samples.

This is the first study to describe urinary lactoferrin in pregnant women.

Further studies are required to better understand variation in urinary cytokines over time, gestation and in response to events such as symptomatic UTI and antibiotic treatment for UTI. Future work may benefit from more intense metabolomics approaches to understand complex cytokine interactions, and well-defined clinical outcomes.

11.5.7 Urothelial cell analysis

11.5.7.1 Introduction

Analysis of shed urothelial cells has helped understanding of the pathophysiology of chronic UTI outside pregnancy. Here I summarise the findings and critically appraise the role of the test in pregnancy, along with a review of more recent literature.

11.5.7.2 Data from this study

Median clue cell proportions were not different between groups in any study. Proportions cannot be compared between studies as different methods were used.

The presence of any clue cells can be compared across the studies, however. 111/157 (70.7%, 95% CI 62.8-77.6) patients in the Abdominal pain study had clue cells compared with 72/80 (90%, 95% CI 80.7-95.3) of those in the PTB study and 32/34 (94.1%, 95% CI 79.0-99.0) voided samples in MSU-CSU study.

When I compared the method of counting to 100 cells versus counting to 20, the correlation between them was strong ($r_s=0.826$, $p<0.001$, $n=69$). If I excluded the PTB study slides that I had analysed early on in the study, the correlation was stronger ($r_s=0.929$, $p<0.001$, $n=59$). This suggests that there was a learning effect whereby my counting was more consistent once I was performing it regularly. I did not double-count the slides owing to time restraints, and have not assessed inter-observer variability, although this should be assessed to validate the study method.

11.5.7.3 Newer contributions from the literature

A previously-described study comparing sampling method in patients with chronic LUTS and controls, found that the proportion of uroplakin-3 positive cells was not different, whether standard MSU, CSU or the MSU device Peezy (Collins, Sathiananthamoorthy et al. 2020). Contamination should not be assumed from the presence of epithelial cells unless uroplakin-staining has confirmed that they do not arise from the urinary tract.

Clue cell proportions were higher in 24 (non-pregnant) patients with overactive bladder symptoms compared with 22 asymptomatic controls in a longitudinal study (Gill, Kang et al. 2018). Controls had lower symptom scores, and lower scores for bacterial load, white cells and epithelial cells. Clue cells in this setting were felt to be a marker of chronic infection.

A urine-dependent human urothelial organoid was developed and infected with uropathogenic *E. faecalis* (Horsley, Dharmasena et al. 2018). This confirmed the previous in-vivo hypothesis that bacteria-associated urothelial cells (clue cells) are shed in response to infection (Horsley, Malone-Lee et al. 2013).

11.5.7.4 Conclusions

We have demonstrated the urothelial origin of shed epithelial cells in pregnancy. We have also demonstrated that shed epithelial cells have associated bacteria, in differing proportions. This supports sediment culture as a type of enhanced culture. This also supports the hypothesis that urinary sediment is of pathophysiological interest in understanding urinary pathology.

There was no relationship between proportions of associated bacteria and other measurements, including symptoms, fresh microscopy cell counts, urinary ATP, standard culture, enhanced culture, and urinary cytokines. There was no relationship with demographic features or obstetric outcomes. This suggests that it is not a useful marker of UTI in pregnancy.

11.6 What does it mean to diagnose UTI?

There has been a recent change towards not treating asymptomatic bacteriuria outside pregnancy (Nicolle 2016). Asymptomatic bacteriuria may be protective. Bacterial interference may prevent UTI, with evidence from *E. coli* and *Lactobacillus* (Darouiche and Hull 2012). Following the Dutch asymptomatic bacteriuria study (Kazemier, Koningstein et al. 2015), a reduction in asymptomatic bacteriuria treatment in obstetrics may occur.

Our analysis of symptoms compared with microbiological and host response markers challenges the definition of asymptomatic in pregnancy. Most had some symptoms, with little correlation with outcomes, microbiology, or host response. Any study on asymptomatic bacteriuria in pregnancy should explore symptoms as standard.

This next issue concerns dichotomisation. The Kass criteria was a cut-off chosen for statistical reasons; this nuance may be lost in modern practice. In all tests studied here, there was a wide spectrum in symptoms, microbiology and host response markers with no evidence of dichotomisation.

Clinicians need practical guidance. Language influences clinical reasoning. Current standard culture interpretation is 'infection' versus 'not infection'. An alternative might be 'more likely to benefit from antibiotics' versus 'less likely to benefit from antibiotics'. Despite dichotomising, it might encourage a focus on outcomes rather than laboratory findings.

In chapter 3.3.4 I discussed an alternative microbiological perspective, that of the damage-response framework (Pirofski and Casadevall 2018). Given the variety in bacteria, host responses and symptoms this would seem a potential model for understanding UTI diagnosis. The damage-response framework has started to be applied to urinary tract pathology (as described by (Long 2017)).

I discussed statistical epistemological issues in the introduction. In particular, the potential benefits of a Bayesian approach rather than classical frequentist statistics, that considers the prior probability. Although I have not used this analytical method in this thesis, this may be worth exploring in future.

Since Kass defined UTI, no other gold standard has been identified. New tests have always been tested against the Kass standard. With recent evidence

challenging the underlying assumptions, it may be time for a new approach to UTI in pregnancy.

Cardiology underwent a change when cardiac biomarkers were introduced into definitions of myocardial infarction (MI) (Goodfellow 2012). A dichotomous ECG-based definition became a spectrum involving varying combinations of symptoms, ECG changes and cardiac biomarkers. While the classical MI (ST-elevation MI) remained a category, inclusion of other syndromes acknowledged risk and benefit of treatment for alternative presentations. This could be adapted for urinary pathology in pregnancy. This could range from acute UTI syndrome (symptoms of UTI), to non-culture-positive UTI syndrome (symptoms plus evidence of inflammation or culture findings that might be consistent with UTI, such as below-threshold or mixed growth), to culture-positive UTI syndrome (symptoms plus 10^5 CFU/ml culture evidence). This would allow comparison with previous research, without excluding women with symptoms or non-positive cultures.

Another approach, inspired by obstetric growth charts, would be to use probability centiles. Hypothetically, culture counts of 10^5 CFU/ml might represent the 95th centile of colony growths, while 10^4 CFU/ml may be 80th centile. A 95th centile result could be treated on that basis alone, whereas an 80th centile result would need additional evidence of UTI such as symptoms, biomarkers or combination of pathogens. This could allow customisation for pregnancy, non-pregnancy, men, renal anomalies etc. With automated laboratory processes, this might be more feasible than it would have been with manual counts.

It would be useful to explore these concepts further with future study designs.

11.7 Do current tests correctly identify patients who need antibiotics?

This observational study was not able to examine whether current tests correctly identified patients needing antibiotics – this would require an interventional study design.

In the pooled data, 64/273 (23.4%) participants had at least one positive MSU in the index pregnancy, with no difference between studies (Chi square = 1.55,

$p=0.46$) and likely would have been offered antibiotics. Unfortunately, detailed antibiotic prescribing data were not collected. This may be an important confounder, particularly when looking at subsequent outcomes for mother and baby.

11.7.1 Case example

Let us take an example of a patient in this study – a recruit to the PTB study with previous preterm birth, who was 17 weeks gestation at recruitment. She was high risk for preterm birth having previously had a late miscarriage and an early preterm birth, and no term deliveries. She was a smoker, with non-white ethnicity, in the second lowest decile of socio-economic deprivation, with a history of recurrent UTI in pregnancy. Her booking urine culture showed 'no significant growth'. On the day she was recruited, urine dipstick was 1+ protein and trace leucocyte esterase, and standard culture was reported as 'screening culture negative', with no standard microscopy performed. By routine care, she would not have been considered to have a UTI. However, her Artemis LUTS inventory score was 32 (the highest value in this study). Daytime frequency was 10-11/day and nocturia >6/night with incontinence 3-4 times per day and 0-1 times per night. On fresh microscopy she had urinary white cell count of $100\mu\text{l}^{-1}$, red blood cells of $660\mu\text{l}^{-1}$ and epithelial cells of $800\mu\text{l}^{-1}$. ATP was relatively low at 1041RLU. Urinary cytokines were raised with IL-6 56.9pg/ml, IL-8 23160pg/ml (the highest value in this study), Lactoferrin 803.9ng/ml. Her sediment culture total colony count was 2699CFU/ml, and 2 isolates were identified in her urine, with colony counts of *Corynebacterium propinquum* 2555CFU/ml, and *Staphylococcus haemolyticus* 145 CFU/ml. She had clue cells present at 5%.

She had a history-indicated cerclage inserted at 18 weeks. She had 4 subsequent standard cultures sent during the pregnancy; these were all 'screening culture negative'. Her shortest cervical length was 27mm, her highest fetal fibronectin was 24ng/ml and she went on to labour spontaneously at term, delivering at 37+4 days.

Given her history, urinary symptoms, fresh microscopy findings and urinary cytokines, was it safe to define her as not having a UTI? She had a cervical

cerclage 9 days after recruitment to this study; it is not known what the natural history of the pregnancy would have been without this.

