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**A Study of Urinary Catheter Encrustation in Patients
with *Proteus* Urinary Tract Infection**

**Thesis presented to the University of London for the degree of Doctor of
Medicine by Sunil Mathur MBBS**

Bristol Urological Institute

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Abstract

The most common cause of encrustation and blockage of long term urinary catheters is colonisation of the urinary tract by *Proteus* spp. However, the degree of encrustation experienced by those with *Proteus* colonisation differs markedly between individuals. This study assessed the range of problems experienced by those colonised by *Proteus* and the factors which may differentiate severe from mild encrustation in this group.

21 long term catheter users found to have *Proteus* on urine screening were followed for approximately 3 months with weekly microbiological and chemical urine analysis and examination of their catheters.

There was considerable variation in catheter lifespan within and between individuals. Some with persistent *Proteus* colonisation had no encrustation problems, others experienced frequent catheter blockage. *Proteus* was usually a stable component of urinary and catheter flora.

Rapid encrustation was associated with a lowered nucleation pH (pH_n). There was no clear difference in voided pH (pH_v) between rapid and slow encrusters, but rapid blockers had a lower mean safety margin ($\text{pH}_n - \text{pH}_v$). pH_v was not a useful predictor of catheter blockage.

pH_n was variable within and between individuals, but was higher in those who encrusted slowly. It was dependent on the calcium concentration and, to a lesser extent, the magnesium concentration of urine, with rapid encrusters having higher urinary calcium.

Proteus isolates were assessed for urease activity. Strains with higher urease activities produced more alkaline urine, but this did not clearly result in shorter catheter lifespans.

Proteus isolates from catheter users were found to have greater antibiotic resistance, particularly to trimethoprim and amoxicillin, than other local urinary tract isolates.

Courses of antibiotic appeared ineffective at altering the urinary flora.

The source of *Proteus* urinary colonisation, examined using Dienes typing and pulsed field gel electrophoresis of bacterial DNA, was commonly found to be the subject's own intestinal flora.

List of Contents

Abstract	2
List of Contents	4
List of Tables	8
List of Figures	11
Acknowledgements	14

Section 1 - Introduction

1.1	Overview	16
1.2	Urinary Catheterisation	18
1.2.1	Development of the Urinary Catheter	18
1.2.2	Modern Urinary Catheters	24
1.3	Long Term Catheterisation	28
1.3.1	Indications for Urinary Catheterisation	28
1.3.2	Prevalence	30
1.3.3	Morbidity	32
1.3.4	Financial Costs of Long Term Catheterisation	41
1.4	Catheter Encrustation	44
1.4.1	Urinary Tract Pathogens	44
1.4.2	Bacterial Biofilms	45
1.4.3	Catheter Associated Urinary Tract Infection	47
1.4.4	Strategies to Control Catheter Associated Infection	50
1.4.5	Crystal Formation	61
1.4.6	Strategies to Control Encrustation	68
1.5	<i>Proteus mirabilis</i>	77
1.5.1	Classification	77
1.5.2	Epidemiology	78
1.5.3	Strain Discrimination	79
1.5.4	The Urease Enzyme	81
1.5.5	Morphology and Physiology	83
1.6	Aims of the Study	85

Section 2 - Methodology

2.1	Protocol Summary	87
2.2	Sample Collection	88
2.2.1	Recruitment	88
2.2.2	Sample Collection	89
2.3	Chemical Analysis	90
2.3.1	Voided pH	90
2.3.2	Nucleation pH	90
2.3.3	Urinary Calcium and Magnesium Concentration	91
2.3.4	Encrustation Analysis	92
2.4	Microbiological Analysis	93
2.4.1	Urinary Culture	93
2.4.2	Catheter Biofilm Culture	94
2.4.3	Faecal Sample Culture	94
2.4.4	Storage of Bacterial Specimens	94
2.4.5	Antibiotic Sensitivities	94
2.4.6	Urease Assay	95
2.4.7	Comparison of Urine And Stool Cultures	98
2.5	Statistical Analysis	101

Section 3 - Results

3.1	The Prospective Study	103
3.1.1	Summary of Data for each Study Participant	104
3.1.2	Variation in Catheter Lifespan	139
3.2	Urinary Constituents and Encrustation	142
3.2.1	Variability in voided pH and nucleation pH	142
3.2.2	Catheter Encrustation Rate	155
3.2.3	Catheter Specific Safety Margin	157
3.2.4	Investigating the 'Blocking pH'	158
3.3	Factors Influencing Nucleation pH	164
3.3.1	Simple Linear Regression	164
3.3.2	Multiple Linear Regression	169
3.4	Urease Activity	172
3.5	Behaviour of Bacterial Populations	176
3.5.1	The Persistence of <i>Proteus</i> Urinary Tract Colonisation	176
3.5.2	Frequency of Co-infections Associated with <i>Proteus</i>	177
3.5.3	Comparison of Cultures from Urine and Catheters	179

3.6	Antibiotic Sensitivities	180
3.6.1	Sensitivity of <i>Pr. mirabilis</i> Isolates from Study Participants	180
3.6.2	Sensitivity of <i>Pr. mirabilis</i> Isolates from a Local Population	182
3.6.3	Differences in Antibiotic Sensitivity between Study Participants and the Local Population	184
3.6.4	Effects of Antibiotic Use on Urinary Bacterial Populations in the Study Group	186
3.7	Comparison of Urine and Faecal Cultures	189
3.7.1	Dienes Typing	189
3.7.2	Pulsed Field Gel Electrophoresis	192

Section 4 - Discussion

Refining our Understanding of Catheter Encrustation	195	
4.1	The Prospective Study	196
4.1.1	Noteworthy Participants	196
4.1.2	Variation in Catheter Lifespans in Individuals	206
4.2	Urinary pH and Encrustation	210
4.2.1	Variability in Measured Values	210
4.2.2	Catheter Encrustation Rate	212
4.2.3	Catheter Specific Safety Margin	217
4.2.4	The Blocking pH	218
4.3	Factors Influencing Nucleation pH	221
4.3.1	Crystal Formation	221
4.3.2	Determinants of Nucleation pH	227
4.3.3	Calculated Theoretical Variability of Nucleation pH	229
4.3.4	Theoretical Inaccuracies of the Nucleation pH Method	234
4.3.5	Alternative Methods of Estimating the Potential for Crystal Precipitation from Urine	235
4.4	Urease Activity	238
4.5	Behaviour of Bacterial Populations	241
4.6	Antibiotic Sensitivities	243
4.6.1	Resistance of Urinary Isolates	243
4.6.2	Effects of Treatment	247
4.7	Comparison of Urine and Faecal Cultures	251

Section 5 - Conclusions

5.1	Conclusions	256
5.2	Future Work	258

Section 6 - Appendices

Appendix 1:	Example Calculation of Nucleation pH	262
Appendix 2:	Analysis Excluding Participants 03 & 11	265
Appendix 3:	Analysis Using Encrustation Rate	266

Section 7 - References

References	268
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List of Tables

- 3.1** Demographic details, catheter details and length of involvement in the trial of the main study participants
- 3.2** Descriptive statistics representing the variability in catheter lifespan, measured in days, for each study participant
- 3.3** Distribution of mean values of pH_v and pH_n of study participants
- 3.4** t test - comparison of mean safety margin between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.5** t test - comparison of mean voided pH between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.6** t test - comparison of mean nucleation pH between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.7** Mann-Whitney U test - comparison of mean urinary calcium concentration between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.8** Mann-Whitney U test - comparison of mean urinary magnesium concentration between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.9** Mann-Whitney U test - comparison of mean total calcium and magnesium concentration between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.10** Encrustation rate (mg calcium and magnesium deposited per day) of catheters in the study, shown as minimum, maximum, mean and sample standard deviation values for each study participant.
- 3.11** List of those participants characterised as rapid encrusters using cut off values of mean catheter life < 28 days or mean encrustation rate > 0.7 mg/day
- 3.12** t test - comparison of pH_v at the start and end of a catheter's lifespan in all blocked catheters
- 3.13** t test - comparison of pH_v at the start and end of a catheter's lifespan in catheters from 28 day blockers
- 3.14** t test - comparison of pH_v before and after catheter blockage in all blocked catheters

- 3.15 t test - comparison of pH_v before and after catheter blockage in catheters from 28 day blockers
- 3.16 Mean magnitude of pH_v changes during a catheter lifespan
- 3.17 Multiple linear regression - a model of the effect of mean urinary calcium and magnesium concentrations on nucleation pH
- 3.18 Multiple linear regression - a model of the effect of mean urinary calcium and magnesium concentrations on nucleation pH with an interaction term included
- 3.19 Mean urease activity of isolated urinary *Proteus* strains from three replicated assays
- 3.20 Mann-Whitney U test - comparison of mean *Proteus* urease activity between those catheter users with a mean catheter lifespan greater than and less than 28 days
- 3.21 Number of participants with persistent *Proteus* colonisation from which other species were isolated from urine samples, either at any point during the trial or for three consecutive weeks
- 3.22 Antibiotic resistance summary for *Proteus* isolates from the study group
- 3.23 Antibiotic sensitivities of clinical isolates
- 3.24 Antibiotic resistance of *Proteus* samples from 145 non-catheterised individuals submitted to a local hospital pathology laboratory
- 3.25 Antibiotic resistance of *Proteus* samples from 36 catheterised individuals submitted to a local hospital pathology laboratory
- 3.26 Resistance of organisms isolated from the local population to second line antibiotics. These isolates would have already proved resistant to some first line antibiotics.
- 3.27 Comparison of the resistance of organisms isolated from the local population and the study population to common antibiotics
- 3.28 Fisher's exact test – differences in antibiotic sensitivities for trimethoprim and amoxicillin between trial isolates and those seen in the local hospital pathology laboratory
- 3.29 Effects of antibiotic use on urinary flora, showing the species isolated immediately before and after the course of antibiotic shown
- 3.30 Dienes typing results, showing isolate combinations which formed demarcation lines and combinations which merged
- 4.1 Percentage of *Proteus* isolates resistant to various antibiotics from the studies of Muder *et al* (1992), Smith *et al* (2000), Wazait *et al* (2003), and the present study.

- 6.1** Comparison of the p-values for statistical tests comparing rapid and slow encrusters using data from all participants, and for tests excluding data from participants 03 and 11
- 6.2** Comparison of the p-values and Pearson's correlation coefficient r between correlation tests using data from all participants, and for tests excluding data from participants 03 and 11
- 6.3** Comparison of the p-values for statistical tests comparing the grouping of participants into rapid and slow encrusters as defined by either mean catheter lifespan or encrustation rate

List of Figures

- 1.1** The position of a urethral catheter in a female
- 1.2** The position of a suprapubic catheter in a female
- 1.3** Representation of the difference in cross section between 100% silicone and latex based catheters
- 3.1** Example of a segment from a participant summary graph
- 3.2** Participant 02 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.3** Participant 03 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.4** Participant 04 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.5** Participant 05 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.6** Participant 06 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.7** Participant 07 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.8** Participant 08 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.9** Participant 09 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.10** Participant 10 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.11** Participant 11 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.12** Participant 12 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.13** Participant 13 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.14** Participant 14 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period

bacterial isolates over the course of the study period

- 3.15** Participant 15 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.16** Participant 16 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.17** Participant 17 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.18** Participant 18 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.19** Participant 19 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.20** Participant 20 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.21** Participant 21 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.22** Participant 22 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period
- 3.23** Mean catheter lifespan for each study participant
- 3.24** Mean safety margin for each study participant
- 3.25** Variability of voided pH and nucleation pH over the course of the study in each catheter user
- 3.26** Linear regression to illustrate correlation between mean catheter lifespan and mean urinary calcium
- 3.27** Linear regression to illustrate correlation between mean catheter lifespan and mean urinary nucleation pH
- 3.28** Linear regression to illustrate correlation between mean catheter lifespan and mean urinary safety margin
- 3.29** Linear regression to illustrate correlation between catheter specific safety margin and individual catheter lifespans
- 3.30** Linear regression to illustrate correlation between mean urinary calcium concentration and mean urinary nucleation pH
- 3.31** Linear regression to illustrate correlation between mean urinary magnesium concentration and mean urinary nucleation pH
- 3.32** Linear regression to illustrate correlation between the sum of mean urinary calcium

and magnesium concentrations and mean urinary nucleation pH

- 3.33** A scatter plot to illustrate the correlation between the mean urinary voided pH of an individual and the urease activity of their colonising *Proteus* strain
- 3.34** A negative Dienes test result - two strains of the same Dienes type merge when they meet
- 3.35** A positive Dienes test result - two different strains repel each other forming a Dienes demarcation line between them
- 3.36** PFGE patterns of *Pr. mirabilis* DNA restriction fragments from pairs of isolates of urinary and faecal origin
- 4.1** Predicted effect of changing calcium concentration in the presence of various magnesium concentrations
- 4.2** Predicted change in nucleation pH as concentrations of calcium and magnesium are varied in proportion from the mean figures for participants 02, 16 and 06
- 6.1** Changes in optical density of a urine sample as it is alkalinised
- 6.2** Regression lines for the straight segments of the trace shown in figure 6.1 for pH values immediately above and below the abrupt change in optical density. The intersection of these lines gives the nucleation pH

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Section 1 - Introduction

1.1 Overview

Introduction to general concepts

1.2 Urinary Catheterisation

Development and technology

1.3 Long Term Catheterisation

Prevalence and associated morbidity

1.4 Catheter Encrustation

Urinary tract infection and the formation of crystalline biofilms

1.5 Proteus mirabilis

Microbiology and urease production

1.6 Aims of the Study

A summary of hypotheses to be tested

1.1 Overview

Many people find that the only way to manage their bladder problems adequately is to use an indwelling urinary catheter for bladder drainage as a long term solution.

Although for many people this is a relatively successful approach, about half of long term catheter users will experience recurrent complications which can have a serious impact on their quality of life.

The most common and troubling of these complications is catheter encrustation.

Crystalline deposits build up on the surfaces of the catheter, leading to blockage of the lumen and drainage eye holes. This causes painful retention of urine or incontinence, needing an emergency visit by a District Nurse or attendance at an Accident and Emergency department to change the catheter. To try to prevent this occurring, catheters have to be changed very frequently. This is inconvenient for the patient and expensive for the health care provider.

In most cases encrustation is due to the presence of urease producing bacteria in the urinary tract. Urease is an enzyme which hydrolyses urea to form ammonium ions, leading to an increase in the voided pH of the patient's urine above the value at which certain calcium and magnesium phosphates can remain in solution. This value has been examined experimentally and termed the nucleation pH. In other cases patients seem to form catheter encrustation without urease producing bacteria present, at a relatively normal voided urinary pH, because they seem to have an abnormally low nucleation pH.

Bacterial colonisation of the urinary tract, usually asymptomatic, is common in patients with urinary catheters and is universal in those with long term catheters.

Organisms adhere to and colonise the catheter's surfaces and, embedded in an adhesive polysaccharide matrix, they form bacterial biofilm communities. In this biofilm mode of growth they are extremely resistant to conventional antibacterial treatments. The organisms in the biofilm attached to the catheter do not cause a symptomatic infection, but if they produce urease the urinary pH will rise. Crystals will precipitate and are then incorporated into the biofilm to form catheter encrustation. Pieces of the crystalline biofilm may detach from the catheter and act as foci for bladder calculus formation.

The most potent urease producing organism commonly found in these patients is *Proteus mirabilis*. It is an uncommon urinary tract pathogen in the general population, but is much more common in those with catheters and can be a very difficult organism to eradicate.

To date, no successful strategy has been established which extends the lifespan of catheters in the face of catheter blockage caused by these bacteria.

1.2 Urinary Catheterisation

A urinary catheter is a tube designed to passively drain the urinary bladder, avoiding the need for normal bladder contraction, bladder capacity, and urinary sphincter function. Catheters can be inserted into the bladder through the urethra, or suprapubically through a surgical tract in the anterior abdominal wall. This is shown diagrammatically in figures 1.1 and 1.2.

1.2.1 Development of the Urinary Catheter

It has been claimed that urinary catheters may have been used by the oldest civilisations known [1], and that catheterisation would have been utilised by prehistoric peoples much as other simple medical techniques such as applying pressure to bleeding wounds must have without any need felt to document such a mundane matter once writing systems were developed [2]. Firm evidence for this is hard to come by.

Retention of urine is referred to as a disorder in the ancient Egyptian medical text known as the Ebers papyrus (dating from around 1500 BCE), but pharmacological therapy is suggested as treatment and catheterisation is not mentioned [3]. Catheters are seemingly shown in ancient Egyptian engravings of surgical tools, such as those depicted in the tomb of the dentist physician Hesi-Re at Saqqara (dated at around 2600 BCE) and on the wall of the twin temple of Kom Ombo (commenced in the second century BCE) [4], but these diagrams are open to other interpretations.

Translations of cuneiform texts from the ancient civilisations of Mesopotamia, dating from the first and second millennia BCE, indicate that medications were applied to the

body or eyes or into bodily orifices by means of metal tubes [5]. RC Thompson's translation of the British Museum's holdings, *Assyrian Medical Texts*, contains the following passage:

(By means of) a tube of copper, into his penis you shall blow (the medication) with your mouth.

Words in parenthesis were added by the translator for clarity, and are not direct translations of equivalent terms in the original text. MJ Geller's translation of other texts [6] mentions a similar process:

If a man's urine constantly drips and he is not able to hold it back, his bladder swells and he is full of wind, his urine duct is full of blisters; in order to cure him, bray (untranslatable), crush it in pressed oil, and (blow) it into his penis through a bronze tube.

It is unclear whether these tubes were passed into the bladder or merely a short distance into the penile urethra, and these do not seem to be descriptions of urinary drainage, so is not unambiguous evidence of catheterisation in the usual sense.

The Indian *Vedas* (compiled between 1500 and 1000 BCE) and the surgical text *The Sushruta Samhita* (dated between 1000 and 600 BCE) describe the use of hollow tubes of metal or wood lubricated with butter or animal fat [7], although some controversy exists as to the accuracy of the translation. It is possible that they were only used to treat urethral strictures rather than to drain the bladder. The *Yadiguiar* of Ibn Cherif from Asia Minor reveals a passage describing the insertion of plant twigs

into the urethra, which some have interpreted as a reference to catheterisation [1], but may again simply indicate urethral stricture dilatation.

More detailed descriptions of these devices are only found in the latter half of the first millennium BCE. The pioneering Hellenistic anatomist Erasistratus of Ceos, probably working in Egyptian Alexandria in the first half of the third century BCE, is credited with treating urinary retention with a metal tube which he named a 'catheter' (from the Greek 'to send down' or 'to lower into'), although the evidence for this comes from a first century CE text [8]. Whether or not Erasistratus first coined the term catheter, evidence is found in the Hippocratic Corpus of writings from around 400 BCE that catheterisation was well known before his time, with it being considered a deficiency of a physician not to be able to succeed in inserting a tube into the bladder [8,9]. A detailed description of types of catheter and instructions for their use in both men and women is found in *On Medicine*, a survey of current medical knowledge written in around 30 CE by the Roman encyclopaedist Celsus Aurelius Cornelius. From this time on catheterisation is frequently mentioned in medical texts for dislodging impacted bladder stones, instilling medicines into the bladder, and relieving retention of urine. Bronze catheters in a variety of curvatures were found in the home of a surgeon during the excavation of the ruins of Pompeii, buried during the volcanic eruption of 79 CE.

Soft catheters made from treated or wire-reinforced parchment or textiles were used in the 16th and 17th centuries, but the rigid silver catheters available at the time were still more popular. This is the time when side drainage eye holes may have been introduced to reduce trauma on insertion. Further improvements came with the development of new materials technologies in the 18th century, specifically the discovery of natural rubber. Bernard and Theden used this to produce pliable elastic gum catheters. This material was, however, sensitive to extremes of temperature,

becoming either too brittle or too adherent to use effectively. This prompted further development of metal catheters, with John Weiss producing a flexible jointed model before newer rubber products established dominance [10]. Using the treatment process developed by Charles Goodyear in 1839, Auguste Nélaton introduced a far more effective device made of vulcanised rubber.

The origin of the modern catheter came with the development of the retention balloon. Until that time, catheters were usually used intermittently, either by the physician or the patients themselves. Some catheters were designed to be externally fixed in place by tapes or sutures from the 16th century, being constructed from soft materials such as wax impregnated cloth to aid comfort, and inserted over a metal stylet. However, these often collapsed or fell out after a short time [11]. This method of catheter retention was still in use into the 20th century [12]. Inflatable balloons made from ox intestine had been used by Theodore Ducamp to treat urethral strictures in 1822. In 1853, JF Reybard described his design of a rubber catheter which had a balloon inflated through a separate lumen to hold it in place inside the bladder, and this has been suggested as the origin of the balloon as a retention device [13]. An alternate method of retention developed by several different physicians in the late 19th century was the wing-tipped catheter, as balloons were unreliable until the development of latex rubber. Examples were produced by Wright, De Pezzer and Malecot [1]. At the turn of the century a large choice of catheter types were available in surgical instrument catalogues [2]. The balloon design was eventually developed into a two-piece dipped latex catheter by Frederick Foley and the CR Bard Company in 1930, with the inflation channel and balloon cemented and tied into a groove in the catheter. The original purpose of this device was to reduce haemorrhage following a prostatectomy operation by compressing the bleeding site with an inflatable balloon at the tip of the catheter. This was soon seized upon as an ideal method of retaining the catheter within any bladder. Following a final refinement

to the design in 1936 to produce the catheter in a single piece [14], the self-retaining Foley catheter has seen little change.

Figure 1.1: Diagram to show the position of a urethral catheter in a female

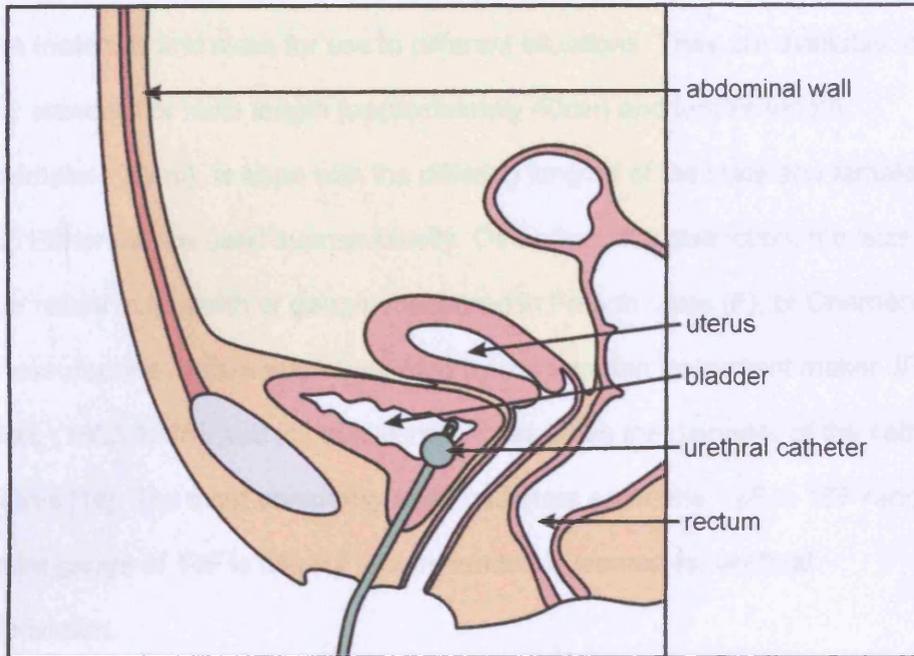
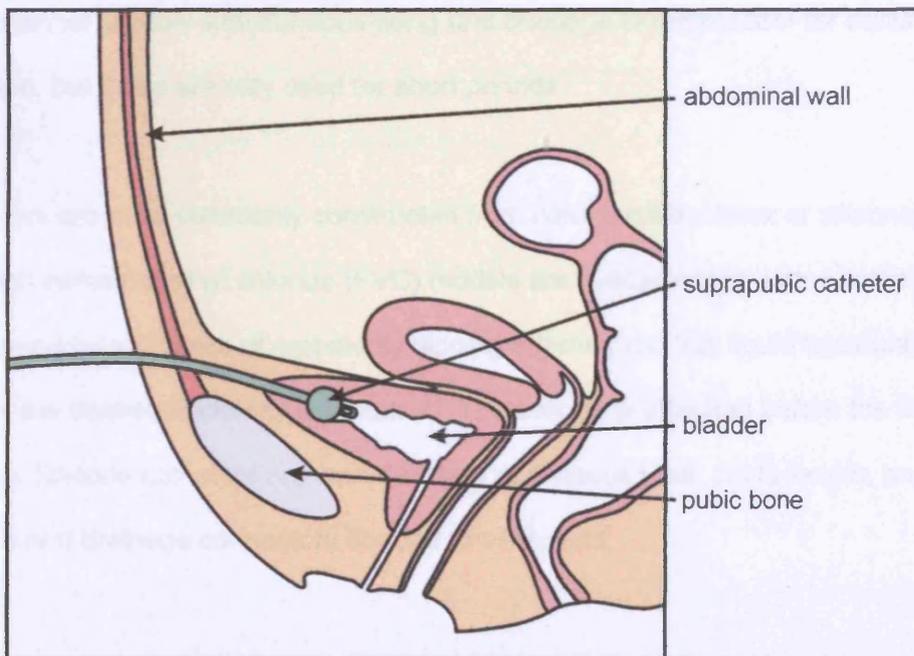


Figure 1.2: Diagram to show the position of a suprapubic catheter in a female



1.2.2 Modern Urinary Catheters

Although similar in basic design, Foley catheters are currently available in a variety of different materials and sizes for use in different situations. They are available in two lengths: standard or male length (approximately 40cm) and female length (approximately 25cm), to cope with the differing lengths of the male and female urethra. Either can be used suprapubically. Other than this distinction, the 'size' of a catheter refers to its width or gauge, measured in French scale (F), or Charrière (Ch). This measurement system was developed by the Parisian instrument maker JFB Charrière (1803-1876), and is equivalent to three times the diameter of the catheter in millimetres [15]. The most commonly used catheters are in the 12F to 16F range. A maximum gauge of 14F is usually recommended in women for urethral catheterisation.

The majority of catheters have a two lumen structure: a large channel to drain the urine, and a smaller one to inflate the retention balloon. Some larger catheters have a third channel to allow simultaneous filling and drainage of the bladder for continuous irrigation, but these are only used for short periods.

Catheters are most commonly constructed from natural rubber latex or silicone, although some polyvinyl chloride (PVC) models are available [16]. Latex catheters are formed by a process of repeatedly dipping a former rod into liquid latex until a tube of the desired thickness is produced. The balloon is attached before the final dipping. Silicone catheters are extruded as a continuous tube, cut to length, and the balloon and drainage connectors bonded to either end.

Various coatings and treatments can be used to try to improve the surface properties of catheters, largely directed at reducing friction. This is especially important for the

latex variety as it encounters more friction on insertion than silicone. Siliconised latex catheters are treated with silicone oil to reduce friction, although this only provides a temporary surface modification which is lost after insertion. Alternatively, a layer of silicone elastomer can be bonded to the surface. Polytetrafluoroethylene (PTFE) is a commonly used low friction coating in many applications, although PTFE coated catheters are actually coated with a layer of polyurethane (PU) containing a suspension of PTFE particles. Both latex and silicone can be coated with a hydrogel, a polymer that absorbs water when in contact with the urethral mucosa to form a layer of soft, low friction gel. More recently, coatings containing antibiotics or metal ions have been developed in an attempt to reduce infection or encrustation. These will be discussed later, in section 1.4.4.

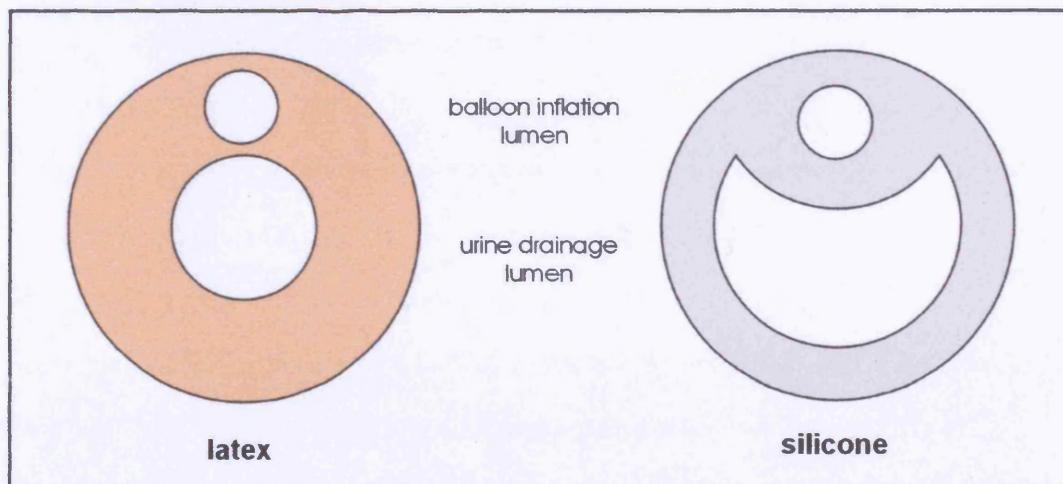
Hydrogel coated latex is the most popular type of catheter used in long term catheterisation. Almost all patients who don't use these use 100% silicone catheters.

Electron microscopy studies have shown that catheter surfaces are very uneven, especially around the catheter eye holes. It has been proposed that surface irregularities on biomaterials may be the sites of bacterial adhesion or crystal nucleation [17]. 100% silicone catheters are smoother than coated or plain latex, except for those latex catheters with hydrogel coatings which expand and smooth out in their hydrated state. While it is known that surface roughness has an effect on bacterial adhesion, this is not a linear relationship. Very smooth surfaces perform well in resisting colonisation and in lowering the shear force needed to detach adherent bacteria, but studies have shown that small increases in roughness aid bacterial colonisation more than larger increases, at least in those materials tested [18,19]. These experiments have not been performed using catheter materials, so it is unclear whether alterations in surface roughness within the physical limits of the currently available materials will affect bacterial adhesion in any way. Given that

chemical composition also affects adherence [20], altering surface roughness by changing the material present at the catheter surface may have unpredictable effects.