11.8 Revisiting Kass

Our work has demonstrated a problem with the Kass culture as a gold standard for urinary pathology in pregnancy. It is an oversimplification of a complex situation that we are far from understanding. What is normal and what is pathological in urinary tract infection in pregnancy? How does urinary pathology relate to pain, to pyelonephritis, and preterm birth? It is worth revisiting Kass' work with this perspective.

Ultimately, Kass described a picture of chronic bacteriuria in pregnancy which commenced in the first trimester. In women with a high burden of bacteriuria, the risk of progression to pyelonephritis was high. In these women, continuous antibiotic removed this risk entirely (Kass 1960).

The descriptions of method in Kass' work are limited. We know the numbers involved – which were small. We know the colony counts. We know that he was concerned about contamination and required significant bacteriuria to be confirmed on two consecutive samples. What is not clear are the features that would be required for any modern interventional study, including details of recruitment, the demography of the samples, random allocation, and blinding.

Did Kass use a different method of culture to what we use now? This is not clear. He did report 95% correlation of colony counts between catheter samples and voided samples (Kass 1957), compared with 80% between repeat voided samples. This is surprising given that we found a much lower colony count in catheter specimens, both in pregnancy and in chronic LUTS patients (Collins, Sathiananthamoorthy et al. 2020).

Did Kass use an enriched sample of participants? He did not describe his recruitment technique. It is implied that all patients attending the prenatal clinic in a single centre were included in the studies (Kass 1960). In the initial sample the rate of bacteriuria was 10% at term, although this was lowered to 6-7%. Pregnant patients with bacteriuria were allocated to placebo and treatment on an alternating basis. There is no description of blinding to

placebo. Note that bacteriuria required two consecutive above-threshold cultures; this is likely to represent a more extreme end of the spectrum of bacteriuria compared to a single measurement.

Did Kass use a different method of treatment? Yes – he essentially used continuous antibiotics. The initial plan was to give 0.5g sulfamethoxypyridazine daily for one or two weeks; if bacteria were still present they were treated according to sensitivities. It was however found that bacteria returned, or patients developed symptomatic UTI, and so all treatment was carried through to term (Kass 1960).

Did Kass have different outcome criteria? Firstly, the outcome he described was pyelonephritis during the last trimester or in puerperium, which he considered to last three months. In the original study, 90 women were described, with 48 receiving placebo, of whom 20 developed pyelonephritis, and 42 being treated, with none developing pyelonephritis (Kass 1960). There is no explanation for the lower number of women being treated, given they were allocated alternately. A subsequent description of the data, from 1962, describes 179 women, with 95 receiving placebo, and 84 treated (Kass 1962). There is no explanation for the increased numbers, or the lower number of treated women. 18 of the placebo-treated women developed pyelonephritis, compared with none of the treated women. What is interesting is that women were followed up post-partum. Of the placebo-treated women, a further 13 developed pyelonephritis. However, in the treated women, 28 had recurring bacteriuria, and 7 developed pyelonephritis.

The description of premature births is vague. The same numbers were used to describe the impact on prematurity. Of the 95 placebo-treated women, 26 were born prematurely, although this included three sets of twins. There were 13 perinatal deaths, including twins, congenital malformations and a death at seven weeks. In the treated group, 6/84 were born prematurely with no perinatal deaths. This was compared against 1000 non-bacteriuric women, with 88 premature births and 20 perinatal deaths. The source of data about the 1000 non-bacteriuric women is not stated.

We are not the first to challenge the Kass dogma (which I should add does not represent the nuanced interpretation in Kass' own papers). This has been questioned decades ago (Maskell 1989), through the work of the UCL chronic UTI lab (e.g. (Kupelian, Horsley et al. 2013) and more recently following discovery of the urinary microbiome (Brubaker and Wolfe 2015). However, it has been less common to question this in pregnancy, where the relationship Kass described between asymptomatic bacteriuria and pyelonephritis has been so well respected.

While pyelonephritis remains an important problem in pregnancy, it is far rarer than it was in Kass' studies (Kazemier, Koningstein et al. 2015). There was no episode of pyelonephritis in this study, in 273 women. Is this because anyone with asymptomatic bacteriuria in the first trimester was successfully treated with one week of antibiotics? Or are there other factors at play that make a comparison with Kass' work inappropriate? The association between asymptomatic bacteriuria and preterm birth has also been challenged, as described previously. Again, it is not clear why this relationship appears far weaker than it did then.

An important message from this is that outcomes are what matter to patients and clinicians. Kass' work was driven by improving outcomes. For more than half a century, approaches to diagnosis of UTI in pregnancy have focussed on comparison against a proxy outcome of standard culture. Outcomes have been measured secondarily, if at all. It is time to revisit Kass' philosophy and research urinary pathology from the point of view of maternal and neonatal benefit and harm.

11.9 Conclusions / Returning to the research questions

In this chapter, I revisited the problem of urinary pathology in pregnancy taken at the start of this thesis. I demonstrated the importance and complexity of problems affecting urinary pathology and its treatment. I evaluated data from the three studies in this body of work against eight assumptions that are made in diagnosing UTI in pregnancy, to demonstrate that they are mostly fallacious. I appraised the alternative diagnostic approaches that I studied here, to see where they add to our understanding of urinary pathology. Finally, I considered

some alternative diagnostic approaches. While I have not been able to generate a new, testable hypothesis of urinary pathology in pregnancy on the basis of 273 pregnant women, I have established the need to re-evaluate our diagnostic approaches based on outcomes rather than arbitrary rules.

12 Conclusion

12.1 Why this work matters

Urinary pathology in pregnancy is important because it is common, it affects women's quality of life, and it is associated with negative outcomes for mother and baby, including pyelonephritis, which can lead to sepsis or intensive care admission, and preterm birth, which is a major cause of infant morbidity and mortality.

Although we can treat infection with antibiotics, we need to be certain that we are treating the correct women, to avoid unnecessary risks to women and their offspring. We are increasingly worried about antimicrobial resistance, and appropriate antibiotic prescribing is one part of tackling this.

The health and economic implications of an inaccurate test for UTI in pregnancy are substantial. To introduce a new test, we would need to put it through rigorous trials. I conducted a critical appraisal of the assumptions made in diagnosing UTI in pregnancy and demonstrated multiple shortcomings. Nevertheless, I do not underestimate the difficulties in challenging well-established practices.

To explore urinary pathology in pregnancy, I studied three settings: women presenting with abdominal pain in second and third trimesters, women at increased risk of preterm birth, and women being catheterised for caesarean section or cervical cerclage. I used parallel methods, to enable pooling of data to study relationships between tests, and to study outcomes including standard culture positive, pyelonephritis, and preterm birth.

The alternative approaches I used to study urinary pathology in pregnancy were derived from research into patients with chronic LUTS, with evidence of chronic UTI not detected by standard culture. These were: (1) objective structured assessment of urinary symptoms using the Artemis LUTS inventory, (2) quality of life assessment using EuroQol EQ-5D-5L, (3) fresh unspun microscopy for cell counts, (4) ATP, (5) enhanced sediment culture using analytical profile index analysis for polymicrobial species identification, (6) ELISA for urinary IL-6, IL-8 and lactoferrin, (7) fluorescent microscopy for

proportions of bacteria-associated epithelial cells (clue cells), (8) fluorescent microscopy with uroplakin-3 staining for identification of shed urothelial cells.

12.2 Summary of findings from the Abdominal pain study (chapter 0)

In this study I compared women with abdominal pain (pain cases) with women presenting with other acute problems (acute controls) and women attending routine appointments (normal controls). Both control groups were gestation-matched.

There were few differences between the pain cases, acute controls and normal controls. In terms of demographics and risk factors, pain cases were more likely to have a lower socio-economic status, with no other differences.

The only difference in LUTS was pain cases reported more nocturia and pain symptoms. Pain cases also reported a lower quality of life. Pain cases were more likely than acute controls to believe they had a UTI, and more likely to be diagnosed with UTI.

The only differences in alternative tests were: (1) ATP differed between groups (highest in acute controls); (2) there was a different distribution of genera and species between the groups; (3) urinary IL-8 and lactoferrin differed between groups.

In those who were diagnosed clinically with a UTI, there were higher white cell counts, IL-8 and lactoferrin; epithelial cell counts and enhanced culture colony counts trended higher but not significantly. This was despite no differences in dipstick and standard culture.

Another notable finding was the variance in results seen. Symptoms, fresh microscopy cell counts, ATP, cytokines, and enhanced culture colony counts, all exhibited a wide spectrum, defying dichotomisation.

Enhanced culture, while not revealing differences between the groups, was notable for demonstrating polymicrobial growth, with a median of 3 isolates identified. This demonstrates the non-sterile nature of urine in pregnancy.

Documentation about LUTS was minimal, even in those diagnosed with UTI. This may reflect the culture-centric approach to UTI in pregnancy.

12.3 Summary of findings from the PTB study (chapter 8)

In this study I compared women attending a preterm birth clinic, with higher risk of preterm birth owing to previous late miscarriage or preterm birth (HR-PTB), with women with a higher risk of preterm birth for other reasons (HR-non-PTB) and women at lower risk of preterm birth because of previous term deliveries (low risk). Groups were gestation-matched.