Both latex and silicone catheters have relatively thick walls, resulting in the urine drainage channel being quite narrow in comparison to the external diameter of the device, especially given that the balloon inflation channel must also be accommodated within the available space. The walls of silicone catheters are slightly thinner, meaning that for a given external diameter they have a wider drainage channel lumen. These catheters also have a drainage channel with a crescent moon shaped cross section, while that of a latex catheter is circular (figure 1.3). Latex based 14F catheters can have a drainage lumen diameter of approximately 1.5 mm compared to 2.5 mm for the 14F 100% silicone catheters [21]. These figures, and the ratio between them, vary considerably across manufacturers, and between different catheters produced by the same manufacturer.

Figure 1.3: Diagrammatic representation of the difference in cross section between 100% silicone and latex based catheters



One potential problem with latex based catheters is the possibility of the user developing a latex allergy, and this may explain the increasing use of silicone devices despite their greater cost. Silicone does have noticeable disadvantages as a catheter material, however. Catheter balloons deflate slowly during use due to permeability of the balloon material to water. In some cases this can lead to the catheter being expelled. Silicone is more water permeable than latex, leading to a more rapid deflation of the catheter balloon [22]. Another problem associated with catheter balloons is that they tend to deflate incompletely after a prolonged period of filling, leaving a cuff of material around the catheter tip which may make the catheter more difficult to withdraw. An audit study of the nurses managing 154 community based catheter users has shown this problem to be reported more commonly with silicone catheters, especially when used suprapubically, despite latex based catheters being used in 86% of patients [23]. In the same study, the cuffs on a variety of 100% silicone catheters were also found to offer more resistance to withdrawal through a simulated abdominal wall than hydrogel coated latex catheters.

The role of catheter material properties and drainage channel width in resisting encrustation is examined in section 1.4.6.

1.3 Long Term Catheterisation

Indwelling urinary catheters are used in two main ways: on a short term basis for a few days or weeks, or on a long term basis for many months or years. Although there is no clear cut off between these two groups, it is conventional to define long term catheterisation as the use of a catheter for a period of over three months [24], although it is sometimes defined as over 28 days [25]. In practice, the term usually indicates that catheterisation is considered to be the definitive treatment for that individual's bladder disorder, and that they will remain catheterised for the rest of their life.

1.3.1 Indications for Catheterisation

Short term catheterisation is most often used in an acute hospital setting. A catheter may be inserted to monitor the rate of urine output in the critically ill patient as a surrogate marker for perfusion of vital organs with an adequate supply of oxygenated blood. The catheter may also be used to drain the bladder in those individuals temporarily unable, because of illness, to void normally. It is also used after various forms of bladder surgery to decompress the bladder or allow bladder irrigation to prevent the build up of clotted blood. While short term catheterisation is usually urethral, suprapubic catheters are sometimes used to prevent the risk of urethral injury and stricture formation which can occur with urethral catheters [26].

Long term catheterisation is more likely to be used in the community. About 10% of long term catheters are used suprapubically. There are several types of bladder disorder for which long term catheterisation may be considered, but it is usually regarded as a measure to be used only when other treatments have failed, are not

appropriate, or are not acceptable to the patient. Many bladder disorders may be treated surgically, but these disorders often occur in the very elderly or frail who have a high risk of complications with such procedures. In general, a patient may be treated with a long term catheter for a chronic condition leading to either incontinence of urine or retention of urine.

Incontinence may be the result of several different pathological processes [27]. In some cases, it is due to a bladder disorder such as *detrusor overactivity*, where the bladder involuntarily contracts to minimal stimulus leading to loss of urine. This can be caused by neurological damage such as in multiple sclerosis or spinal cord injury [28], but is also a common idiopathic condition. Another problem, known as *stress incontinence*, occurs when the sphincter mechanism at the neck of the bladder cannot maintain enough pressure to retain urine against the normal passive rises in abdominal pressure caused by movement or coughing. Neurological damage can affect sphincter function as well as bladder function. Idiopathic detrusor overactivity and stress incontinence are both common conditions, being more common and more severe in elderly women, and they can occur together. Long term catheterisation would normally be considered only in the most severe cases, when large volumes of urine are being lost involuntarily. Neurological causes may create more severe conditions than an idiopathic cause. *Functional incontinence* is the term used to describe loss of urine due to decreased mobility to such a degree that a person cannot reach a toilet in a timely manner. In mild to moderate detrusor overactivity a person often has enough warning to reach a toilet in time, but this is less likely if that individual has a mobility problem. Mobility problems, like detrusor overactivity, are more common with increasing age.

Retention of urine may be due to *detrusor underactivity* to such a degree that the bladder cannot contract sufficiently to fully empty itself. It is also caused by conditions

which increase the pressure needed to be generated to void urine through the urethra, such as the enlarged prostate commonly found in an elderly man [29]. These disorders can occur in combination, and are again more common with increasing age. Neurological damage can cause detrusor underactivity. It can also result in the sphincter at the neck of the bladder failing to relax when the bladder contracts, which also leads to urinary retention.

1.3.2 Prevalence

A survey by Roe showed a prevalence of long term catheterisation of 0.03% of the population aged over 18 years of age served by the health centres, hospital out-patient departments, a urology department and the continence advisory service in one United Kingdom district health authority [30]. A larger survey conducted over two years by Kohler-Ockmore and Feneley, and also including the populations of nursing homes not served by the district nursing service, showed a prevalence of 0.07% in the over 16 age group [24]. It was shown that the prevalence increases appreciably with increasing age. In those aged over 75 years it increases to 0.5% and is 2% in those 85 years old [31].

Long term catheterisation is common among those needing long term health care. As can be seen in section 1.3.1, although the conditions responsible for catheterisation can occur in otherwise fit individuals, they are often caused by a generally disabling condition such as spinal cord injury or multiple sclerosis, or are found in people who are disabled through chronic disease or old age. Of those people with long term catheters, 87% have a chronic medical condition and about a third are resident in nursing homes [24].

Warren *et al* reported that 9.4% of women and 6.4% of men (overall 7.5% of residents) were catheterised in a survey of nursing homes in the United States [32]. A study in Denmark by Zimakoff *et al* showed 4.9% of nursing home residents and 3.9% of those receiving home care had catheters [33].

It is likely that this prevalence data will misrepresent the true extent of catheter use among the general population. There is no central register of individuals using catheters. Data for these studies have been collected from the records of those medical services attending the catheter users, and expressed as a proportion of the local population. Not all catheter users are likely to have been recorded, depending on the various services surveyed. For example, there is no data from care home residents in the Roe study as these records were not sought. This would lead to a significant underestimate, as these individuals are often not attended by community nurses but by the care home staff themselves. As shown above, care home residents have a high rate of catheter use. The higher prevalence in the Kohler-Ockmore and Feneley study compared to the Roe study is therefore likely to be due to the inclusion of the nursing home population. However, including care homes could produce an error if these facilities were concentrated in certain locations which may be under or overrepresented within the sampled area. The catchment areas of the various medical teams from which data was taken may not necessarily overlap, and no attempt was made to match demographic and social factors in the sampled regions to the UK population as a whole. Data for these studies were also collected in areas where these investigators worked. Given their particular interest in the problems of catheterisation it is possible that these regions would manage continence problems in a different fashion from other regions, perhaps resulting in a lower rate of catheterisation. The Zimakoff study followed a campaign in Denmark to reduce catheterisation in hospitals, and this may have resulted in a reduced rate of

catheterisation in care homes leading to difficulties in applying these figures to other populations.

It is clear that catheterisation is not uncommon among older people and those with disabilities. This group of people often have little alternative but to use a catheter. A lack of manual dexterity or a degree of cognitive impairment prevents intermittent self catheterisation. Condom catheters in males cannot be used by those with retention of urine, and many men find them difficult to attach securely. General immobility makes the regular changing of diapers difficult and leaving urine in contact with skin increases the risk of pressure sores. Long term catheterisation is likely to be seen more commonly as the proportion of the population aged over 80 increases [34].

1.3.3 Morbidity

Catheters are associated with considerable morbidity. The most common problems are encrustation, bladder calculi, catheter bypassing, and symptomatic infection. The mechanism of infection, encrustation and calculus formation will be dealt with in section 1.4 in more detail.

Urinary Tract Infection

Bacteriuria is an almost inevitable consequence of long term catheterisation [35], but this does not inevitably lead to the development of symptomatic urinary tract infection. On average, long term catheter users will develop one day of febrile illness for every 100 days of catheterisation. These episodes usually last for less than 24 hours and resolve without antibiotic treatment [36,37]. Even so, episodes of cystitis, upper urinary tract infection and potentially life threatening systemic bacterial sepsis do cause problems in many catheter users [38]. Catheters are the largest cause of

nosocomial infection in the developed world [32,39]. These infections are often more difficult to eradicate than in a non-catheterised patient due to factors discussed in later sections.

The changes of chronic upper urinary tract infection are also seen more commonly in those with indwelling catheters. Warren *et al* surveyed the post mortem findings of all residents who died aged 65 or older in a large long term care facility over a period of two years [40]. They found a significantly higher rate of chronic renal inflammation (43% versus 18%) and chronic pyelonephritis (10% versus none) in those residents who had been catheterised for a total of > 90 days in their last year of life compared to those who had not. Chronic pyelonephritis was defined as chronic renal inflammation with renal scarring and distortion of the renal calyces. These findings are seen in those with dysfunctional urinary tracts who are not catheterised, however, and it is possible that the disease which required catheterisation contributed to the renal damage, rather than just the catheter itself. In a similar autopsy study, the changes of acute pyelonephritis were found more commonly among those with catheters in situ at the time of their death than those without (38% versus 5%) [41]. It should be noted that chronic asymptomatic bacteriuria is not uncommon in the elderly, even in the absence of catheterisation. The prevalence of bacteriuria is 5-10% of men and 10-20% of women in the community, and between 25 and 50% of elderly residents in long term care facilities [42]. The non-catheterised elderly population also has a correspondingly higher incidence of symptomatic urinary tract infection than the general non-catheterised population.

Symptomatic infection of the bladder and upper urinary tract is understandably found in both urethral and suprapubic catheter users. There does seem to be a lower incidence of urethritis, prostatitis, epididymitis, and orchitis in suprapubic catheterisation [43], the explanation being that these are a group of infections of

those structures connected to the male urethra, and a catheter in the urethra would be a more direct source of infection than one inserted into the bladder through the abdominal wall.

Catheter Encrustation

Most manufacturers recommend that long term catheters are changed routinely every twelve weeks, although in many people they do not last this long because of encrustation related catheter blockage. Around 40 to 50% of catheter users are troubled with this problem [24,44,45]. Deposits of phosphate containing crystals, in association with bacterial communities, build up on those surfaces of the catheter which are in contact with urine. This leads to blockage of the catheter lumen, and the need for more frequent catheter changes, sometimes as often as twice a week.

Crystalline deposits on the tip of the catheter can cause urethral trauma when the catheter is removed, or can prevent removal completely, usually in the case of a suprapubic catheter where the scar tissue track is less pliable than a urethra.

Catheter users have been divided into two groups – ‘blockers’ and ‘non-blockers’ [46]. Blockers are those whose catheters are obstructed by encrustation in a period of less than six weeks, and are usually treated by crisis management, having their catheters changed as an emergency when they block. Non-blockers are those whose catheters last longer than this, and they usually have their catheters changed at routine intervals. This is a reasonable clinical distinction between those with a significant medical problem and those without, but it is not clear that this dichotomous classification is appropriate for scientific study. It has sometimes been assumed in the literature that those whose catheters last longer than six weeks are not forming encrustation. It is probable that these two groups are not distinct, but simply opposite ends of a continuum of very fast and very slow encrustation. It is certainly the case

that some catheters are regularly changed every twelve weeks according to manufacturer's guidelines and never cause any difficulties, yet show signs of encrustation at removal. At the other end of the spectrum are those whose catheters block every two or three days. It is likely that those who form encrustation, whether severe or trivial, are a distinct group from those who do not, due to differences in urinary bacterial flora (discussed in section 1.4). It does not seem to be the case, however, that those who block their catheters before they were due to be changed, or within any other set period of time, are a distinct group from those whose catheters last longer than this. In studies using this definition the 'non-blocker' group will include both non-encrusters and slow encrusters, who are probably quite different physiologically.

Bladder Calculi

Long term catheter users are at increased risk of forming bladder calculi, which, like encrustation, are composed of phosphate crystals and bacterial communities [47,48]. Calculi are possibly formed when pieces of encrustation are dislodged from the catheter surface, although they could form *de novo* by the same mechanisms as in non catheterised individuals. Encrustation on the catheter balloon is often left in the bladder when the balloon is deflated, forming what are known as 'egg shell' calculi. Further crystallisation occurs on the fragment surface and the calculus can increase in size. The calculus acts as a permanent source of bacteria in the bladder [49] and may physically block the catheter lumen. It also causes bladder irritation and may increase detrusor spasm.

Catheter Bypassing

Bypassing of urine around a urethral catheter often occurs when bladder drainage is blocked, either by encrustation of the catheter or by kinking or compression of the drainage tubing. Bypassing can also occur in an individual with a freely draining catheter as a result of detrusor overactivity [50]. This can cause an involuntary bladder contraction which creates a flow of urine exceeding the capacity of the thin catheter drainage lumen, resulting in fluid flow around the catheter. In the case of a suprapubic catheter, urine can leak urethrally. In women the urethra has relatively low resistance and bypassing is a common problem. Urethral leakage from women with suprapubic catheters can occur if urethral damage prevents normal urethral closure. This can result from long term urethral catheterisation or from repeated expulsion of urethral catheters with the balloons inflated [51]. Since catheters do not fully empty the bladder, some female suprapubic catheter users find themselves permanently incontinent. Surgical closure of the urethra is a possible solution, but is a significant procedure in a generally infirm population [52].

Chronic Pain

Most catheter users with intact sensation find their catheter uncomfortable to some degree. When asked, 69% of patients considered their catheter uncomfortable, with 19% rating the pain greater than 50% on a visual analogue scale [24]. Painful bladder sensations and perineal pain are not uncommon, and can be distressing.

Occasionally this is due to an allergy to the catheter material, often latex, but is usually simply a pressure effect. Discomfort can sometimes be reduced by decreasing the diameter of the catheter, and using softer latex catheters rather than silicone. In a person with a urethral catheter this discomfort can be helped by changing to a suprapubic catheter, although this is not always successful given that the catheter balloon is still in the bladder and possibly causing mechanical irritation.

A suprapubic catheter is inserted through a surgically created tract sited on the anterior abdominal wall, and this area often remains permanently tender. The advantage of the suprapubic site is that the patient does not normally sit or lie on it, so it is subject to little pressure other than from the catheter itself. Studies have shown them to be more comfortable and acceptable to patients than urethral catheters in short term post-operative situations [53,54]. In a survey of spinal cord injured patients fitted with a long term suprapubic catheter, 27 out of 32 stated they were satisfied with this form of management, despite the expected levels of encrustation and bypassing being present among the group [50]. However, larger, more rigid catheters such as the wider 100% silicone varieties can still be quite uncomfortable.

Urinary Tract Trauma

Long term catheters also physically damage the urethra and bladder. Peri-urethral glands are blocked and scar leading to urethral strictures [55-57]. While this is not a problem in a male who will be catheterised for the rest of his life, in a female this scarring can occur in the urethral sphincter area and prevent full urethral closure. This can lead to increased urinary bypassing and urethral leakage. In males an erosion of the ventral aspect of the penis can occur due to pressure necrosis of the urethra. This causes the appearance of a hypospadias after a prolonged catheterisation. Some authorities recommend that long term catheters should always be placed suprapublically to avoid this, although it may be a more important consideration in those who are expected to return to normal micturition at some stage [58,59].

Lesions of red, polypoid 'catheter cystitis' are commonly found on the bladder mucosa of catheter users [60]. These are thought to be due to the siphoning effect of a column of urine in the catheter drainage tubing sucking bladder mucosa into the catheter eye holes, as these lesions have been eliminated in a catheterised rabbit model by allowing air inlet into the drainage system [61].

Haematuria

While not often severe enough to cause clinical problems such as catheter blockage, occasional blood in the urine is very common, with 30% of catheter users complaining of this, often after a catheter change [24]. Microscopic haematuria is present in almost all patients who are tested. Enlarged prostates and the tracks through which suprapubic catheters are inserted often have small surface blood vessels which are easily damaged by the catheter, and the polypoid lesions of 'catheter cystitis' in the bladder may also bleed.

Malignancy

Several studies have suggested an increased risk of squamous cell carcinoma of the bladder among spinal cord injured patients, especially among those using long term catheters [62-64]. Squamous cell carcinoma has a less favourable prognosis than transitional cell carcinoma, which is the more common tumour in the normally functioning bladder. Kauffman *et al* studied a group of 62 spinal cord injured patients with urethral and bladder biopsies [65]. The incidence of squamous metaplasia was higher in those with long term catheters than those without, and among the catheter users, was more common in those who had been catheterised for longer than 10 years. Of those with squamous cell carcinoma, four out of five had indwelling catheters. A study by Locke *et al* found 2 cases of squamous cell carcinoma out of 25

spinal cord injured patients who had been catheterised for over 10 years [66], although these patients were those admitted to the authors' hospital unit for various reasons rather than being randomly selected from the catheterised spinal cord injured population. The mechanism responsible for this increased risk is hypothesised to involve either chronic mechanical irritation of the bladder epithelium by the catheter, or the production of carcinogenic compounds by the bacteria which colonise the catheterised urinary tract. The numbers involved in these studies are small, however, and given the known increase in squamous cell carcinoma among all those with spinal cord injury it is difficult to determine how much of the increased risk is due to the catheter and how much to other factors.

This suggested mechanism for the carcinogenic effect of urinary tract infection is bacterial production of *N*-nitrosamine compounds, which are well recognised carcinogens in animal models [67] and thought to be important in the development of several human tumours [68,69]. *N*-nitrosamine compounds can be endogenously produced in infected human urine. Nitrate is a normal constituent of urine. The majority of urinary pathogens can reduce urinary nitrate to nitrite, including those organisms such as *Pseudomonas*, *Klebsiella*, *Proteus* and *Escherichia coli* which are commonly found colonising catheterised urinary tracts [70]. This reaction forms the basis for dipstick testing of urine for urinary tract infection. The nitrosation reaction of these nitrites with urinary amines, such as dimethylamine, to form *N*-nitrosamine compounds can also be catalysed by various common uropathogens such as *E coli*, *Morganella* and *Pseudomonas* [71]. *N*-nitrosamines need to be further metabolised by the target organ cells to produce the DNA reactive species which cause malignancy. This certainly occurs for some *N*-nitrosamines in rat and mice bladders as these are the basis for a commonly used animal model of bladder cancer [72]. A study on non-catheterised individuals with *E. coli* or *Pr. mirabilis* urinary tract infections showed that both nitrite and *N*-nitrosamines were formed [73]. The levels

were positively correlated and both rapidly decreased when the infection was treated with antibiotics. *N*-nitrosamines were not formed in the absence of nitrite. Another clinical study showed that the colonised urine of 29 out of 30 paraplegic subjects contained nitrosamine compounds, although not all of these individuals used indwelling catheters, while the sterile urine of control subjects did not [74]. Although it is documented that some rubber products, including urinary catheters, can be contaminated with *N*-nitrosamine compounds which could confound the results of studies such as this, this was controlled for in this particular trial by testing one of the drainage devices in sterile urine to see if leached *N*-nitrosamines could be detected, which they could not. It should be noted that there are a large number of different *N*-nitrosamines which are known to be carcinogenic in animal models, but different compounds cause different cancers and the dose needed and the cancers caused vary between species, so it is difficult to predict the effect of the particular compounds produced in these studies on the human bladder.

A large case control study from the United States has suggested urinary tract infection as a risk factor for the development of bladder cancers, including squamous cell carcinoma [75]. In this study, Kantor *et al* used interview data from 2982 people with recently diagnosed bladder cancer to look for a past history of urinary tract infection, among other factors, which may have contributed to the development of the disease. Similar interviews were conducted with 5782 randomly selected non-sufferers. It was found that those with bladder cancers were 1.6 times more likely to report previous infections than control subjects, and twice as likely to report more than three infections. The risk of squamous carcinoma was even higher, but the number of cases of this tumour in the study was small. The investigators attempted to control for the possibility that the infections were caused by the cancers by not including infections which had occurred in the year prior to diagnosis, but no maximum limit was placed on how long before diagnosis the infection could occur. It

is difficult, intuitively, to imagine how a single uncomplicated lower urinary tract infection lasting no more than a few days could give rise to a bladder cancer, but even more so when the infection occurred decades before the cancer. Also, the investigators did not look for proof of infection, only that the subject recalled being given the diagnosis by a physician. Urinary tract infection can be misdiagnosed, with many transient non-infective lower urinary tract symptoms being attributed to infection when none is present. By the time the laboratory culture results are available the symptoms have resolved and the patient may never realise they did not have an infection. The contrary is also true, with infections caused by bacterial counts so low as to be dismissed by laboratories as sample contamination. It therefore cannot be certain that the correct number of infections was attributed to each participant. An even more important source of inaccuracy is the phenomenon of recall bias. It is quite possible that those recently diagnosed with a serious disease would look to attribute blame for their condition on previous events in their life, and so may be more likely to recall episodes of infection than the control subjects, possibly explaining a large proportion of the observed difference in infection rates. It would seem that the case for infection as a risk factor for bladder cancer remains unproven.

1.3.4 Financial Costs of Long Term Catheterisation

The high complication rate of this form of bladder management involves considerable time on the part of health care staff, usually district nurses, and presumably a significant cost to health care providers.

Getliffe determined that the care of those with long term catheters represented 4% of district nurse's patient caseload in one United Kingdom health district [76]. A survey by Sassoon *et al* showed that a group of 145 community catheter users needed 1000 district nursing visits in one year and took up 500 hours a month of nursing time [77].

What is not clear from either of these surveys is the amount of nursing time these patients required relating to the illnesses and disabilities responsible for their catheterisation in the first place. A large proportion of district nursing time, and therefore cost, is spent on travelling to visit a patient. If the patient required visiting for other reasons, the additional time spent attending to a catheter would not justify the entire visit being classified as a cost of catheterisation. However, this is not the case for emergency visits for blocked catheters. In the Kohler-Ockmore and Feneley study, 457 catheter users generated 149 emergency hospital referrals for catheter complications in 6 months, 104 of which were dealt with by Accident and Emergency departments, and 60% of which were for blocked catheters [24]. An even greater number of emergencies were dealt with in the community by District Nurses.

Evans *et al* performed a 3 month pilot study of 7 community patients with long term catheters in the United Kingdom in 1999. Participants were chosen to represent a range of catheter complication rates, underlying pathologies and degrees of dependency on care [34]. Costs taken into account were those directly incurred by the patient, such as laundry bills, as well as those incurred by health care and social service agencies. Indirect costs, such as loss of earnings, were not measured.

Managing those catheter users who did not suffer complications was inexpensive, the cheapest amounting to £119 over 3 months. When complications occurred the costs increased substantially, with one user costing £2585 over the same period, mostly in National Health Service expenditure. Again, it should be noted that some of these costs would have been incurred as a result of the underlying illness rather than as a result of the catheter. An allowance was made for this in terms of general nursing care for the more disabled patients in this study, with only a proportion of the cost of each visit being ascribed to the catheter, although the authors admit that they considered this costing a 'best guess' rather than an accurate figure. Patient costs may also have been overestimated. Catheter users may be frequently incontinent

due to catheters blocking and bypassing, but may be totally incontinent without a catheter. Alternatives, such as pads and external drainage devices, also leak urine. The catheterised person may have increased laundry costs compared to an individual with normal continence, but these may be equivalent or even reduced compared to any realistic alternative. However, the difference in cost between uncomplicated and complicated catheters, and the fact that 83% of the costs in this study were those incurred by hospital treatment for catheter problems, suggests that the catheter itself was contributing significantly to overall health care costs in addition to basic care for the catheter user's underlying medical problem.

While larger studies will be needed to develop a more accurate financial model, it is clear, given the high complication rate, that long term catheterisation is not a cheap form of continence management. This should provide the incentive needed to investigate improvements in health technologies and management strategies in this area.

1.4 Catheter Encrustation

Half of all long term catheter users experience a phenomenon known as catheter encrustation [24]. Scanning electron microscopy has shown that the encrusting material is composed of large crystals of struvite (magnesium ammonium phosphate hexahydrate) and a poorly crystalline apatite (a form of calcium phosphate) embedded in a matrix [78]. Large numbers of bacterial cells are also seen in this matrix [79]. These structures of crystal, bacteria and matrix are known as *crystalline biofilms*. They can develop rapidly as a coating on those catheter surfaces in contact with urine, obstructing the flow of urine through the catheter eye holes and drainage lumen, and so causing a catheter to block.

1.4.1 Urinary Tract Pathogens

In uncatheterised, otherwise healthy individuals resident in the community, 80% of urinary tract infections are caused by *Escherichia coli*, and 10 to 15% are caused by *Staphylococcus saprophyticus*, this last being especially prevalent among young women. *Enterococcus faecalis*, *Klebsiella* and *Proteus* infections do occur in this group, but not commonly [80].

In those with urinary catheters the pattern changes, reflecting the organisms seen in nosocomial infection: that is, those infections acquired in hospitals and long term care facilities, or as a result of a medical procedure. *E. coli* are present in 50% of cases, but *Proteus* and *Klebsiella* infections make up a significant proportion of the remainder. In individuals with long term catheters or structural abnormalities of the urinary tract, up to 44% of urine cultures contain *Pr. mirabilis*. *Pseudomonas aeruginosa*, *Ent. faecalis*, and *Staphylococcus aureus* are also seen quite commonly, along with *Citrobacter*, *Serratia*, *Providencia* and *Candida* spp. Colonisation with

multiple bacterial species is found in up to 95% of urine cultures from long term catheter users [81-84].

1.4.2 Bacterial Biofilms

Until relatively recently, the study of bacteria was concerned largely with the study of the organisms as they exist in their *planktonic* state. Planktonic bacterial forms are those which are present as individual organisms suspended in a liquid medium. It is now recognised that bacteria predominantly exist in communities attached to solid surfaces, both in the environment and in their infections of human hosts. These communities are known as *biofilms* [85].

A biofilm is defined as a group of microbes immobilized at a solid surface, in single or multiple layers, embedded in or surrounded by an organic polymer matrix, primarily of microbial origin [86]. Biofilms play a central role in the pathogenesis of human disease [87]. They form dental plaque leading to periodontal inflammation [85], form on the urothelium of the kidneys as a prelude to invasion of the renal parenchyma in pyelonephritis [88], are found on many implanted surgical prostheses [89], and are responsible for haematogenous infection from intravascular catheters [90]. They have been observed on the majority of ureteric stents [91] and urinary catheters [92] removed from both symptomatic and asymptomatic individuals.

The formation of a biofilm on an implanted device such as a catheter takes place in several steps. When a biomaterial is introduced into a body fluid, such as urine, blood or saliva, macromolecules rapidly adsorb onto the biomaterial's surface to form a thin organic layer known as the conditioning film. The molecules in the film equilibrate with those in suspension until the most stable, most adhesive layer is formed. It is to this layer which the first micro-organisms will attach, and several proteinaceous

compounds in the conditioning film play a role in bacterial adhesion [93]. Bacteria possess *fimbriae*: long protrusions which extend beyond the bacterial capsule to provide specific adhesion molecules for attaching to defined ligands on host epithelial cells, and these ligands may be present in conditioning films. The *glycocalyx* of a planktonic bacterium forms its thin capsule. It is comprised of a sticky polysaccharide layer, which allows non-specific adhesion to solid surfaces lacking receptor ligands.

Once a bacterium is temporarily attached to a surface in this manner, it upregulates gene expression to synthesize new adhesion molecules to consolidate surface adhesion. This is accompanied by an increase in glycocalyx production to form a diffuse extracellular polysaccharide slime layer, rather than a thin capsule. Part of this conversion from reversible surface attachment to permanent surface adhesion is mediated by signalling molecules released from the bacteria. In a location with a high density of surface attached organisms, the local concentrations of these secreted signalling molecules increase to a level which triggers the gene expression responsible for conversion to the surface adherent state. With a low density of organisms this does not occur, making it more likely that they will detach from the surface. This response to local population density is known as *quorum sensing*. In this case it allows preferential attachment of bacteria in microcolonies, rather than as individual cells. It has been shown that catheter associated biofilms of *Pseudomonas aeruginosa* release acylated homoserine lactones (AHLs), a class of these quorum sensing signal molecules [94]. In some species, such as *Pr. mirabilis*, the altered gene expression triggered by these signal molecules causes an even more radical change in phenotype to produce a *swarmer cell*, which can move rapidly across surfaces to extend the biofilm (this is described in more detail in section 1.5).

These microcolonies of surface adherent organisms, phenotypically distinct from their planktonic cousins, coalesce to form a biofilm. The colonies grow within a dense

matrix of extracellular polysaccharide. The proportion of the volume of the biofilm taken up by matrix as opposed to bacteria varies from 75 to 90%, depending on the organism [95]. Microcolonies and biofilms may be comprised of single or multiple species of bacteria.

A mature biofilm is conventionally described as comprising of three layers [96]:

- The linking film, comprising the conditioning film and the initial layer of bacteria which adhere to it.
- The base film, consisting of slowly growing compact organisms.
- The surface film, which is made up from loosely attached bacteria and can give rise to planktonic organisms.

There appear to be water filled channels between the cell clusters which may act to allow transport of nutrients into, and waste products out of, the biofilm. The surface film seems to be involved in nutrient transport, and can reduce the diffusion of detergents and antibiotics into the lower layers of the biofilm [97].

It should be noted that the presence of a biofilm does not indicate a disease state. Catheter and ureteric stent biofilms can exist without any symptoms of local or systemic infection.

1.4.3 Catheter Associated Urinary Tract Infection

The urinary catheter is the leading cause of infections acquired as a result of a medical intervention [32,98], due to the large number of devices in use [24,33], and the fact that they connect an area of the skin frequently colonised with pathogenic organisms [99] to a warm body cavity containing an ample nutrient supply.