Participants in the HR-PTB group were more likely to have a history of UTI. Those in the HR-non-PTB were more likely to be of white ethnicity, have lower BMI; this group also included some nulliparous women.

Cervical cerclage occurred in the HR-PTB group only, with 13/37 already having one and 5/37 who went on to have one inserted. The HR-PTB group were more likely to have progesterone treatment, and go on to have higher fetal fibronectin levels, with no difference in cervical length.

Preterm birth was more common in HR-PTB and low risk groups with three cases of PPRM in HR-PTB. There were no other differences in maternal or neonatal outcomes.

Other than having more MSU samples sent during pregnancy, there were no differences in standard microbiology throughout the pregnancy, or at time of recruitment. 27.5% of participants had at least one positive MSU in pregnancy, suggesting a high potential exposure to antibiotics.

There were very few differences in alternative tests. In enhanced culture there was a list of species detected only in HR-PTB patients, however the significance of this cannot be determined in a study of this size.

Including pilot study data and additional low risk controls from the Abdominal pain study did not substantially alter the analysis.

Very similar to the Abdominal pain study, there was a wide variance in results in all alternative tests; LUTS and polymicrobial bacteriuria were very common.

12.4 Summary of findings from the MSU-CSU study (chapter 9)

In this study I compared CSU samples with paired MSU samples in women having catheters for caesarean section or cervical cerclage.

Dipstick leucocyte esterase, and standard culture, were more likely to be positive in MSUs.

Fresh unspun microscopy white cell and epithelial cell counts were higher in MSUs. Enhanced culture showed higher colony counts in MSUs and more isolates. Many species identified in MSU samples were not seen in CSU samples, including *E. coli*; some isolates in CSUs were not seen in paired MSUs.

IL-8 and lactoferrin were higher in MSU (whether normalised or not), and IL-6 proportion below LLOD was lower.

The number of uroplakin-3 positive epithelial cells in MSU samples was higher than CSU, however there was no difference in the proportion of these compared with total epithelial cells. The proportion of clue cells was higher in MSUs.

Red cell counts and protein were higher in CSUs. ATP was markedly higher in CSUs.

12.5 Summary of findings from pooled data (chapter 10)

I combined findings from all MSU samples (n=273). This was to: (1) gain understanding about alternative tests in pregnancy; (2) describe the relationship of tests to each other; (3) compare alternative tests against standard culture; (4) compare tests against clinical outcomes.

LUTS were very common, with wide variance. LUTS correlated negatively with quality of life. While there was correlation between groups of LUTS, LUTS did not correlate with any of the urine tests.

Standard microscopy white cell counts correlated with fresh microscopy counts and dipstick leucocyte esterase, however did not discriminate. Negative dipstick was sometimes associated with pyuria. Standard microscopy epithelial cell counts correlated with fresh microscopy counts, and dipstick protein correlated with total protein.

Enhanced culture showed bacteriuria in nearly all samples. Semi-quantitative categories of the standard culture report correlated with enhanced culture

colony counts. Enhanced culture identified all organisms identified in positive standard cultures, plus additional organisms. Of particular concern, enhanced culture identified GBS in many participants in whom it was not detected by standard tests.

ATP did not correlate with any other measures. Enhanced culture total colony counts weakly correlated with fresh microscopy cell counts and the cytokines.

Fresh microscopy white cell and epithelial cell counts correlated positively with urinary cytokines, particularly IL-8. IL-8 and lactoferrin correlated strongly with each other.

There were no cases of pyelonephritis, thus no comparison against this outcome could be made.

Standard culture was positive in 22/266 cases. Assuming standard culture is the gold standard for UTI, none of the other tests performed as well. However, the only alternative test differences between standard culture positive and standard culture non-positive were white cell and epithelial cell counts, enhanced culture total colony counts, and one marker of quality of life. Only enhanced culture total colony counts remained significant in a logistic regression model.

Preterm birth by all causes occurred in 23/222 women recruited before 37 weeks. Total colony counts, one marker of quality of life, mixed growth at booking, cervical cerclage, mixed growth during pregnancy, and number of MSUs sent during pregnancy were different. Only total colony counts remained significant in a logistic regression model. Performing an analysis of only those preterm births classified as spontaneous gave similar findings.

Additional to the planned analyses, it was noted that MSUs performed as part of the study, with instructions about how to do a clean-catch MSU, had a lower rate of mixed and non-significant growth than all other MSUs in pregnancy. This warrants further exploration.

12.6 Key implications of findings

Women with abdominal pain in pregnancy had urinary pathology undetected by standard tests, but this was no more frequent than in pregnant women with other acute problems or normal controls.

We studied women with abdominal pain in pregnancy because this is a common presentation, but under-researched. We hypothesised that some women with abdominal pain may have urinary pathology undetected by standard tests. We were correct in this hypothesis, however unable to explain the implications of this given that the same effect was seen in the other groups.

Similarly, women with previous preterm birth had urinary pathology undetected by standard tests, but this was no more frequent than women with other risk factors for preterm birth or low risk controls.

Compared with CSU samples, MSU samples had higher white cell and epithelial cell counts, higher colony counts at enhanced culture, and higher IL-8 and lactoferrin. This was despite having similarly high proportions of urothelial cells within the sediment, showing that this was not caused by increased contamination. This fits with the hypothesis that urinary sediment contains shed urothelial cells, and that catheters do not sample as much of the sediment ((Collins, Sathiananthamoorthy et al. 2020)). This flips the usual story, that catheter specimens protect against contamination, and instead raises the idea that they might be less good at detecting urinary pathology. Given the evolving body of urinary microbiome research using urinary catheters to avoid contamination e.g. (Jacobs, Thomas-White et al. 2017), as well as the common use of CSUs to investigate intrapartum sepsis, this point needs careful appraisal.

Most voided samples had bacteriuria using enhanced culture, regardless of symptoms or standard culture results. This is further evidence that urine in pregnancy is not sterile. In addition, polymicrobial enhanced culture isolates were almost universal, evidence against mixed growth representing contamination.

There was wide variance in all variables including symptoms, without a way to discern what was normal and what was pathological. It is not clear exactly how symptoms, in a cross-sectional study, relate to diagnosis and subsequent outcomes, but this requires further investigation. Of the women diagnosed with UTI in the Abdominal pain study, only two thirds had documentation about their symptoms, with only a third documented in all the pain and acute cases. This suggests urinary symptoms are undervalued by clinicians.

Shed epithelial cells were largely urothelial in origin, and correlated with white cell counts, IL-8 and lactoferrin. This is evidence that epithelial cells are not a marker of contamination, rather they may reflect pathophysiological processes in the bladder.

Uropathogens and commensals did not differentiate between study groups. There is no evidence from this study that the so-called uropathogens are specifically pathogenic, rather than part of the bladder microbiome.

Compared with MSU samples, CSU samples had higher red blood cell counts, higher total protein and higher ATP. Haematuria may be secondary to trauma. The higher protein is curious and may have implications for use of CSUs in diagnosing pre-eclampsia. The high ATP is also intriguing and invites speculation as to how it may be released by urothelial cells in the process of catheterisation. ATP did not correlate with any other signals in the urine, suggesting it is not useful as a marker of urinary pathology in pregnancy, contrary to previous evidence outside pregnancy.

Standard culture positivity was associated with higher colony counts by enhanced culture. This is consistent with a spectrum approach to culture where Kass criteria detects the most severe end of the spectrum.

The lack of cases of pyelonephritis is important. At the time of Kass this was a common and morbid condition. Our study is consistent with modern studies showing a much lower prevalence. The low prevalence may be caused by treatment for asymptomatic bacteriuria at booking, however prevalence was low even in a setting without such screening (Kazemier, Koningstein et al. 2015). This phenomenon warrants further investigation.

Preterm birth by all causes was associated with higher colony counts by enhanced culture. This suggests microbial burden may be important in preterm birth.

The finding that mixed growth at booking, and mixed growth during the pregnancy, was associated with preterm birth, has implications for the role of mixed growth in pregnancy. Again, this may be related to microbial burden; mixed growth is rarely treated, and it may be that patients with mixed growth were less likely to receive antibiotics.

The relationship between UTI and preterm birth is confusing, as I have outlined previously. The correlation seen in early studies of asymptomatic bacteriuria has not been seen in modern studies, however studies of symptomatic UTI continue to report an association. The findings here may point to a way forward in exploring the microbial burden in the bladder and how it relates to preterm birth, rather than the simple dichotomisation by Kass criteria.

12.7 Limitations

In terms of study design, the Abdominal pain study was recruited as planned with adequate numbers in each study group, and well-phenotyped study groups. However, the PTB study had inadequate recruitment of low-risk women, with only 13. Initially I had planned to incorporate low risk cases from the student pilot study that preceded this work, but felt I could not guarantee the quality of the data. Subsequently I performed a re-analysis including this data, but very few of the low risk patients were of the required gestation so the group remained undersized, and not all alternative tests used in the other studies had been performed.

It would be pertinent to carry out further studies in women recruited via the preterm birth clinic, particularly to understand more about women who have had, or go on to have, cervical cerclage, and the effect of urinary pathology present in these women.

I have described how the PTB study functioned as a pilot for the Abdominal pain study. This might have affected accuracy of fresh microscopy cell counts owing to a learning effect, or determination of genus / species of enhanced

culture isolates. It did however allow the Abdominal pain study protocol to be optimised, which in turn benefitted the MSU-CSU study.

A great omission is data about the use of antibiotics. Although prescriptions for antibiotics were noted at the time of review for pain cases and acute controls in the Abdominal pain study, we did not collect data about prior or subsequent antibiotic use in the pregnancy, or compliance with antibiotics. This limits the analysis. This is information that should be collected in future UTI in pregnancy studies.

There was some loss of follow-up in the study. Some is inevitable in all clinical studies, however ideally we would have sought permission to collect email addresses for contacting patients for follow-up information, as it is our observation that email addresses remain consistent over time despite patients moving house. We tried to minimise this issue in the Abdominal pain study by recruiting locally booked women, however some did not deliver at UCLH. Recruitment in the preterm birth clinic, as a tertiary service, was not always local.

Standard results were affected by the lack of standard microscopy. This might have been corrected via negotiation with the laboratory.

In terms of alternative methods, the Artemis LUTS inventory worked well for symptoms assessment, as critiqued above, however it was hard to interpret at a single point in time. Other than the pain cases and acute controls in the Abdominal pain study, we did not ask patients whether they thought they had a UTI, or seek information about how bothered they were by their symptoms. We did not ask them to compare with pre-pregnancy. To help understand the implications of their scores, such questions would have been helpful.

There were two techniques involving subjective counting where we were not able to measure inter-observer variability owing to reasons described previously. These were fresh microscopy and fluorescent microscopy. This is frustrating and limits the reliability of the work described here. In future studies I would incorporate this into the study design as a requirement. In the case of cell counts, using proxy measures of dipstick and standard microscopy it can

be seen that counts were largely of the correct order of magnitude which is partly reassuring.

The API method used for species identification in enhanced cultures has inadequacies, as described above, and the inability to identify the species of *Lactobacillus spp* leaves a gap in the work in terms of comparisons with urinary microbiome work. Subsequent work should include analysis of the microbiome of these samples, allowing comparison with the advanced culture.

In terms of outcome measures, I was unable to assess risk of pyelonephritis as there were no cases. Regarding preterm birth, I chose to combine both spontaneous and medically-indicated PTB as both may be contributed to by urinary pathology. This is challengeable, however numbers of spontaneous PTB were low, such that analysis may have lacked power to identify differences. Literature review highlighted pre-eclampsia as being associated with UTI. I did not collect outcomes relating to subsequent pre-eclampsia so was unable to comment on this.

In terms of the comparison against outcomes, these were secondary analyses which were intended but not statistically defined at the outset; this could be criticised. Bonferroni correction was used to reduce the risk of multiplicity, however this is at the risk of false negatives. It should be remembered that the pooled data included 273 participants, which limits the conclusions that can be drawn. Whilst data can contribute to future meta-analysis, we cannot draw meaningful conclusions about rarer outcomes from these data.

This study did not collect post-neonatal period data about infant outcomes; this should be a feature of any research involving PTB (van 't Hooft, Duffy et al. 2016).

12.8 Original contributions to knowledge

12.8.1 Novel use of tests

In this research I have studied tests that have not previously been described in pregnancy, in particular epithelial clue cells, uroplakin-3 staining of epithelial cells, urinary lactoferrin, urinary ATP, and sediment culture as a form of

enhanced culture. I have also studied the Artemis LUTS inventory as an approach to symptomatology.

I have also studied tests that previously have been looked at in isolation in pregnancy, rather than in comparison with standard culture, such as fresh unspun microscopy, urinary IL-6 and IL-8, and EuroQol quality of life assessment.

I developed a modified clean catch mid-stream urine collection kit, validated a commercial IL-8 kit for urine, and validated a faster method of counting bacterial-associated epithelial cells.

Even if these have not led to a better test for UTI, however it is conceived, they contribute to a greater understanding of bladder pathophysiology in pregnancy, with relevance to future systematic review and meta-analysis to avoid research wastage.

12.8.2 New interpretations

Central to this thesis is a questioning approach to the assumptions underlying diagnosis of UTI in pregnancy, including a re-appraisal of Kass from a modern obstetric perspective. Proposal of a move away from a culture-centric model has been described before, however framing it within obstetrics is a new approach.

The consideration of the part of the urine that is being sampled has been described before in chronic LUTS patients; we are the first to demonstrate in pregnancy that MSUs contain the same proportion of shed urothelial cells as CSUs but in greater quantities, which correlates with higher cell counts, enhanced culture colony counts and urinary cytokines. We have re-shaped the hypothesis from CSUs avoiding the problem of contamination to CSUs potentially missing infection owing to not sampling the sediment.

We have established both epithelial cells and polymicrobial growth as experimental tools worthy of further consideration in pregnancy. We have suggested a change from considering epithelial cells and mixed growth as markers of contamination, and thus exclusion from studies, to being included in research on urinary pathology in pregnancy.

12.9 Implications

This body of work demonstrates the importance of going back to clinical basics; taking a history from a woman and listening to her symptoms. Clinical diagnosis of UTI correlated with pathophysiological signals including white cell counts and urinary cytokines, suggesting there is some merit to this, regardless of the standard culture result. We are unable to say from this work what are normal symptoms and what are not.

I have argued that UTI is a spectrum condition, however in my statistical analysis I have searched for dichotomy. This may be an area that would in future benefit from more sophisticated analysis such as machine learning.

The Kass criteria was originally conceived to predict pyelonephritis, at the severe end of the UTI spectrum. We have demonstrated that microbial burden may indeed be important, but rather than a dichotomy caused by a diagnostic threshold, there may be a spectrum whereby microbial burden plus other pathological factors combine to increase risk. This may particularly be an issue in preterm birth risk.

12.10 Impact on practice

This study involved a unique approach to a problem that many might not even consider germane. By combining expertise in obstetrics, medicine, urogynaecology, microbiology and cell biology we have been able to advance the field. Such cross-specialty collaborations invite questions, disagreements, and exploring assumptions such as 'it's always like this' or 'this is the way we do things'.

Obstetrics has a culture-centric approach to UTI owing to the dogma about asymptomatic bacteriuria, established in Kass' writings and perpetuated over the following sixty-plus years. This inhibits a normal clinical approach of taking a history, examining a patient and deriving a differential diagnosis, let alone a more complex approach such as Bayesian analysis or the damage-response framework. This risks missing UTI where it is present, and over-treating it where treatment is not required. It has inhibited research in the field as well as clinical practice. To reshape thought in this area would be challenging.

UTI in pregnancy has been viewed as an extension of UTI in general. This study shows that UTI in pregnancy has specific attributes, not least that treatment is directed towards pregnancy outcomes. This study examines urinary pathology in pregnancy in its own right.

The urinary tract is an ecological niche in the body with a specific microbiome. A continuous process of shedding of umbrella cells from the urothelium is part of the host response to infection. Bacteria are present in the urinary tract, and either enter acutely or persist chronically. They have mechanisms to evade detection such as entering cells or forming biofilms. The host response to bacteria varies and some people may mount an immune response involving release of neutrophils and increased urothelial cell shedding. Some patients may be symptomatic, which may or may not relate to the combination of bacteria, the bacterial load and the inflammatory response. In pregnancy there are changes in anatomy, physiology, pregnancy hormones, immune system and behaviour (of both patients and clinicians) which may all contribute to effects on symptoms, bacterial behaviour and host immune response.

This study has demonstrated that diagnosis is a complex process. UTI in pregnancy is considered simple – if the culture is positive, the patient needs antibiotics. However, we have seen that perhaps we do not really know what we mean by UTI, what its implications are for women and their babies, and how best to test for it. I would argue that the most important message is the need for greater thoughtfulness about the diagnostic process. This has to be balanced against health economics and the logistical challenges of assessing all pregnant women. Clinicians and patients should be aware that diagnosis is based on probability and not certainty, is vulnerable to bias, and that there can be false positives and false negatives (Sbrojavacca 2012).

12.10.1 Suggestions for recommendations for future practice for clinicians

What follows are some simple suggestions for improving clinical practice around current urinary testing in obstetrics, while awaiting development or validation of alternative tests, based on the conclusions described so far:

1. Assess for urinary symptoms in pregnant women; this will affect your interpretation of the test results.
2. Facilitate a clean-catch mid-stream urine specimen – provide instructions, make it normal to do labial cleaning, and use a large enough sterile container that women can more easily use. This may reduce excess mixed growth rates.
3. Get a fresh sample, and get the sample to the laboratory for microscopy and culture as soon as possible, for the most meaningful results.
4. Do not simply use a negative dipstick or culture to rule-out UTI; consider the likelihood of a UTI or consequences of a UTI for the patient in front of you.
5. Pay attention to microscopy when interpreting urine culture results, including epithelial cell counts; is there evidence of inflammation as well as bacteriuria? Do not assume epithelial cells indicate a contaminated sample.
6. If a patient is symptomatic of UTI, or at high risk of the consequences of a UTI (such as previous renal or urinary tract problems, immunocompromised, at high risk of preterm birth), a standard culture threshold might not be adequate as lower counts may be clinically significant. These clinical details should be highlighted on the request form and may require discussion with a microbiologist.
7. If a patient has recurrent mixed or non-significant growth, consider whether she has a UTI, particularly if she is symptomatic or at increased risk from a UTI. This may require discussion with a microbiologist.
8. Be mindful, and at times sceptical, of dipstick, microscopy and culture results that do not seem to match the patient. No test is perfect.