Catheters can be drained by either a closed or open method. Closed drainage involves the connection of a sterile catheter to a sterile drainage bag before catheter insertion. This connection is not subsequently broken, and care is taken to avoid introducing infection through the drainage tap of the bag by using unwashed or ungloved hands or letting it touch the floor. Using this method, bacteria should not have an opportunity to colonise the catheter lumen or the interior of the drainage bag. An open drainage system originally described the use of an open ended catheter emptying into a separate receptacle, but in modern practice this does not occur. Even so, few long term catheter users experience a truly closed system. Drainage bags are usually changed on a weekly basis leading to almost inevitable contamination of the catheter lumen, urine sampling can lead to bacterial introduction, and leg bags are sometimes disconnected so a larger 'night bag' can be attached overnight.

The mechanism of bacterial colonisation of the bladder is best studied in the context of short term catheterisation, where a catheter is inserted into an uninfected bladder for the first time. In an open drainage system, 93% of those given indwelling catheters develop bacteriuria within 4 days [100]. With the development of closed drainage, rates of bacteriuria can remain below 20% after two weeks in the best series [101,102]. While this has been a great advance in the use of catheters for brief periods, the rate of colonisation has been calculated as between 3% and 10% per day [103-105], suggesting that the development of bacteriuria is practically inevitable for long term catheter users. Of those undergoing long term catheterisation using closed drainage, 50% had bacteriuria after 10 days [103], and over 90% after 4 weeks [106].

The low rates of bacteriuria found in the first few days after catheter insertion suggests that organisms are not introduced with the catheter if a proper aseptic

catheterisation technique is used. The bacteria mostly gain access to the bladder in the days following catheterisation. The two suggested routes for bacteria to ascend to the bladder are either through the urine drainage channel of the catheter, or in the periurethral space between the exterior surface of the catheter and the urethral mucosa.

Garibaldi *et al* assessed the rate of development of bacteriuria in a group of 1213 hospitalised patients undergoing short term catheterisation using closed drainage [99]. All patients had a swab of the urethral meatus cultured on the day of catheterisation. They found that the chance of developing bacteriuria was significantly greater in those with a positive meatal culture compared to those without, and in 85% of cases the same bacterial species was found in urine as on the meatus. In a trial comparing standard closed drainage with the technique of sterilising drainage bags with chlorhexidine, Gillespie *et al* showed that despite the contents of the bags remaining sterile, bacteriuria still occurred [107]. It was noted that the infections were caused by species typically found on the perineal skin. This suggests the importance of the periurethral route in strict closed drainage situations.

Nickel *et al* studied the mechanism of bacterial ascension along catheter surfaces in an animal model using *Pseudomonas aeruginosa* [108]. They showed that if the closed drainage system was broken, bacteria ascended rapidly by the intraluminal route, reaching the bladder in 32 to 48 hours. If the system remained closed, bacteria ascended periurethrally on the outer catheter surface, reaching the bladder more slowly, in 72 to 168 hours. In both cases the infection was mediated by *Pseudomonas* biofilms creeping over the catheter surfaces. In an *in vitro* model developed to look more closely at the intraluminal *Pseudomonas* biofilm [109], they showed that the leading edge of the film was thin and was preceded by individual adherent bacteria. They noted that ascent of the biofilm was slowed, but not

prevented, by the use of antibiotics in concentrations known to be bactericidal to planktonic *Pseudomonas*. They theorised that turbulent urine flow within the catheter lumen would deposit some planktonic bacteria on the catheter wall ahead of the biofilm. These would form microcolonies and eventually become coherent with the advancing main biofilm, thus speeding its ascent. In the presence of antibiotic these individual planktonic organisms would be killed, and the bacterial colony could only advance by division of cells within the protection of the extracellular polysaccharide matrix covering the main biofilm. This division may even be slower than normal, as rapid division could make the organisms more sensitive to antibiotic. On the periurethral catheter surface there would be no turbulent urine flow, so the advance would also be slow compared with the intraluminal route.

It was shown in a study of 15 nursing home residents having monthly urine samples over the course of a year, that there was considerable change in the bacterial species present [81]. It seems that long term catheter users experience repeated re-infection, at least in a long term care facility environment.

1.4.4 Strategies to Control Catheter Associated Infection

Bacterial colonisation of the urinary tract, whether by planktonic or biofilm-resident organisms, does not necessarily lead to symptoms of cystitis or pyelonephritis. It is accepted medical practice not to treat bacteriuria in catheter users unless accompanied by symptoms [80]. As well as biofilms being commonly found on individuals with unproblematic urinary catheters and urethral stents [91,92], a study of spinal cord injured patients has shown the presence of biofilms on bladder mucosa in individuals who were asymptomatic [110]. This does not mean that colonisation of the urinary tract is harmless in all cases. Bacteria are responsible for symptoms of catheter encrustation and the possible development of squamous cell carcinoma of

the bladder. They are also a source of organisms which can go on to produce symptomatic infection.

Antibiotics are ineffective at preventing febrile infection in long term catheter users if bacteriuria is screened for and treated before the onset of symptoms. This method was employed in a randomised controlled trial of long term catheter users by Warren *et al* [111]. Subjects in the treatment group were screened for the presence of bacteriuria, and treated with a 10 day course of cefalexin if susceptible organisms were isolated. The incidence of febrile episodes when antibiotics were being used was similar to that during periods when they were not, but cefalexin resistant organisms did emerge in the treatment group patients.

A randomised double blind crossover placebo trial by Rutschmann and Zwahlen has shown that long courses of fluoroquinolone antibiotics can reduce the infective complications of catheterisation [112]. Symptomatic infection and catheter blockage was reduced with a three month course of norfloxacin, as compared to the placebo group. While gram negative organisms were reduced in urinary isolates, gram positive norfloxacin resistant organisms flourished. It would seem only a matter of time before resistance developed in gram negative organisms, or symptomatic infection with gram positive organisms became prevalent. Three months cannot be considered an adequate representation of the treatment period required to make a difference to the life of a long term catheter user in respect of these issues.

Viable organisms have certainly been seen in biofilms from catheterised patients receiving antibiotics, and these could cause symptomatic infection as soon as the antibiotic is stopped, or the organisms develop resistance [79].

Catheter associated infection is known to be particularly difficult to treat with antimicrobials. The planktonic bacteria may be cleared, and the symptomatic infection may settle, but urine cultures following the cessation of therapy will most likely be positive. This can be explained by the organisation of the bacteria into biofilms.

As has been mentioned, the extracellular polysaccharide in the surface film limits diffusion of some antimicrobial agents into the lower layers of the biofilm [113]. This barrier is not the only method of protection that the organisms have, as the surface film produced by some species is quite permeable to certain antibiotics. Anderl *et al* developed a method in which biofilms were allowed to develop on permeable membranes [114]. A second permeable membrane was placed on the surface of the mature biofilm and a filter disc placed on this. These assemblies were incubated on antibiotic containing agar. The biofilms were sampled to determine biofilm organism viability and the filter discs tested for antibiotic penetration by determining if they produced zones of inhibition when transferred to agar growing a test *E. coli* strain. This *E. coli* system had been calibrated to measure antibiotic concentration. The researchers found that *Klebsiella pneumoniae* biofilms which produced the antibiotic inactivating enzyme β -lactamase were impermeable to, and therefore understandably resistant to, the antibiotic ampicillin. However, a biofilm of a strain of *Kl. pneumoniae* which did not possess β -lactamase was still resistant to ampicillin, even though it was penetrated within 10 minutes by antibiotic at 250 times the minimum inhibitory concentration (MIC) determined for the planktonic form of this strain. After 40 minutes the penetrating antibiotic concentration increased to 1000 times the MIC, but without any reduction in viable biofilm organisms. Similar results were found with ciprofloxacin. The antibiotic penetrated the biofilm in concentrations higher than those needed to eradicate planktonic organisms, but produced little reduction in the

numbers of viable organisms in the biofilm. This suggests that biofilms use other mechanisms in addition to a diffusion barrier to resist antimicrobials.

Selection of an appropriate antibiotic is based on the culture of bacteria, in most cases from urine. These, however, are the planktonic organisms, not those sessile bacteria resident in the biofilm. Bergqvist *et al* [115] observed that organisms isolated from urine aspirated suprapubically did not match the organisms cultured from urine drawn through the catheter. In a quarter of cases, organisms not present in the suprapubically sampled urine were cultured from the catheter urine, suggesting the presence of biofilm organisms which, while shed in their planktonic state sufficiently to appear in urine sampled through the catheter, did not ascend to reach the bladder. Ramsay *et al* demonstrated that in 11 of 16 catheter and urine pairs studied, the organisms in the urine did not fully match those grown from the catheter biofilm [82]. It is often unclear from the polymicrobial urine cultures often obtained from catheter users which of the several species present is responsible for the symptomatic episode. It may be that treatment failure is a result of the antibiotic prescribed not being targeted at the correct organism.

As has been described, biofilm organisms are phenotypically distinct from planktonic bacteria of the same species. As an example, the proteins of the *Ps. aeruginosa* cell envelope have been shown to differ by 30 to 40% between the two phenotypes [116]. Antibiotics are active against specific pathways of cell metabolism that may be vital in the planktonic phenotype, but may not even be present in the biofilm phenotype, leading to the antibiotic being ineffective against this form.

Many antimicrobials have a considerably higher activity against fast growing, dividing cells. Slow growing organisms do not express some proteins necessary for the

binding of these agents [117]. It is known that the growth rate of organisms is very low in the deeper layers of a biofilm, giving the colony resistance to antibiotic therapy.

It has been shown that these methods enable organisms in biofilms to resist antimicrobial substances at concentrations 1000 to 1500 times greater than those which would be lethal to planktonic bacteria [95]. With organisms being bathed by sub-therapeutic doses of antibiotics, it is likely that selection of resistant organisms will occur. With multiple species in close proximity, the transfer of plasmid mediated resistance between organisms or between species is possible [118,119]. While antibiotic therapy can penetrate tissues and eradicate planktonic bacteria, once the therapy is stopped, new planktonic cells released from the surface of the catheter biofilm soon repopulate the urine. These cells tend to be more resistant to antibiotics than the original population [70,111].

Preventing the colonisation of the urinary tract in the first place would be a significant step in improving the usefulness of long term catheterisation. In short term catheterisation, where it is known that bacteriuria can theoretically be delayed for several weeks, and where the dominant complication is symptomatic febrile infection, any further delay in the rate of colonisation may entirely prevent complications within the time frame that the catheter is required for. Several methods of achieving this have been tried:

Meatal cleansing

In a closed drainage system, colonisation of the bladder is presumed to occur from bacteria on the urethral meatus. Regular cleansing of the meatus with soap, antiseptic, or antibiotic solutions has not been shown to delay the onset of bacteriuria. Burke *et al* compared green soap or povidone-iodine solution meatal cleansing in

samples of roughly 200 short term catheter users against no cleansing in a randomised controlled trial [120]. Bacteriuria rates were found to be slightly higher in the treatment groups, and this was statistically significant in the high risk female patients. Subsequent randomised trials using 1% silver sulfadiazine cream [121] and polyantibiotic cream [122] also failed to show benefit from meatal cleansing, although they did not show an increase in risk with treatment. Worryingly, a survey of periurethral skin flora in patients receiving meatal cleansing with the antiseptic chlorhexidine following spinal cord injury showed a rapid change in flora from gram positive organisms to gram negative strains such as *Pr. mirabilis*, *Ps. aeruginosa* and *Providencia stuartii* [123,124]. As well as being highly resistant to chlorhexidine, these organisms are more resistant to other common antibiotics should they cause a urinary infection.

Drainage bag sterilisation

Gillespie *at al* randomised short term catheter users between the standard closed drainage technique and a technique where chlorhexidine was added to the drainage bag [107]. Despite the contents of the chlorhexidine drainage bags remaining sterile, bacteriuria occurred with similar frequency in the bladders of the treated group (51%) as in the control group (45%), presumably because the route of infection was periurethral. Similar findings have been reported using other methods of sterilising drainage bags, such as a study of hydrogen peroxide use in those requiring catheters for longer than five days [125]. In this case, as well as bacteriuria not being prevented, episodes of febrile infection happened with equal frequency as in the control group and bacterial growth of several common nosocomial pathogens occurred in the drainage bags. The source of bladder organisms seemed to be the drainage bag flora in this case.

Bladder washouts

Another technique used to treat or prevent bladder colonisation is repeated irrigation of the catheter with antiseptic or antibiotic solutions. To administer these, the drainage bag is disconnected and 50 to 100 ml of the solution is flushed into the bladder through the catheter. The catheter may then be clamped and the solution left in place for 10 to 15 minutes, before being allowed to drain out. It has been pointed out that exposure of bacteria to an antimicrobial agent by this method is very different to the exposure used to determine the minimum inhibitory concentration (MIC) of an agent. This is done by culturing organisms over 24 hours in the presence of the antimicrobial in a laboratory. The effectiveness of such short exposures to the popular chlorhexidine bladder washout solution was examined in an artificial bladder model by Stickler *et al* [126], against a variety of bacterial strains obtained from catheterised individuals. It was found that in the biofilms that formed, even strains of bacteria which were determined to be chlorhexidine sensitive in conventional MIC tests survived the washouts. Further similar work by this group has shown the ineffectiveness of various other washout compounds such as povidone-iodine, phenoxyethanol, chlorhexidine supplemented with EDTA and Tris, noxythiolin and neomycin [127].

Davies *et al* conducted a randomised clinical trial of chlorhexidine versus normal saline as a bladder washout in those catheterised for longer than ten days [128]. Neither treatment produced a reduction in bacterial counts, but there was an increase in *Proteus* spp isolated from the chlorhexidine group. A clinical study using a neomycin-polymyxin antibiotic washout solution carried out by Warren *et al* showed no difference in the rate of acquisition of bacteriuria [129]. This group proposed that any effect of the antibiotic was outweighed by the influx of organisms when the

closed system was broken. It was also shown that the bacteriuria in the treated group had higher levels of antibiotic resistance.

Comprehensive Antiseptic Policies

A combination of the above approaches has also been implemented. The Southampton Control of Infection Team instituted a comprehensive policy of chlorhexidine use to prevent urinary tract infection in hospital based catheter users [130]. Chlorhexidine was used to clean the urethral meatus before catheter insertion, and was present in the lubricant gel used to introduce the catheter. Chlorhexidine was used for regular drainage bag sterilisation. A chlorhexidine solution was used for daily meatal cleansing, after which a chlorhexidine containing cream was applied to the periurethral area. Following the introduction of this policy, an outbreak of chlorhexidine resistant *Pr. mirabilis* developed in the hospital, infecting 90 patients [131]. The outbreak strain, as well as being resistant to chlorhexidine, was also resistant to the antibiotics trimethoprim, sulphafurazole, ampicillin, azlocillin, carbenicillin, gentamicin and tobramycin [132]. The authors recommended that the antiseptic policy should be abandoned.

Systemic Antibiotic Prophylaxis

Prophylactic antibiotics given at the time of initial catheterisation, before bacterial colonisation occurs, do not seem to prevent urinary colonisation.

Giving antibiotics at the time of the first catheter insertion may delay the onset of bacterial colonisation for a few days, but the reduction in bacteriuria is short lived [103]. It is controversial whether such limited effect has a benefit which outweighs the risk of spreading antibiotic resistance even in short term catheterisation [133].