12.11 Future research priorities

This research has demonstrated the lack of relevant clinical outcomes in research in UTI in pregnancy. It is desirable to establish core outcome sets for research on UTI in pregnancy, to reduce research waste. This would be following a standardised process, involving prospective registration, systematic review of existing outcomes, patient and public involvement, stakeholder

prioritisation and dissemination via a core outcome sets database (Williamson, Altman et al. 2017).

Mixed growth at booking or during the pregnancy was associated with preterm birth in this study. This is a new finding as mixed growth has so frequently been excluded from studies because of contamination concerns. Large database-based studies investigating the relationship between mixed growth and outcomes should be conducted to investigate this relationship.

Additionally, translational research should investigate in greater detail what 'mixed growth' entails, using enhanced culture and next generation sequencing methods on a larger scale, and relating this to symptoms and clinical outcomes.

Further to this, the relationship in this study of the abundance of bacteriuria with preterm birth risk needs further assessment. A quantitative approach to culture, not dichotomous, again in relation to symptoms and outcomes, would be essential.

The data in this thesis were cross-sectional. A longitudinal analysis of symptoms in pregnancy, alongside a detailed assessment of microbiological and immunological components of the urine, is warranted to understand what is normal for pregnancy. Understanding how symptoms, cultures and cellular or inflammatory response markers change over hours, days, weeks and months may also help assess the reproducibility and reliability of these data.

The dogma of UTI in pregnancy always comes back to asymptomatic bacteriuria at booking. This needs to be re-examined, taking into account symptoms, pathophysiological signals in the urine, treatments applied, and detailed clinical outcomes for mother and baby. Ultimately, a UK-based randomised controlled trial may be warranted to assess the benefits of screening for and treating asymptomatic bacteriuria at booking.

There is a growing body of research into bladder microbiome, and in its wake, bladder metabolomics. These are noble avenues of investigation and it is important to explore the benefits of new technology. However, it needs to be performed alongside routine and low-tech analyses, in well-phenotyped studies with good outcome assessment. Any new test will have to be well-

justified if it is to displace the standard culture. UTI in pregnancy is a global problem and it is important to consider the development of affordable, accessible tests for all settings.

12.12 Conclusions

In this research I have questioned the current gold standard of UTI diagnosis and examined the assumptions underlying this.

I have demonstrated a high prevalence of urinary pathology in pregnant women in different settings, using alternative tests; these detected more pathology than standard culture. However, the significance of this in terms of outcomes is not clear.

I have been able to combine multiple approaches to apply a damage-response framework approach to UTI investigation.

I have explored assumptions about contamination in voided samples and questioned the belief that catheter samples are 'better' – rather, they may be sampling less of the urinary sediment which contains shed urothelial cells.

My original contribution to knowledge is in the novel and parallel application of alternative tests in pregnancy. In particular, Artemis LUTS inventory, ATP, fresh unspun urine microscopy, enhanced sediment culture, urinary IL-6, IL-8 and lactoferrin, and clue cell proportions.

13 Appendices

13.1 Artemis LUTS inventory

Artemis History Form

Study Number:

Day Time Frequency	1-2	5-6	9-10	13-14	17-18
How often do you pass urine during the day?	2-3	6-7	10-11	14-15	18-19
	3-4	7-8	11-12	15-16	19-20
	4-5	8-9	12-13	16-17	>20
Nocturia	0	1-2	3	4-5	6
How often do you get up to pass urine at night?	0-1	2	3-4	5	>6
	1	2-3	4	5-6	
Daytime Incontinence	0	0-2D	1-3D	2-4D	4-5D
How often do you leak urine during the day?	0-1D	1-2D	2-3D	3-4D	>5D
	1-2W	1-3W	2-3W	4-5W	4-6W
	1-2M	1-3M	1-4M	1-2Y	1-3Y
	3-4Y	3-5Y	3-6Y	PMD*	
Nocturnal incontinence	0	0-2N	1-3N	2-4N	4-5N
How often do you leak urine at night?	0-1N	1-2N	2-3N	3-4N	>5N
	1-2W	1-3W	2-3W	4-5W	4-6W
	1-2M	1-3M	1-4M	1-2Y	1-3Y
	3-4Y	3-5Y	3-6Y	PMD*	

Duration Of Symptoms:	Years	Mths
Pad dependant Incontinence (Do you need to wear Pads to prevent leakage?)	Yes	No
CISC (Do you perform clean intermittent self catheterisation?)	Yes	No
Permanent indwelling catheter (Do you have or had a permanent urinary catheter?)	Yes	No
Stress incontinence symptoms	Some	None
Cough Sneeze incontinence (Do you leak when you cough or sneeze?)	Yes	No
Exercise incontinence (Do you leak when you exercise?)	Yes	No
Lifting incontinence (Do you leak when you lift something?)	Yes	No
Laughing incontinence (Do you leak when you Laugh?)	Yes	No
Standing incontinence (Do you leak on Standing up?)	Yes	No
Positional incontinence (Do you leak when you change Positions?)	Yes	No
Passive incontinence (Do you leak urine for no reason and without the feeling that you want to go?)	Yes	No
Overactive Bladder Symptoms	Some	None
Urinary urgency (Do you have a sudden need to rush to the toilet to urinate?)	Yes	No
Urinary urge incontinence (When rushing to the toilet, do you leak before you reach the toilet?)	Yes	No
Cold weather Exacerbation (Does cold weather make your urgency worse?)	Yes	No
Running water Urgency (Does the sound of running water make your urgency worse?)	Yes	No
Running water incontinence (Have you leaked urine on hearing the sound of running water?)	Yes	No
Latchkey urgency (Do you have urgent need to pass urine when you put the key in your front door?)	Yes	No
Latchkey incontinence (Do you leak urine when you put the key in your front door?)	Yes	No

Waking rising urgency (Do you have to rush to the toilet on waking up?)	Yes	No	
Waking rise incontinence (Do you have urgency and leak urine on waking up?)	Yes	No	
Anxiety fatigue aggravation (Does your urgency worsen when you are tired or anxious?)	Yes	No	
Premenstrual aggravation (Does your urgency worsen prior to a period?)	Yes	No	
Leaking when coughing with urgency (If you had urgency and coughed, would you leak?)	Yes	No	
Voiding Symptoms	Some	None	
Hesitancy (Is there a delay before you start to urinate?)	Yes	No	
Reduced stream (Do you feel the urine stream is reduced compared to before?)	Yes	No	
Intermittent Stream (Do you stop and start more than once when you urinate?)	Yes	No	
Straining to void (Do you have to push or strain to pass urine?)	Yes	No	
Terminal Dribbling (At the end of your urination, do you dribble?)	Yes	No	
Postmicturition dribbling (Do you dribble urine straight after you have finished urinating?)	Yes	No	
Double Voiding (Do you need to sometimes go twice in a short space of time to urinate, e.g. 5 min apart)	Yes	No	
Incomplete emptying (Do you feel that you have emptied your bladder fully?)	Yes	No	
Pain Symptoms	Some	None	
Bladder pain on Filling (Do you experience any bladder pain or discomfort when it is full?)	Yes	No	
Bladder pain relieved by voiding (Is this pain or discomfort relieved after emptying?)	Yes	No	
Bladder pain partially relieved by voiding (Is this discomfort relieved slightly after emptying?)	Yes	No	
Bladder pain unrelieved by voids (Is this pain or discomfort not relieved after emptying?)	Yes	No	
Bladder or suprapubic pain (Do you suffer from pain in the bladder area?) (point to the area)	Yes	No	
Loin pain (Do you suffer from pain in the kidney area?) (point to the area)	Yes	No	
Dysuria (Do you suffer from pain during urination in urethral area?) (point to the area)	Yes	No	
Urethral pain (Do you suffer from pain in the urethral area?) (point to the area)	Yes	No	
Pain or discomfort referred to genitals (Do you have pain going to the genital area?)	Yes	No	
Left or right iliac fossa pain (Do you have pain in the lower part of your tummy?) (point to RIF or LIF)	Yes	No	
Pain radiating to legs (Do you have pain going down the tops of your thighs?)	Yes	No	
Bladder pain during micturition (Do you have pain while passing urine?)	Yes	No	
Pain after micturition (Do you have pain after urinating?)	Yes	No	

PMD* Postmicturition dribbling

13.2 STROBE Checklist for case-control studies

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls (b) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed

(d) If applicable, explain how matching of cases and controls was addressed

(e) Describe any sensitivity analyses

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results

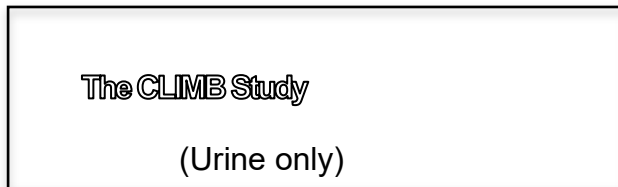
Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

13.3 CLIMB Study participant information leaflet



Version 4: Date: 6th March, 2012 Project ID: 09/H0714/66

The CLIMB Study:

Cervical Length, Inflammation and Bacterial species and pre-term birth:

Researchers:

Professor Donald Peebles, Consultant in Obstetrics and Maternal Fetal Medicine

Dr Anna David, Consultant in Obstetrics and Maternal Fetal Medicine

Dr Catherine James, Clinical Research Associate

Dr Patricia Hunter, Senior Research Fellow

Ms Frida Forya, Research Nurse

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and to decide whether or not you wish to take part. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

6% of babies in the UK are born pre-term (before 37 completed weeks of pregnancy) and there are very few ways to predict which women are at risk of going into labour early. The survival rate for babies that are born very early is improving, but many develop long-term health problems.