In a placebo controlled trial sometimes quoted as supporting antibiotic prophylaxis, Van der Wall *et al* showed that the use of low dose ciprofloxacin in post operative patients needing catheterisation for 3 to 14 days led to significantly reduced rates of bacteriuria and symptomatic urinary infection in the treatment group [134]. These patients, however, were considered at higher than normal risk of infection due to their surgery, and this is reflected in the very high rates of symptomatic infection found. The authors of the study do not suggest that these results should lead to prophylaxis for all catheterisations. Over the course of the study, although total rates of bacteriuria and rates of gram negative bacteriuria were certainly reduced in the treatment group, ciprofloxacin resistant gram positive urinary tract colonisation developed. Their urinary tracts were also repopulated with ciprofloxacin sensitive gram negative organisms once the catheter was removed and ciprofloxacin stopped.

Antibacterial Coated Catheters

Catheters have been developed which release antibiotics from their surface coatings in an attempt to prevent bacterial colonisation. Laboratory studies have shown that they consistently release concentrations of antibiotic sufficient to produce zones of inhibition on agar plates against many common uropathogens. Johnson *et al* showed that nitrofurazone catheters were active against *E. coli*, *Kl. pneumoniae*, *Citrobacter freundii*, *Staph. aureus*, coagulase-negative *staphylococci*, and non vancomycin resistant *Enterococcus faecium* [135]. Similar studies on a norfloxacin releasing catheter by Park *et al* showed that inhibition of *E. coli*, *Kl. pneumoniae* and *Pr. vulgaris* was still present after 10 days use [136]. A chlorhexidine impregnated catheter showed activity against *E. coli*, *Pr. mirabilis* and *Staph. epidermidis* in a study by Whalen *et al* [137]. While these studies failed to account for some important uropathogens, such as *Pseudomonas*, such results have encouraged development

and marketing of antibacterial catheters. It should be noted, however, that bactericidal activity against lawns of bacteria on agar cannot necessarily be extrapolated to activity against biofilm resident organisms *in vivo*. These catheters also lose activity with time, and so have a useful lifespan which is shorter than the twelve weeks which would ideally be expected from an uncomplicated long term catheter.

Ciprofloxacin catheters have been tested in a catheterised rabbit model [138]. A statistically significant increase in the time for bacteriuria to develop after *E. coli* were applied to the urethral meatus was seen with the antibiotic compared to the standard catheter, from 3.5 to 5.3 days. A delay of this magnitude may be useful for short term catheterisation, but would be of no benefit for long term catheter users.

In a human study on post operative short term catheterisation by Darouiche *et al*, a catheter impregnated with minocycline and rifampicin was used [139]. This successfully reduced the rate of colonisation with gram positive organisms over a mean catheterised period of two weeks, although 7% of patients were still colonised with the antibiotic catheters. There was no reduction in the rate of colonisation with gram negative organisms, however, with 46% of treated subjects being colonised. Unfortunately, some of the most common uropathogens such as *E. coli*, and the most troublesome, such as *Pseudomonas* and *Proteus*, are gram negative. There is also the possibility of promoting the emergence of predominantly gram negative infections and resistant gram positive organisms in the local population. The experience with chlorhexidine use detailed above suggests that the use of catheters releasing this compound may be particularly unwise.

Silver Coated Catheters

Silver is an effective antimicrobial agent which does not promote bacterial resistance and is active against a broad spectrum of bacteria. It has been used in a range of medical devices outside the urinary tract. Silver ions have a bacteriostatic action at low concentrations by binding to a wide range of biological molecules and displacing essential metal ions. At higher concentrations, silver-DNA binding has a bactericidal effect [140].

Silver has been shown to delay ascending infection from drainage bag to bladder reservoir in an artificial bladder model of short term catheterisation [141]. A silver releasing device in the drainage tubing prevented *Pr. mirabilis*, *E. coli* and *Ps. aeruginosa* from colonising the bladder for the 10 days in which the experiment ran. In the models with no silver, the bladders were colonised in 5.7 to 7.7 days.

Silver can be applied to catheter surfaces as either an alloy or an oxide. Several randomised clinical studies have been performed measuring rates of bacteriuria with these catheters compared to normal catheters in short term catheterisation of hospitalised patients. While these studies have had very varied results, a meta-analysis showed that silver alloy, but not silver oxide, catheters did result in significantly lower rates of bacteriuria [142]. The studies analysed have been criticised, however, and the efficacy of silver catheters is still considered unproven [143].

The release rate of free silver ions appears to be the important factor in determining the activity of a silver device [144]. Studies have shown that the effectiveness of silver is reduced in the presence of some common constituents of biological fluids. Reduced activity has been shown with horse serum, bovine serum, chloride ions and

albumin [140,145]. These observations may explain the controversy among the trial results.

These devices have not been tested in the context of long term catheterisation, but *in vitro* studies show that *Pr. mirabilis* will migrate across, and form biofilms on, commercially available silver catheters [21,146]. Again, the possibility arises that intervention will eradicate easy to treat organisms which rarely cause symptoms, to replace them with antibiotic resistant, encrustation promoting species.

Conclusion

None of the techniques discussed offers the realistic chance of preventing urinary tract colonisation in those with long term indwelling urinary catheters. It seems that the best that can be achieved is the delay of colonisation for, at most, a few weeks. Most of this benefit can be obtained by using the simple and well established principle of closed drainage. Additional techniques run the risk of promoting an increase in prevalence of difficult to manage pathogens while providing only a small additional benefit to the individual catheter user. In conclusion, while the methods described above may have some use in the short term hospital setting, perhaps for high risk individuals, it would seem that the chronic use of antibacterial agents in long term catheterisation is both ineffective and prone to select for problematic, antimicrobial resistant bacterial species. Further trials are needed to establish the risk/benefit ratio before these become accepted practice.

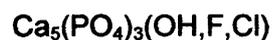
1.4.5 Crystal Formation

Encrustation describes the deposition of crystals onto the surfaces of a biomaterial when in contact with urine. It is a common occurrence on urinary catheters, both

urethral and suprapubic, and on ureteric stents. An electron microscopy study of encrusted urinary catheters by Cox *et al* revealed that the deposits are made up from large crystals of struvite and aggregates of small apatite crystals, embedded in thick layers of bacteria and matrix [147]. Encrustation was therefore shown morphologically to be a crystalline biofilm.

Apatite and Struvite

Various apatite minerals exist, being composed of an anhydrous lattice of calcium and phosphate with either a hydroxyl or halide group attached:

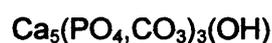


One of the strongest, found in teeth and bones, is hydroxyapatite:



The associated hydroxyl or halide groups alter the strength of the mineral, with fluoride providing more strength than the hydroxyl groups found in the urinary tract forms.

The weaker mineral carbonate apatite is more commonly found in catheter encrustations, attached to hydroxyl groups:



The replacement of a varying proportion of the phosphate groups with carbonate groups results in a more poorly defined crystalline structure.

Struvite is a mineral composed of hydrated magnesium, ammonium, and phosphate:



Both struvite and carbonate apatite are found in the large 'staghorn' calculi found in the kidneys of those with chronic urinary tract infection. These stones are simply large crystalline biofilms, and form in much the same way as catheter encrustation, although without the presence of a foreign biomaterial, the initial crystals probably form on biofilm debris from the infected renal papillae. Apatite stones can also form in the absence of infection.

Effects of Urease

Calcium and magnesium salts are normally found dissolved in urine. In most individuals, crystal precipitation does not occur, even on the surface of a foreign body in the urinary tract such as a ureteric stent. Calcium and magnesium salts are held in solution in urine even when quite concentrated. The factor mainly responsible for the encrustation of urinary devices and the formation of staghorn calculi is the presence in the biofilm of organisms, such as *Pr. mirabilis*, which possess the enzyme urease. This acts on the abundant urea present in urine.

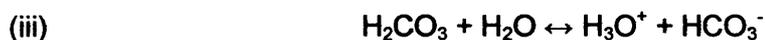
Urease catalyses the hydrolysis of urea to ammonia and carbamic acid:



Carbamic acid spontaneously decomposes to ammonia and carbonic acid:



In aqueous solution, the carbonic acid and two ammonia molecules produced from these reactions exist in equilibrium with the ions hydrogencarbonate and ammonium:



The net result of this ammonium production is an increase in the ratio of OH^- to H_3O^+ ions: the urine becomes more alkaline. Normal urine has a pH of about 6 to 6.5, but in the presence of an infection with a urease positive organism, this can increase to 8 or 9. The reason that this is important is that the substrates which form struvite and carbonate apatite are more readily available at high pH.

Effects of an Alkaline Environment

Le Chatelier's principle states that when an external change is made to a system, the system will respond so as to oppose the change. In other words, if more substrates for carbonate apatite are made available, more carbonate apatite will form to remove those substrates and attempt to rebalance the system. The reason that more substrates are available at high pH is also explained by Le Chatelier's principle. Consider the complete dissociation of carbonic acid to hydrogencarbonate and then to carbonate, expanded from equation (iii):



In an alkaline environment, the many available OH^- ions will react with any H_3O^+ ions produced and effectively remove them from the system:



The equilibrium system will respond so as to oppose this: in other words try and replace the lost H_3O^+ ions by further dissociation of carbonic acid. The more H_3O^+ ions removed from the right side of equation (v), the further to the right that equilibrium will shift. In other words, a greater quantity of the molecules in the system will be in the dissociated state described by the right side of the equation. At the very high alkalinity produced by the abundant ammonia, significant dissociation of hydrogencarbonate to carbonate occurs.

Phosphates are found readily in the urine as part of the urinary buffer system. The buffer step of the reaction is the dissociation of dihydrogenphosphate to hydrogenphosphate, but the high alkalinity removes enough H_3O^+ ions to drive the production of phosphate ions:



As ammonium is produced directly from the urease reaction, it can be seen how several of the substrates for the production of struvite (NH_4^+ , PO_4^{3-}) and carbonate apatite (CO_3^{2-} , PO_4^{3-} , OH^-) are formed more abundantly in an ammonia-rich alkaline environment. This encourages their precipitation out of solution to form the crystals of encrustation in the presence of urease.

Nucleation pH

Choong *et al* studied the differences in urine chemistry between catheter blockers and non-blockers [148]. They experimentally determined the pH above which crystals

began to precipitate from solution, called the nucleation pH (pH_n), using an optical density method validated by ion selective electrodes. They then compared this to the pH of the urine voided by the patient (pH_v), and the presence or absence of urease positive organisms in the urine.

They found that they could divide patients into three groups. In the two largest groups, non-blockers and urease-positive blockers, the nucleation pH was similar, around 7.4, but the blockers had urease positive organisms in their urine raising the voided pH to almost this level, while the non-blockers had no urease activity and their voided pH was around 6.5, well below pH_n . They also found a small group of urease-negative blockers. In this group the pH_n was abnormally low, allowing precipitation at normal voided urinary pH. They concluded that other urinary factors must be responsible for this lowering.

Although providing useful data, this study raises several questions. As in other published studies of encrustation [24,45,46,149-151], the authors initially divided patients into two groups on the basis of the time the catheters took to block. Quoting from the methodology:

The patients were subdivided into two groups; 'blockers' were those whose catheters regularly became blocked by encrustation within a 6-week period, requiring replacement; and 'nonblockers' were those whose catheters could be changed at intervals of longer than 6 weeks.

As stated earlier, there appears clinically to be a continuous spectrum of severity of catheter encrustation, including those who block catheters in greater than 6 weeks and those who do not block their catheters, but have encrustation present at the

routine 12 week change suggesting that if the catheters were left beyond the limit allowed by the manufacturer they would eventually block. While it is true that those who block catheters in less than 6 weeks suffer the most inconvenience from their catheters, in order to determine the medical cause of the problem it is more important to differentiate encrusters from non-encrusters, rather than blockers from non-blockers. If the aim is to differentiate those who encrust quickly, and therefore have the most severe clinical problem, from those who encrust slowly, and who rarely, if ever, block their catheters, then those without strongly urease positive bacteria in their urinary tract need to be excluded as their urinary chemistry will not lead them to encrust in the absence of these organisms. Although urease activity was looked at in this study, it was done as a *post hoc* analysis of the 'blocker' group, and not used as part of the initial categorisation. There was no mention of the urease status of the 'non-blockers'. Bacterial species are also not mentioned, and many urease positive organisms do not produce enough urea to alter urinary pH. Once the dichotomous variable of the presence or absence of urease has been accounted for, since encrustation severity is a continuous variable, data analysis would be better carried out using regression techniques rather than arbitrarily dividing the patients into two groups and comparing them using a Mann-Whitney or Student t-test.

The results were also based on single samples of urine. It is possible that urinary parameters, including pH_v and pH_n , vary. This may have a significant effect on the propensity of the urine to precipitate crystals, and may also suggest that if pH_n varies naturally, it may be manipulated therapeutically.

1.4.6 Strategies to Control Encrustation

As well as the techniques described in section 1.4.4 to prevent bacterial colonisation of the catheter, other work has focused on the prevention of encrustation even if a biofilm does eventually form.

Acidification

The chemical reactions which form the struvite and carbonate apatite crystals are in equilibrium. In theory, acidifying the environment in which the reactions takes place should allow dissolution of the crystals. There are two main methods of achieving this: taking acidifying agents systemically which will be excreted and concentrated in the urine, or flushing the catheter directly with an acidic solution. These solutions used to flush the catheter are often referred to as bladder washouts, but are more correctly termed *catheter maintenance solutions* [152].

The addition of acid (H_3O^+ ions), by any route, is countered chemically in two ways. Firstly the natural buffering ability of a solution, in this case urine, which contains ammonia and ammonium ions:



The addition of acid effectively removes OH^- ions from the right side of this equation (see equation (vi)). By Le Chatelier's principle, this causes the reaction to shift to the right, counteracting the loss of OH^- ions, and removing ammonia from the solution. The equilibrium constant of this reaction is such that it has a very effective buffering capacity at the alkaline pH present in infected urine, so markedly reducing the pH change caused by added acid.

Removing ammonia from solution also encourages the catalysed hydrolysis of more urea (the intermediate carbamic acid step from equations (i) and (ii) has not been shown for simplicity):



Again, by Le Chatelier's principle, the reaction shifts to the right to replace the reaction products removed from the right side of the equation. As the amount of urea present in urine is large, ammonia can be replaced very easily.

Over and above this, the activity of the urease enzyme itself is greater in more neutral environments, as it works quite inefficiently at higher pH [153,154].

The combination of the highly efficient ammonia/ammonium buffer, ready supply of replacement ammonia, and additional capacity of the urease enzyme is extremely effective at maintaining the alkaline environment. An in vitro study by Bibby and Hukins using purified urease in artificial urine demonstrated the effectiveness of this buffering capacity [155]. They calculated that to prevent a pH rise from the conversion of the urea in a day's urine would require 2.7 litres of 0.1 M monobasic acid. This would be impossible to supply orally, due to its toxicity. Clinical studies have shown that while it is possible to acidify normal urine by ingesting ascorbic acid, this strategy is ineffective if the urine is infected with a urease producing bacteria [156].