We are trying to understand why some women give birth to their baby early. Previous studies have suggested that the way that the mother's immune system behaves in pregnancy may be important. We would like to better understand how the components of the immune system in the urine behave in pregnancy.

Why have I been chosen?

We are inviting all pregnant women booking to have their baby at this hospital to take part in this study including those with no risk factors for preterm birth.

>

Do I have to take part?

No. It is up to you to decide. If you do take part you will be given this information sheet to keep and asked to sign two copies of a consent form. *If you do decide to take part you are still free to withdraw (change your mind) at any time and without giving a reason. This will not affect the standard of care you receive.*

What will happen to me if I take part?

- We would like to carry out additional tests on the urine sample that is examined during your routine antenatal care.

What tests will be done on the sample?

We will examine the components of the immune system in the sample and we will determine which bacteria are present.

What do I have to do?

You do not need to do anything different if you agree to take part in the study.

What are the possible benefits of taking part?

It is unlikely that there will be any benefit to you but the information gained by this study may help future pregnant women and their babies at risk of being born pre-term.

What are the possible disadvantages/risks of taking part?

We do not expect any risk to you from the sample that you would provide.

What if something goes wrong?

We do not expect any risk from the sample that you would provide. If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns of this study, the normal National Health Service complaints mechanisms should be available to you.

Will my taking part in this study be kept confidential?

All information collected about you during the research will be kept strictly confidential and samples will be anonymised. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. All data will be kept safe and secure in accordance with the Data Protection Act 1998 and will be collected, stored and handled by the researchers listed at UCL.

Who is organising and funding the research?

The research is funded by Wellbeing of Women, Action Medical Research and The Wellcome Trust.

What will happen to the results of the research study?

The results will be analysed, presented in scientific meetings and published in peer-reviewed journals. Your identity will not be revealed in any report or publication. You may obtain a copy of the results from the UCL Institute for Women's Health.

The local Research Ethics Committee has reviewed this study and given its approval.

For further information, please call a member of the research team on

■■■■■■■■■■.

Thank you for considering taking part in this study!

13.4 Appendix: Data collection proforma

Demographic information

CLIMB number		Date of birth	
Date of recruitment		Occupation	
Postcode		Ethnicity	
Partner occupation		Partner ethnicity	
Telephone number		Email address	
Consents to contact?	Telephone	Email	Neither
Setting	MFAU	ANC / US	Other

Social history

BMI	Height	Weight at booking	
Smoking status	Current (amount)	Ex-smoker (amount, when stopped)	Never smoked
Alcohol during pregnancy	No	Yes	If yes, amount
Other drugs during pregnancy	No	Yes	If yes, detail

Current pregnancy

EDD		Gestation at recruitment	
EDD by scan or LMP	First trimester scan	Dated by LMP	Dated by later scan
Multiple pregnancy?	Singleton	Multiple - twins	Multiple – higher order (state)
Conception	Spontaneous	Assisted	If assisted, details
Obstetric complications to date (see list on page 2)		Gestation at booking	
Booking urine	Dipstick	Microscopy	Culture
Triage (MFAU) attendances this pregnancy inc current	Number	Reasons	

Previous obstetric history

Gravidity (number of pregnancies including this one)		Parity (number of babies born >24 weeks or 500g + other pregnancies)	+

Year	Outcome (e.g. Livebirth, Stillbirth, Miscarriage, Termination, Ectopic pregnancy)	Gestation at end of pregnancy	Mode	Birthweight	Obstetric complications (see list on next page)

Obstetric complications:

Complication	Year(s)	Gestation(s) of onset	Details (e.g. severity, nature, type)	Treatment (if applicable)	Impact on outcome (if applicable)
Pre-eclampsia (PET)					
Pregnancy-induced hypertension (PIH)					
Preterm prelabour rupture of membranes (PPROM)					
Threatened preterm labour					
Shortened cervix on scan					
Cervical cerclage					
Steroids for fetal lung maturity					
Preterm delivery (spontaneous onset)					
Preterm delivery (iatrogenic onset)					
Genital tract infection					
Group B strep carriage					
Antepartum haemorrhage					
Small for gestational age fetus antenatally					
Birthweight below 2.5kg					
Urinary tract infection requiring antibiotics					
Kidney infection / pyelonephritis					
Antibiotics for any other reason					
Gestational diabetes					
Other 1 (describe)					
Other 2 (describe)					
Other 3 (describe)					
Other 4 (describe)					
Other 5 (describe)					

If affecting more than one pregnancy, complete the details above for each pregnancy.

Other preterm birth risk factors

Risk factor	Year(s)	Details (e.g. severity, nature, type, amount of cervix removed)
LLETZ		
Cone biopsy		
Other cervical surgery		
Uterine evacuation		
Other uterine surgery e.g. myomectomy, hysterotomy		
Uterine anomaly e.g. bicornuate uterus		

Renal and urinary tract

Problem	Yes	No	Details
Have you ever had a UTI?			How many times? During pregnancy? During this pregnancy? Details of UTI(s) in this pregnancy including treatment:
Other renal problem			
Other bladder problem			

Medical history

Medical problem	Date of onset	Details	Treatment

Investigations this pregnancy
Fetal fibronectin (result and date):
Other microbiology results:

Scan findings in this pregnancy			
Cervical length		Date	Funnelling/sludge?
Cervical length		Date	Funnelling/sludge?
Cervical length		Date	Funnelling/sludge?
Cervical length		Date	Funnelling/sludge?
Cervical length		Date	Funnelling/sludge?
Anomaly scan	HC	AC	FL
	EFW	Cervical length	Gestation
Last growth scan	HC	AC	FL
	EFW	Cervical length	Gestation

What does the patient think is the diagnosis?

Does the patient think she has a urinary tract infection?

Clinician's clinical diagnosis

Management plan

Current symptoms

Current symptoms?	Yes	No	Details
Abdominal/pelvic pain / discomfort			Character: Severity/10: Location: Duration:
Tightenings / contractions			Frequency (/10mins): Painful: Speculum findings: Fetal fibronectin result:
Vaginal bleeding			Amount: Coitus within 24 hours: Yes / No Placental site: High / Low covering os
Water leaking			Details: Confirmed: Speculum / Pad / Scan / Not confirmed
Urinary symptoms			Details:
Change in discharge			Details:
Reduced fetal movements			Details:
Chest pain			Details:
Symptoms of pre-eclampsia			Details: Booking BP: Current BP: Proteinuria:
Cold/flu-like symptoms			Details:
Fever			Details: Suspected source: Temp: Pulse: Current BP:
Nausea or vomiting			Details:
Change in bowel habit			Details:
Chest pain			Details:
Shortness of breath			Details:
Headache			Details:
Itching			Details:
Other(s) (please specify)			Details:
Other(s) (please specify)			Details:
Other(s) (please specify)			Details:

Outcome data

Diagnosis on CDR from index presentation	

Microbiology results (inc date and detail)	
Blood results (WBC, CRP)	
Scan if done (inc date and findings)	
Any episodes of pyelonephritis during this pregnancy? (Y/N/ details)	
Steroids given in pregnancy? (Y/N/details)	
Magnesium sulphate given? (Y/N/details)	
Placental histology? (Y/N/results)	
T21 screening?	PAPP-A (MoM) NT (MoM)

Date of birth		Gestation at birth	
Induction of labour?	Yes/No	If induction, reason?	
Mode of delivery	Elective CS	Emergency CS	Instrumental
	SVD	Reason for operative delivery:	
Intrapartum sepsis?	Maternal tachycardia?	Fetal tachycardia?	Pyrexia?
	Intrapartum antibiotics?	Suspected source?	Microbiology results:
Postnatal maternal sepsis?	Maternal tachycardia?	Pyrexia?	Details:
	Postnatal antibiotics?	Suspected source?	Microbiology results:
Sex		Birthweight	
Admission to SCBU/NNU?		Apgars at 5 minutes	
Neonatal sepsis?	Antibiotics?	Suspected source?	Microbiology results:

Definitions:

Maternal tachycardia HR > 100

Maternal pyrexia T > 37.5 on two occasions or T > 38 on one occasion

Fetal tachycardia BR > 160 or rise in baseline > 15

Checklist

Info leaflet given?		Notes
CLIMB consent signed?		Notes
CLIMB number allocated?		Notes
Obstetric questionnaire completed?		Notes
Artemis LUTS inventory completed?		Notes
QoL questionnaire completed?		Notes
Follow-up details for 2-week review?		Notes
Clean catch urine specimen?		Notes
Lab MSU sent?		Notes
Urine dipstick done?		Notes
Fresh microscopy within 2 hours?		Notes
ATP reading?		Notes
Routine culture?		Notes
Sediment culture?		Notes
DAPI slide prepared?		Notes
Uroplakin slide prepared?		Notes
Frozen aliquots?		Notes
HVS done?		Notes inc not indicated
CLIMB swabs taken?		Notes
MSU result chased?		Notes
Follow-up review completed?		Notes
Obstetric outcome data completed?		Notes
Placental histology?		Notes
Scan results reviewed?		Notes
T21 screening results reviewed?		Notes
Any outstanding data?		

13.5 CLIMB Study consent form

University College London Hospitals NHS Foundation Trust	
FETAL MEDICINE UNIT Integrated Antenatal service Elizabeth Garrett Anderson Wing University College Hospital 235 Euston Road London NW1 2BU Telephone: 020 7380 9872	
Centre: UCLH Patient ID for this study:	Project ID number: 09/H0714/66 Form version: <u>5: 03/01/15</u>
<u>CONSENT FORM pg 1</u>	
Title of project: Cervical length, inflammation and bacterial species in <u>pre term</u> birth. The CLIMB <u>study</u>.	
Name of Principal Investigator: Prof Donald Peebles	
+	<i>Please initial box</i>
1. I confirm that I have read and understood the information sheet: <u>for the above study and have had the opportunity to ask questions.</u>	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
4. I understand that sections of any of my medical notes may be looked at by responsible individuals from UCL or UCLH or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
5. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
6. I understand that my samples are a <u>gift</u> and agree that the samples can be stored anonymously and used by the custodian Prof. Peebles of UCL for future ethically approved research relating and, as described in the attached information sheet.	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
7. I agree to take part in the above study.	
	<input style="width: 30px; height: 30px;" type="checkbox"/>
	<input style="width: 10px; height: 10px;" type="checkbox"/>

University College London Hospitals

NHS Foundation Trust

Centre: UCLH
Patient ID for this study:

Project ID number: 09/H0714/66
Form version: 5: 03/01/15

CONSENT FORM pg 2

Title of project: **Cervical length, inflammation and bacterial species in pre term birth. The CLIMB study.**

Name of Principal investigator: Prof Donald Peebles

Name of patient

Date

Signature

Name of Person taking consent
(if different from researcher)

Date

Signature

Researcher (to be contacted
if there are any problems)

Date

Signature

Comments or concerns during the study

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.

- 1 form for Patient:
- 1 to be kept as part of the study documentation,
- 1 to be kept with hospital note

13.6 EuroQol Quality of Life - EQ-5D-5L tool

Under each heading, please tick the ONE box that best describes your health
TODAY.

MOBILITY

- I have no problems in walking about ☐
- I have slight problems in walking about ☐
- I have moderate problems in walking about ☐
- I have severe problems in walking about ☐
- I am unable to walk about ☐

SELF-CARE

- I have no problems washing or dressing myself ☐
- I have slight problems washing or dressing myself ☐
- I have moderate problems washing or dressing myself ☐
- I have severe problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

USUAL ACTIVITIES (*e.g., work, study, housework, family or leisure activities*)

- I have no problems doing my usual activities ☐
- I have slight problems doing my usual activities ☐
- I have moderate problems doing my usual activities ☐
- I have severe problems doing my usual activities ☐
- I am unable to do my usual activities ☐

PAIN / DISCOMFORT

- I have no pain or discomfort ☐
- I have slight pain or discomfort ☐
- I have moderate pain or discomfort ☐
- I have severe pain or discomfort ☐
- I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

- I am not anxious or depressed ☐
- I am slightly anxious or depressed ☐
- I am moderately anxious or depressed ☐
- I am severely anxious or depressed ☐
- I am extremely anxious or depressed ☐

- We would like to know how good or bad your health is TODAY.

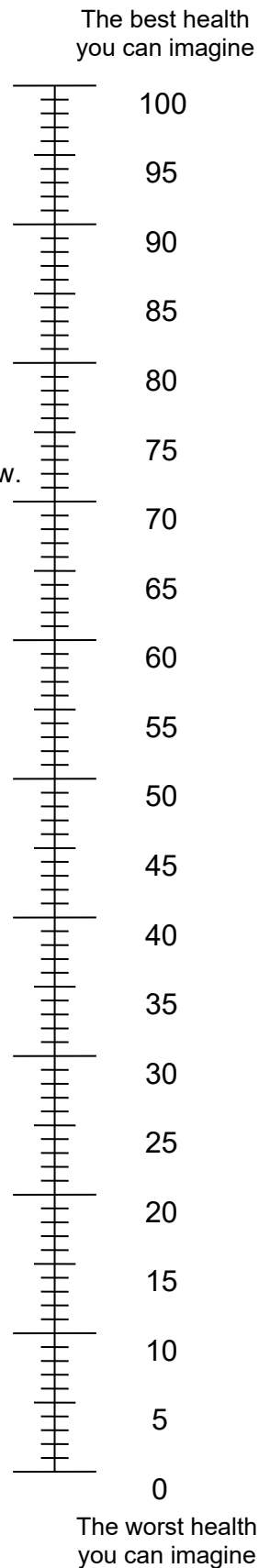
- This scale is numbered from 0 to 100.

- 100 means the best health you can imagine.
0 means the worst health you can imagine.

- Mark an X on the scale to indicate how your health is TODAY.

- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY =



13.7 Technical methods for clue cell analysis and urothelial cell analysis

13.7.1 Fluorescent microscopy method for uroplakin-staining of shed epithelial cells

Fluorescent microscopy was used to count epithelial cells staining positive for uroplakin-III, expressed as a proportion of the total epithelial cells counted. This protocol was originally developed by Harry Horsley (Horsley, Malone-Lee et al. 2013).

1. WGA, DAPI, UP3 and Alexa Fluor 555 goat anti-mouse IgG were stored at -20°C and thawed immediately prior to use.
2. All steps were carried out at room temperature including incubations.
3. A Thermo Scientific, Superfrost Ultra Plus microscope glass slide was labelled and the field area for cells was marked using the filter card (Fisher Scientific) as a guide.
4. The slide was assembled with a cuvette holder, slide, filter and Shandon single funnel cuvette.
5. The assembled cuvette was positioned in the cytocentrifuge (Shandon cytospin 2 cytocentrifuge) and balanced. 100µl of sample was added to the single funnel cuvette assembly.
6. This was spun at high acceleration, at 800rpm (≈ 75 RCF) for 5 minutes. This resulted in a visible disc of urinary particulate, approximately 5mm diameter ($\approx 20\text{mm}^2$), deposited on the slide.
7. The assembled cuvette was promptly removed from the cytocentrifuge and the slide removed without dislodging the deposit.
8. The cellular deposit was circumscribed with a hydrophobic barrier pen (ImmEdge pen, Vector Laboratories).
9. Cells were fixed by adding 100µl of 4% formaldehyde and covering to shield from light for 20 mins at room temperature.
10. The formaldehyde was aspirated, and the slide was washed with 100µl of PBS (phosphate buffered saline) once.
11. Cells were permeabilised using 100µl of 0.2% Triton-X (Sigma-Aldrich), incubated for 5 minutes, and aspirated.

12. Cells were primed with 100µl of 10% goat serum (Sigma Aldrich) and incubated for 40 minutes.
13. The primary antibody stain, 1 in 10 Uroplakin-3 stain (Mouse monoclonal antibody IgG1, Progen Biotechnik), was prepared by adding 10µl of uroplakin-3 stain to 90µl of 1% goat serum (Sigma Aldrich).
14. The primer was aspirated and 100µl of primary antibody was added. This was covered and incubated on an orbital shaker for one hour. It was aspirated and washed 4 times with 100µl of 1% goat serum.
15. The secondary antibody stain with counterstain, containing 1 in 100 DAPI, 1 in 500 Alexa fluor 555 goat anti-mouse IgG, and 1% goat serum, was prepared by adding 1µl DAPI and 0.2µl Alexa fluor 555 goat anti-mouse IgG to 98.9µl 1% goat serum.
16. The primary antibody was aspirated, and the slide washed 5 times with PBS. 100µl of secondary antibody with counterstain was added, covered and incubated on an orbital shaker for one hour.
17. The secondary antibody was aspirated, and the slide washed 5 times with 100µl PBS. The final wash was left on for 2 minutes on the orbital shaker to remove all traces of remaining antibody.
18. The slide was immediately mounted with a drop of FluorSave mounting reagent (Calbiochem). A cover slip was placed gently ensuring no bubbles and secured with clear nail varnish at each corner. The slide was covered from the light and allowed to dry for at least an hour.
19. Once dry the slide was stored at 4°C in a light protective box.
20. The slide was viewed using a fluorescent microscope to count UP3-positive epithelial cells and bacteria-associated urothelial cells. Alexa fluor 555 excites at a wavelength of 555nm and emits at 565nm, giving a red appearance to urothelial cell membranes under fluorescence. DAPI excites at 360nm and emits at 460nm giving a blue appearance to nuclei and bacteria.
21. Images of example cells were captured at 100x magnification with the Infinity 3 Monochrome camera with Infinity Capture, and processed for presentation using Image J.