The second role of acid in catheter encrustation is the use of citric acid 'catheter maintenance solutions', available in concentrations of 3.23% or 6%, the lower concentration being used most often. 50 to 100 mL of solution is flushed into the

catheter and bladder, either regularly or in response to a blockage. The catheter may then be clamped for 10 to 15 minutes, and the solution then drained. This strategy has been shown to decrease encrustation in some artificial bladder models using artificial urine [157,158]. A clinical crossover study, however, has shown no significant benefit of these solutions *in vivo* [159].

The microbiology of the crystalline biofilm provides an explanation. As mentioned before, crystals form within the biofilm and are encased in bacterial cells and extracellular polysaccharide matrix which could protect them from environmental change. McLean *et al* conducted a study using a *Pr. mirabilis* crystalline biofilm created *in vitro* in a flow cell, a device in which urine can be circulated over the biofilm while it is continuously viewed through a microscope. They showed that struvite crystals which formed outside of the biofilm were dissolved by a high flow rate of acidic urine, while those in the biofilm were resistant [160].

Moreover, concern has been raised as to the effects of repeated exposure of the bladder to these acidic solutions. Irrigation of the bladder in general has been shown to increase the shedding of urothelial cells, an indicator of bladder mucosal damage, even when only using 30 to 60 mL of physiological saline [161]. The glycosaminoglycan on urothelial cells which aids in preventing bacterial adhesion is known to be removed by acid [162], and in a rabbit model acidic solutions resulted in significant urothelial injury [163].

Encrustation Resistant Catheter Materials

In addition to studies on catheters which resist bacterial colonisation, work has been done looking directly at the rates of encrustation on different materials. This has been

done looking at crystalline biofilm formation, and the sterile encrustation formed in the absence of infection more commonly found on ureteric stents.

An observational study in 18 catheter users over a period ranging from 8 to 47 weeks by Broklehurst and Brocklehurst revealed no difference in unscheduled catheter changes between silicone coated and latex catheters, but encrustation was not specifically studied [164]. Various antiseptic drug treatments were also being tested during the trial period, although these seemed to have little effect on bacterial populations and all participants received the same protocol. In a study on ten catheter users, Hukins *et al* seemed to suggest that 100% silicone catheters were more resistant to encrustation by struvite than those made from other materials [165]. Electron microscopy of one used catheter from each subject showed that there were more crystal deposits on the silicone elastomer and PTFE coated latex catheters than on the two 100% silicone catheters examined, even though the silicone devices had been in situ for the longest time. It should be noted that each patient may have had a different propensity to encrust, and while deposits of struvite were found on all catheters the paper does not confirm that all of the patients had a clinical problem with catheter blockage. A crossover study by Kunin *et al* showed less catheter blockage using 100% silicone catheters than silicone elastomer coated latex, PTFE coated latex, and simple latex catheters, but they suggested a role for the larger urine drainage channel cross section in the silicone catheter rather than the properties of the material [151]. Each type of catheter was used for 14 days in each subject before being removed, tested for patency, and inspected for encrustation. The silicone catheters were reported as being grossly encrusted as often as any other material, but were less frequently completely or almost blocked. Hydrogel coated latex catheters, which are very popular in modern practice, were not used in these studies.

In a sterile *in vitro* encrustation model alkalinised with purified urease, Cox *et al* showed no significant difference in the encrustation rates of hydrogel coated latex, silicone elastomer coated latex, and 100% silicone catheters in terms of mass of mineral deposition per unit surface area [166,167]. In a static, sterile system there appears to be no effective difference between the commonly used catheter materials in their propensity to encrust, but this may not reflect the bacterially colonised clinical situation.

Morris and Stickler used an *in vitro* bladder model infected with clinically sourced *Pr. mirabilis*, using both artificial and pooled human urine to study the resistance of catheters to crystalline biofilm formation [21,168]. The use of *Proteus* produces both the alkaline urine to encourage crystallisation, and the extracellular polysaccharide matrix on which it can develop. Identical gauge (14Ch) 100% silicone, silicone elastomer coated latex, hydrogel coated latex and silver hydrogel coated latex catheters all encrusted rapidly. In terms of the time taken for the encrusting catheters to block, the 100% silicone catheters lasted the longest, but these authors also hypothesised that this could be adequately explained by the larger drainage channel cross section, rather than the encrustation resisting properties of the material. The 14F latex based catheters tested had a drainage lumen diameter of approximately 1.5 mm compared to 2.5 mm for the 14F 100% silicone catheters.

The lack of difference between the rate of encrustation of the different available catheter materials in sterile test environments, and the observation that all materials subjectively encrust rapidly in infected *in vitro* and clinical settings, suggests that this hypothesis may have some validity. However, while it makes sense that a wider lumen would take longer to block, the degree to which catheter size affects catheter lifespan has not been formally assessed. Catheter size has not been found to be related to catheter lifespan [24,46,149], but it was not investigated as the primary

focus of these studies. Catheter users were also included in these analyses who were classified as non-blockers. They were presumably not colonised by urease producing bacteria and so would never encrust any catheter irrespective of its size, and their inclusion would confound the results. Given the known variability in the severity of encrustation, and that those with severe encrustation may have been changed to larger sized catheters to compensate for this problem, the confounding factors are likely to render observational data useless. A rigorously controlled experimental study is needed, but has not been performed. If the association between catheter lumen diameter and catheter lifespan were pronounced it would indicate that proven catheter blockers would be best served with larger lumen catheters, both by switching to 100% silicone, and by simply using larger gauge catheters. Larger gauge catheters are associated with greater discomfort and bladder spasm when used urethrally, and so need to be fitted suprapubically, a procedure which itself carries a small risk of serious complications [169].

A study by Tenke *et al* examined the use of heparin as a coating on urological devices [170]. They found that although heparin coated 100% silicone catheters blocked as quickly as standard 100% silicone catheters when tested in a *Pr. mirabilis* infected bladder model with pooled human urine, encrustation was only found on the uncoated catheters. The heparin catheters were obstructed by 'plugs of clear gel-like material'. In a clinical study, these researchers found that heparin coated ureteral stents did not obstruct over the course of a year, whereas silicone and hydrogel coated stents blocked in 7 weeks and 5 months, respectively.

Choong *et al* developed an *in vitro* model for urological devices using pooled human urine and validated it against an animal model [171]. They showed, in a sterile test at a slightly acidic pH, that hydrogel coated latex catheters performed better than PTFE coated latex catheters. Looking at ureteric stents, they showed that coating in

hydrogel actually increased encrustation compared to the uncoated material. 100% silicone resisted encrustation more successfully than plain polyurethane, but the best material was polyurethane coated with hyaluronic acid, a naturally occurring hydrophilic polysaccharide which is a type of glycosaminoglycan. It is debatable how accurately these results will transfer to an alkaline, infected situation, but it is certainly a technology worthy of further study.

Urease Inhibitors

Biofilms do not tend to result in encrustation or renal tract calculi without a rise in pH and ammonium concentration caused by the action of bacterial urease. If biofilm formation cannot be prevented, using inhibitors of urease should prevent their development into crystalline biofilms. Known inhibitors of urease include hydroxamic acids, phosphoramides, and thiol compounds [153].

Acetohydroxamic acid (AHA) is a competitive urease inhibitor with a similar molecular structure to urea. It has been shown in a randomised double blind trial to retard growth of struvite renal calculi in those with chronic infection [172], although half of those treated required a reduction or cessation in therapy because of side effects. It can occasionally dissolve existing calculi. In a small clinical crossover study of five catheter blockers, Burns and Gauthier showed a significant decrease in the amount of encrustation on the catheter surface and a lengthening of the time it took for catheters to block [173]. Unfortunately, the toxic side effects of AHA, such as headache, vomiting, diarrhoea, hallucinations and abdominal pain, and more seriously, deep vein thrombosis and haematological abnormalities, are quite common [172,174]. These are a significant risk for the predominantly elderly, immobile population of catheter users. It has also been shown to be teratogenic in animal

studies, and theoretically could be carcinogenic with long term or high dose use [175]. Its clinical use has not been widespread for these reasons.

A potentially less toxic and more potent urease inhibitor, fluorofamide, has been used to prevent calculus formation in a *Pr. mirabilis* infected rat model [176].

Both AHA and fluorofamide have been shown to prevent the rise in pH and crystalline deposition on catheter surfaces caused by *Pr. mirabilis* in an artificial bladder model [177]. Further assessment of these compounds is needed before they are used clinically.

Pre-emptive Catheter Changes

Norberg *et al* found that an individual can be allocated a fairly predictable 'average expected catheter life' based on their past three to five catheter blockages [178], and this allows the catheter to be changed routinely before it blocks. Although it is inconvenient and costly to change catheters frequently, it is cheaper and less bothersome to the patient than waiting for the blockage to occur and sending an emergency nurse. Larger lumen catheters are also used in an attempt to delay blockage. What causes the observed differences in catheter lifespan between individuals with identical catheters is uncertain.

Blocking pH

One of the techniques employed in the Bristol area to deal with catheter encrusters involves the use of a 'blocking pH'. The theory is that when a new catheter is inserted, the patient will initially void acidic urine. The pH will then gradually increase as the catheter remains in place. There is supposed to be a critical value of the

voided pH above which the catheter will block. The voided pH is measured on a weekly basis using commercially available urine dipstick pH strips, and the catheter allowed to block. The last pH reading before blockage is recorded as the blocking pH. This is taken to be an individual value for each catheter blocker. Subsequent catheters are then changed as soon as the voided pH is equal to or greater than the previously determined blocking pH on the grounds that catheter blockage is imminent. It is possible that this blocking pH is related to, or identical to, the nucleation pH.

This technique has apparently reduced the rate of catheter blockage in at least one of the areas in question (unpublished data), but the numbers who use this method have been small and the technique has not been formally trialled. This method has gained popularity in Bristol, and the author is aware that it has spread to other areas of the United Kingdom. It has been suggested as a management technique in the nursing literature [179]. However, there is no published clinical or laboratory evidence to support it, and it is not even certain that such a change in pH occurs with increasing catheter life.

1.5 *Proteus mirabilis*

In some ancient Greek writings, Proteus was described as the immortal Old Man of the Sea, a herder of sea beasts and servant of the sea god Poseidon. He was sought after due to his powers as an oracle, but used his ability to change shape at will to attempt to evade those seeking his council. In Homer's *Odyssey* he is eventually captured by Menelaus and forced to reveal the fates of several heroes of the Trojan War. The name *Proteus* was therefore given to a group of remarkable polymorphic Gram-negative bacteria identified in 1885 by the German bacteriologist and pathologist, Gustav Hauser.

1.5.1 Classification

The genus *Proteus* is classified in the phylum *Proteobacteria*, as part of the family *Enterobacteriaceae* whose members are often found as inhabitants of the gastrointestinal tract of warm blooded animals. *Proteus* spp are gram-negative, facultative anaerobic bacilli. They are not able to ferment lactose, and so are not grouped with many of the other *Enterobacteriaceae* as coliforms.

The currently recognised species of *Proteus* are *Pr. mirabilis*, *Pr. vulgaris*, *Pr. penneri*, *Pr. myxofaciens* and *Pr. hauseri*, with three genomospecies remaining unnamed. The classification has altered over time. *Pr. penneri* and *Pr. hauseri* were originally classified as biogroups 1 and 3 of *Pr. vulgaris*. Organisms once thought of as belonging to the *Proteus* genus have now been placed in other genera: *Pr. morgani*, *Pr. rettgeri*, and *Pr. inconstans* are now classified as *Morganella morgani*, *Providencia rettgeri* and *Providencia* spp, respectively. The similarity of the

organisms in these genera result in them often being considered together in the literature, where they are sometimes referred to as the tribe *Proteeae*.

1.5.2 Epidemiology

Proteus is found widely distributed in soil and water in the environment, where it is involved in the decomposition of animal matter. In humans, *Proteus* is found as part of the normal flora of the gut. Its main pathological role is in infections of the urinary tract, but it is also a cause of wound infection, pneumonia and gram-negative sepsis.

In normal human faeces, *Pr. mirabilis* is found in between 2.7% and 24% of subjects [180-183], although colony counts are low compared to more common species such as *E. coli*. However, *Pr. mirabilis* is responsible for the majority of clinical infections involving *Proteus* spp. A survey by Senior found that 96.5% of urinary tract infections involving *Proteus* or *M. morganii* were due to *Pr. mirabilis*, compared with 1.5% due to *Pr. vulgaris* [184]. *Pr. vulgaris* is isolated from the human gastrointestinal flora relatively rarely, found in between less than 5% of cases [180-182]. This has been proposed as a major factor in the lower frequency of *Pr. vulgaris* urinary tract infection [185]. *Pr. mirabilis* is well known as a cause of nosocomial disease, but can sometimes be found as a community acquired infection in otherwise normal individuals. *Pr. vulgaris* and *Pr. penneri* tend to only be present in individuals with a particularly high risk for nosocomial infection, such as residents of long-term care facilities.

It is thought that *Proteus* bacteriuria is probably acquired from the *Proteus* species present commonly in the gastrointestinal tract, as the majority of urinary isolates of other infection causing bacteria are, but they could also be transmitted between

catheter users by care staff during a catheter user's frequent contacts with the medical profession [42,186].

1.5.3 Strain Discrimination

It is possible to distinguish between different strains of *Pr. mirabilis* by a variety of methods. This is useful for epidemiological studies, as identical strains are likely to share a common source [187]. These methods are usually divided into phenotypic techniques, which examine bacterial behaviour and expressed proteins, and genotypic methods, which examine the genetic material of the organism. Phenotypic methods can be criticised as bacterial strains could express identical proteins while having considerable differences in their genome.

A very simple phenotypic method is Dienes typing, as originally described by Louis Dienes [188]. It was found that if two strains of *Proteus* were inoculated to the same agar plate and incubated overnight, they would swarm across the plate as usual until the leading edges of the two swarms met. If the two strains were distinct, they would inhibit each other. A clearly visible line of demarcation would form between the two swarming strains. If the strains were identical, or at least very similar, their swarming edges would merge, and no demarcation line would be seen.

A more discriminatory but considerably more complicated method is Pulsed Field Gel Electrophoresis, or PFGE. This is a genotypic method which uses enzymes known as *restriction endonucleases*. These enzymes cut DNA at points along its length which contain a certain nucleotide sequence, different for each type of enzyme. This produces a series of DNA fragments of differing lengths depending on where and how often the target nucleotide sequence appears in the original DNA. These fragments can then be sorted according to size by gel electrophoresis. By passing an

electric field across a polyacrylamide or agarose gel in which the DNA fragments are embedded, the negatively charged fragments will move through the gel. In conventional electrophoresis, larger fragments will find it more difficult to pass through the pores in the gel, and so will not move as far in a given time. This produces a characteristic pattern in the gel when the DNA is stained, with fragments being seen further from the start point in inverse proportion to their size. DNA from different bacterial strains will have a dissimilar arrangement of the target nucleotide sequences, so the fragments produced by restriction enzyme digestion will be of different sizes and give a different pattern in the gel. Comparing these patterns enables different strains to be distinguished.

However, conventional gel electrophoresis only works up to a certain size of DNA fragment. Above this limit the fragments rotate to line up with the electric field and travel through the gel lengthwise, so differences in length no longer make a difference to speed of travel, and these fragments cannot be separated.