13.7.2 Fluorescent microscopy method for clue cell staining

Fluorescent microscopy was used to count epithelial cells associated with bacteria, expressed as a proportion of the total epithelial cells counted. This protocol was originally developed by Harry Horsley (Horsley, Malone-Lee et al. 2013):

1. WGA, DAPI were stored at -20°C and thawed immediately prior to use.
2. All steps were carried out at room temperature including incubations.
3. A Thermo Scientific, Superfrost Ultra Plus microscope glass slide was labelled and the field area for cells was marked using the filter card (Fisher Scientific) as a guide.
4. The slide was assembled with a cuvette holder, slide, filter and Shandon single funnel cuvette.
5. The assembled cuvette was positioned in the cytocentrifuge (Shandon cytospin 2 cytocentrifuge) and balanced. 80µl of sample was added to the single funnel cuvette assembly.
6. This was spun at high acceleration, at 800rpm (≈ 75 RCF) for 5 minutes. This resulted in a visible disc of urinary particulate, approximately 5mm diameter ($\approx 20\text{mm}^2$), deposited on the slide.
7. Combined WGA (wheat germ agglutinin) and DAPI (4',6-diamidino-2-phenylindole) stain was prepared. WGA had been conjugated to alexa-fluor-488 (Invitrogen Ltd, Paisley, UK); it was used to label the cell membrane to aid cellular identification. DAPI was used for fluorescent counterstaining of host and pathogen DNA. Solution of WGA 1 in 200 dilution and DAPI 1 in 100 dilution in Hank's balanced salt solution (HBSS) was prepared by adding 1µl WGA and 2µl DAPI to 197µl HBSS.
8. The assembled cuvette was promptly removed from the cytocentrifuge and the slide removed without dislodging the deposit.
9. The cellular deposit was circumscribed with a hydrophobic barrier pen (ImmEdge pen, Vector Laboratories).
10. Cells were fixed by adding 80µl of 4% formaldehyde and covering to shield from light for 20 mins at room temperature.

11. The formaldehyde was aspirated, and the slide was washed with 80µl of PBS (phosphate buffered saline) three times.
12. 80µl of the earlier prepared WGA/DAPI stain was added, ensuring cells were all covered. Slides were covered to shield from light and incubated for 20 minutes.
13. The stain was aspirated, and the slide was washed with 80µl of PBS four times, ensuring no fluid was left on the slide.
14. The slide was immediately mounted with a drop of FluorSave mounting reagent (Calbiochem). A cover slip was placed gently ensuring no bubbles and secured with clear nail varnish at each corner. The slide was covered from the light and allowed to dry for at least an hour.
15. Once dry the slide was stored at 4°C in a light protective box.
16. The slide was viewed using a fluorescent microscope to count epithelial cells and bacteria-associated epithelial cells. Alexa fluor 488 excites at a wavelength of 495nm and emits at 519nm, giving a green appearance to cell membranes under fluorescence. DAPI excites at 360nm and emits at 460nm giving a blue appearance to nuclei and bacteria. DAPI is capable of penetrating cell membranes and so is able to label intracellular and adherent pathogens without permeabilisation.
17. Images of example cells were captured at 100x magnification with the Infinity 3 Monochrome camera with Infinity Capture, and processed for presentation using Image J.

13.7.3 Fluorescent microscopy method for combined urothelial cell and clue cell staining

Fluorescent microscopy was used to count epithelial cells staining positive for uroplakin-III, expressed as a proportion of the total epithelial cells counted, as well as those uroplakin-positive cells with associated bacteria. This protocol was originally developed by Harry Horsley (Horsley, Malone-Lee et al. 2013) and modified by Linda Collins (Collins, Sathiananthamoorthy et al. 2020) to produce triple stained slides to facilitate reduced laboratory work and faster slide viewing:

1. WGA, DAPI, UP3 and Alexa Fluor 555 goat anti-mouse IgG were stored at -20°C and thawed immediately prior to use.
2. All steps were carried out at room temperature including incubations.
3. A Thermo Scientific, Superfrost Ultra Plus microscope glass slide was labelled and the field area for cells was marked using the filter card (Fisher Scientific) as a guide.
4. The slide was assembled with a cuvette holder, slide, filter and Shandon single funnel cuvette.
5. The assembled cuvette was positioned in the cytocentrifuge (Shandon cytospin 2 cytocentrifuge) and balanced. 80µl of sample was added to the single funnel cuvette assembly.
6. This was spun at high acceleration, at 800rpm (≈ 75 RCF) for 5 minutes. This resulted in a visible disc of urinary particulate, approximately 5mm diameter ($\approx 20\text{mm}^2$), deposited on the slide.
7. The assembled cuvette was promptly removed from the cytocentrifuge and the slide removed without dislodging the deposit.
8. The cellular deposit was circumscribed with a hydrophobic barrier pen (ImmEdge pen, Vector Laboratories).
9. Cells were fixed by adding 80µl of 4% formaldehyde and covering to shield from light for 20 mins at room temperature.
10. Combined WGA (wheat germ agglutinin) and DAPI (4',6-diamidino-2-phenylindole) stain was prepared. WGA had been conjugated to alexa-fluor-488 (Invitrogen Ltd, Paisley, UK); it was used to label the cell membrane to aid cellular identification. DAPI was used for fluorescent counterstaining of host and pathogen DNA. Solution of WGA 1 in 200 dilution and DAPI 1 in 100 dilution in Hank's balanced salt solution (HBSS) was prepared by adding 1µl WGA and 2µl DAPI to 197µl HBSS.
11. The formaldehyde was aspirated, and the slide was washed with 80µl of PBS (phosphate buffered saline) three times.
12. 80µl of the earlier prepared WGA/DAPI stain was added, ensuring cells were all covered. Slides were covered to shield from light and incubated for 20 minutes.

13. The primary antibody stain, 1 in 10 Uroplakin-3 stain (Mouse monoclonal antibody IgG1, Progen Biotechnik), was prepared by adding 10 μ l of uroplakin-3 stain to 90 μ l of 1% goat serum (Sigma-Aldrich).
14. The secondary antibody stain, containing 1 in 500 Alexa fluor 555 goat anti-mouse IgG and 1% goat serum, was prepared by adding 0.2 μ l Alexa fluor 555 goat anti-mouse IgG to 99.8 μ l 1% goat serum.
15. The WGA/DAPI stain was aspirated and the slide was washed with 80 μ l of PBS four times, ensuring no fluid was left on the slide.
16. Cells were permeabilised using 80 μ l of 0.2% Triton-X (Sigma-Aldrich) in PBS, incubated for 5 minutes, and aspirated. The slide was washed with 80 μ l PBS three times.
17. Cells were primed with 80 μ l of 10% goat serum (Sigma-Aldrich) in PBS and incubated for 30 minutes.
18. The primer was aspirated and 50 μ l of primary antibody was added. This was covered and incubated on an orbital shaker for one hour. It was aspirated and washed 3 times with 50 μ l of 1% goat serum.
19. 50 μ l of secondary antibody was added, covered and incubated on an orbital shaker for one hour.
20. The secondary antibody was aspirated, and the slide washed 5 times with 50 μ l of 1% goat serum, aspirating all fluid at the end.
21. The slide was immediately mounted with a drop of FluorSave mounting reagent (Calbiochem). A cover slip was placed gently ensuring no bubbles and secured with clear nail varnish at each corner. The slide was covered from the light and allowed to dry for at least an hour.
22. Once dry the slide was stored at 4°C in a light protective box.
23. The slide was viewed using a fluorescent microscope to count UP3-positive epithelial cells and bacteria-associated urothelial cells. Alexa fluor 488 (WGA) excites at a wavelength of 495nm and emits at 519nm, giving a green appearance to cell membranes under fluorescence. Alexa fluor 555 (Uroplakin-3) excites at a wavelength of 555nm and emits at 565nm, giving a red appearance to urothelial cell membranes. DAPI excites at 360nm and emits at 460nm giving a blue appearance to nuclei and bacteria.

24. Images of example cells were captured at 100x magnification with the Infinity 3 Monochrome camera with Infinity Capture, and processed for presentation using Image J.

13.8 Recruitment proforma for PTB study

Name:

Hospital Number:

Post code:

DOB:

Ethnicity:

Occupation:

Date of recruitment:

CLIMB number:

Gestation at recruitment:

EDD:

Parity: 0 1 2 3 4 >4 (Specify:)

Previous PTB: N Y (Gestation:)

Previous cervical cerclage: N Y (Gestation at delivery:)

Previous miscarriages <14/40: N Y (How many:)

Previous miscarriages >14/40: N Y (How many: Gestation:)

Previous treatment to cervix: N Y (Year:)

LLETZ Cone biopsy

Uterine anomaly: N Y (Specify:)

Trans-cervical surgery: Y N

TOP (Number:) ERPC (Number:) Other (Number:)

Smoker: Current Ex Number per day:

BMI:

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