Unfortunately, cutting a very large piece of DNA such as a bacterial genome into small enough fragments to separate by conventional electrophoresis would produce so many fragments of very similar size that they would form a continuous blurred band on the gel, rather than a distinct, readable pattern.

PFGE allows much larger DNA fragments to be used in the gel. The direction of the electric field is regularly changed, or pulsed. This forces the DNA fragments to rotate to line up with the field before they can start to move again. This takes more time the longer the fragment, and even very large fragments will separate on the gel. This means that a whole bacterial genome can be cut into a few very large fragments and so produce an easily readable gel pattern.

Both the Dienes typing and PFGE techniques have been used in epidemiological studies of *Pr. mirabilis*. The ability of a typing technique to differentiate unrelated strains is measured as a *discrimination index* (DI), which is the probability that two dissimilar strains will be placed in different groups by the technique [189]. A DI > 0.90 is considered desirable. The DI of these two typing methods in studies of *Pr. mirabilis* was examined by Pfaller *et al* [190]. They calculated a DI of 0.980 for Dienes typing and a DI of 0.992 for PFGE. Sabbuba *et al* calculated similar DI values (0.988 for Dienes typing and 0.993 for PFGE) while using these techniques to demonstrate that the *Proteus* strains in the urine and catheter biofilm of catheter encrusters were identical [191], and that in those with bladder stones, that the stone and catheter biofilm strains were also identical [49].

1.5.4 The Urease Enzyme

Several virulence factors have been characterised for *Pr. mirabilis*, although the most important from the perspective of catheter encrustation is that it is a urease positive organism. It possesses the enzyme urease, which catalyses the hydrolysis of urea to produce ammonia and carbamic acid. The urease test is one of the procedures used to identify bacteria. They are incubated in a medium containing urea and a pH indicator. If the pH increases, the bacteria are classified as urease positive. Although a great many organisms are classified as urease positive on the basis of this test, *Pr. mirabilis* has by far the greatest ability to produce ammonia of any human urinary tract pathogen. A study of urinary isolates from catheterised residents of a long term care facility found that although the urease enzyme of the *Pr. mirabilis* isolates had a relatively low affinity for its substrate, it could hydrolyze urea at a rate 6 to 25 times that demonstrated by the urease enzymes of other species [192]. The lower affinity of the *Pr. mirabilis* urease enzyme for substrate (K_M of urease = 13 mM) is unimportant in practice, as given the extremely high concentrations of urea in urine (up to 500

mM) the active site of the enzyme will still be almost constantly occupied [193]. The urease of *Pr. mirabilis* is a 250 KDa nickel metalloenzyme, located in the organism's cytoplasm [153]. It is the production of ammonia from the abundant supply of urea in urine that is responsible for the dramatic rise in urinary pH found in individuals with a *Pr. mirabilis* urinary tract infection. Urease production is especially high when *Proteus* is in its swarmer state [194], as detailed in section 1.5.5.

As described previously, high urinary pH leads to precipitation of phosphate containing crystals in the form of renal and bladder calculi and encrustations on implanted devices. It could be theorised that these crystalline deposits act to protect the bacterial community in a way which may be superior to the level of defence offered by a non-crystalline biofilm. It should be noted, however, that in the absence of foreign material in the urinary tract, even a chronic *Proteus* infection does not lead to calculus formation in the majority of cases. This would seem to suggest that crystal precipitation is not the primary purpose of urease.

Ammonia is present in normal uninfected urine as an important part of the urinary buffer system. It is produced from glutamine in the proximal convoluted tubule of the kidney in amounts proportional to the acid load in the body. The conversion of this ammonia to ammonium ions in the collecting ducts traps hydrogen ions in the urine and allows for controlled acid excretion from the body. However, extreme alkalinity caused by high levels of ammonia is toxic to all cells. Even *Proteus* itself will not grow once it has raised the pH above a value of around 9 [195].

Experiments in rat and mice models of pyelonephritis have shown the importance of urease production in *Pr. mirabilis* infections ascending from the bladder to the kidney. If urease activity is absent, either because of chemical inhibition by acetohydroxamic acid (AHA) [196] or the use of a urease negative *Proteus* mutant [197], bacterial

counts in the renal parenchyma are reduced, although bacterial counts in the urine may not be. The presence of urease activity also leads to greater renal damage on histology [196]. Even in a model of the haematogenous route of infection, where urease negative mutants caused pyelonephritis as often as urease positive strains, renal necrosis was only seen when there was urease activity [198,199].

Very high alkalinity, which could be produced locally by urease producing organisms, is thought to decrease the activity of complement [200], one of the immune system's key antibacterial measures, and this may confer a survival advantage on *Proteus*.

1.5.5 Morphology and Physiology

Proteus can exist in two distinct morphological and physiological forms, known as swimmer cells and swarmer cells. Although this ability is not unique to the genus, *Proteus* seems able to swarm much more readily, and in a greater range of environmental conditions, than other bacteria.

In liquid suspension *Pr. mirabilis* exists in the planktonic swimmer state. It is a 1 to 2 µm rod with multiple flagella covering its surface for motility, and a coating of fimbriae for adhesion to surfaces. As a swimmer cell, it is morphologically similar to many other members of the *Enterobacteriaceae* family.

On contact with a solid surface, *Proteus* has the ability to convert to the swarmer state. Instead of normal growth by septation and division, the bacterial cells elongate dramatically to form multinucleated, highly flagellated filaments 20 to 80 µm in length [201]. These cells line up in parallel to form rafts that are able to move rapidly over surfaces *en masse*. Physiologically, there is a significant increase in protein synthesis [194]. A new type of flagellin protein is expressed, and potential virulence enhancing

factors such as ZapA metalloproteinase, haemolysin and urease are produced in amounts 30 to 80 times higher than in the swimmer state. Large quantities of glycocalyx are produced to form a loosely attached extracellular polysaccharide slime layer, rather than a thin capsule. This is thought to both enhance adhesion and provide a low friction surface for swarming [202].

On solid surfaces *in vitro*, *Pr. mirabilis* exhibits short, coordinated bursts of swarming activity interspersed with a reversion to a surface bound vegetative swimmer state. This produces characteristic concentric rings on an agar plate, representing periods of swarming to colonise a section of surface, interspersed with consolidation in the vegetative state in which intensive cell multiplication occurs, before another period of expansion by the leading edge of organisms differentiating to form further swarmer cells [203]. Those cells other than those in the leading edge of an expansion are in the vegetative state. The rings represent the differing densities of these vegetative organisms in those areas which were swarmed across, and those areas where cell multiplication occurred.

This ability to form swarmer cells seems to allow rapid colonisation of solid surfaces and the establishment of extensive *Proteus* biofilms. Using a simple laboratory model, Stickler and Hughes demonstrated that *Pr. mirabilis* could swarm rapidly over the surfaces of all the major types of catheter [204]. They suggested that swarming may play a role in both the initiation of catheter associated infection and the subsequent spread of the biofilm over the catheter surface. Recently Jones *et al* reported that a non-swarmer mutant of *Pr. mirabilis* had lost the ability to migrate over 100% silicone catheters [205].

1.6 Aims of the Study

Several studies have examined the differences between catheter blockers and non-blockers. The main difference, however, has clearly been the presence of urease producing organisms, usually *Proteus*, in the urinary tract of the catheter blockers. While it is known from clinical observations that the frequency of catheter blockage by encrustation is extremely variable, both within and between individuals, the factors that modulate the rate of encrustation are not understood, and there is little data in the literature on the variability of the times catheters take to block in patients colonised with *Proteus*.

The aims of this study were:

1. To conduct a prospective study on a group of catheterised subjects who have *Proteus* urinary tract colonisation and record the frequency of catheter blockage by encrustation.
2. To examine the relationship between the nucleation pH of a subject's urine and the rate of encrustation.
3. To investigate the factors influencing the nucleation pH of urine in these individuals.
4. To test the hypothesis that the rate of catheter encrustation is dependent on the urease activity of the colonising strain of *Proteus*.
5. To report on the antibiotic sensitivities of the *Proteus* isolated from the catheterised subjects.
6. To determine whether the source of the *Proteus mirabilis* strains that colonise the catheterised urinary tract is the subject's own faecal flora.
7. To observe the stability of *Proteus* as a component of the bacterial flora of the catheterised urinary tract and its relationship to other species.

Section 2 - Methodology

2.1 Protocol Summary

Overview of the study

2.2 Sample Collection

Ethical approval, patient recruitment and data collection

2.3 Chemical Analysis

Nucleation pH, urine composition and encrustation analysis

2.4 Microbiological Analysis

Bacterial culture, antibiotic sensitivities, strain typing and urease activity

2.5 Statistical Analysis

Data analysis tools used in the study

2.1 Protocol Summary

A group of long term urinary catheter users who were found to have *Proteus* urinary tract colonisation were investigated in a simple prospective observational study.

These patients were followed for a period of several months during which their catheters were analysed to determine the rate of deposition of encrustation on them. Weekly microbiological testing of urine samples was used to measure the variation in urinary flora. Patients were also tested for carriage of *Pr. mirabilis* in their gastrointestinal tract.

The urease production of different *Proteus* strains isolated from different patients was examined, as were rates of co-infection with other bacterial species.

The voided pH (pH_v) and nucleation pH (pH_n) of each patient's urine was measured weekly to establish the variability of these values over time. The possible relation of pH_n to other factors was also assessed with weekly measurements of total urinary calcium and magnesium.

Ethical approval was granted by the Southmead Hospital Local Research Ethics Committee before the study commenced (ref: project 070/02).

2.2 Sample Collection

2.2.1 Recruitment

Patients with long term urinary catheters, either suprapubic or urethral, were approached through the catheter clinic at the Bristol Urological Institute's BioMed Centre, through local nursing homes, or through district nurses in the Bristol area. Both those with catheter problems and those without were approached. A single urine sample was taken as a screening test and cultured using our standard culture techniques (section 2.4) for the presence of *Proteus* spp. If this was shown to be positive the patient was approached for full enrolment into the study. Consent was obtained from the patient or relatives according to the local ethical committee guidelines. The first individuals to consent made up the cohort in the study, but as a significant proportion declined to take part those included cannot be considered a formally random sample of *Proteus* colonised catheter users.

2.2.2 Sample Collection

Urine Collection

Urine samples were obtained on a weekly basis throughout the trial period by a visit to the participant's residence. The participant was usually visited at the same time on the same day each week. The participant's catheter was clamped for 10 minutes prior to collecting the sample and the first 2 ml of urine was discarded, in order to ensure that bladder urine was collected rather than urine which had been pooling in the catheter tubing. Samples were collected in sterile plastic containers without preservative. A pH reading was taken immediately and the sample then sealed and refrigerated over ice for transport. Testing of pH was performed using a hand held

glass electrode pH meter. Urine samples were transported to the laboratory and plated onto culture medium on the day of collection.

Catheter Collection

Catheters were collected whenever they were changed routinely or as the result of an emergency blockage. The catheter was kept refrigerated in a sealed, sterile container from removal until laboratory analysis. The date of catheter insertion and removal, along with the type and size of the catheter, was recorded.

Faecal Samples

A single faecal sample was collected by rectal swab from those participants who consented to this part of the study and cultured using standard techniques. This was compared with a urine sample collected at the same time. Four of the original study participants did not consent for faecal sample collection, so an additional four patients were recruited solely for this part of the trial in the same manner as for the full study.

2.3 Chemical Analysis

2.3.1 Voided pH

The pH of each subject's urine was measured immediately on collection of the sample using a Checker 1 portable glass electrode pH meter (Hanna Instruments, Leighton Buzzard, UK), which measures to a resolution of 0.01 pH units. Calibration of all pH meters used in the study was done using the same pH 4 and pH 7 standards (Fisher Scientific, Loughborough, UK).

2.3.2 Nucleation pH

Evaluation of nucleation pH was based on the method of Choong *et al* [148] as described by Suller *et al* [206]. Experiments were performed in a water jacketed chamber at 37°C. An electronic meter was used to measure pH (FisherBrand Hydrus 300). Urinary pH was first reduced to 4.0 by adding aliquots of concentrated hydrochloric acid to the sample. The sample was then alkalinized in increments of 0.25 pH units with 10 M sodium hydroxide solution up to a pH of 10, or until sufficient data points had been recorded above the supposed nucleation pH to allow a straight line to be plotted. This process took approximately 45 minutes for each sample. At each increment the optical density was measured at 550 nm using a distilled water blank on a Cecil Model CE 1011 spectrophotometer. 1 ml of sample was removed and centrifuged at 3500 rpm for 3 minutes to remove precipitates. The supernatant was diluted in 5% nitric and concentrations of calcium and magnesium remaining in solution measured using atomic absorption spectroscopy as described below. The nucleation pH was determined from the resulting plot of pH versus optical density, or pH versus [Ca] and [Mg].

The nucleation pH (pH_n) was defined by an abrupt change in the slope of the graph showing either an increase in turbidity or a decrease in dissolved [Ca] or [Mg] caused by precipitation of crystalline Ca and Mg containing salts. As described by Choong *et al*, plotting pH versus [Ca], [Mg] or optical density produces two straight line segments which intersect at the pH_n . Regression lines were calculated by least squares analysis for these two portions of the graph and used to determine the pH at their intersection. An example is shown in Appendix 1. This method was found to have good repeatability with small variances. For example, a typical triplicate pH_n determination gave values of 6.74, 6.80 and 6.74 with a mean of 6.76 and standard deviation of 0.03.

The pH_n calculated using both the metal concentration and optical density methods was found to be equivalent on analysis of the initial urine samples, and in samples from healthy volunteers, as in the Choong *et al* study. The optical density method was therefore adopted as standard for the remainder of the study due to its simplicity, and the ability to use smaller urine samples.

2.3.3 Urinary Calcium and Magnesium Concentration

Urine was acidified to a pH of 4.0 using concentrated hydrochloric acid. The sample was centrifuged at 3500 rpm for 3 minutes to remove precipitates. The supernatant was diluted in 5% nitric acid to 1 in 10, 1 in 50, 1 in 100, 1 in 200, and 1 in 400, and concentrations of calcium and magnesium remaining in solution measured using atomic absorption spectroscopy on a PerkinElmer Instruments AAnalyst 200 spectrometer. Air/acetylene gas was used for magnesium at a wavelength of 285.2 nm, and NO_2 /acetylene for calcium at 422.7 nm. Calibration curves for calcium and magnesium were constructed using commercial Spectrosol standards (BDH Chemical Ltd, Poole, UK) diluted in 5% nitric acid, made using analar grade nitric acid

and ultra high purity water, to concentrations of 0.5 - 5.0 µg/ml for calcium and 0.2 - 2.0 µg/ml for magnesium.

2.3.4 Encrustation Analysis

Sample catheters were cleaned of material from their external surface by wiping carefully with a dampened swab, and divided into 2 cm sequential sections starting just distal to the catheter eyeholes. The sections were placed in a solution of 100 ml 5% nitric acid for two hours at 37°C and sonicated for 5 minutes. The resulting solution was filtered using 0.2 µm disposable filters (Sartorius, Goettingen, Germany), and then further diluted in 5% nitric acid and analysed using atomic absorption spectroscopy as above to obtain total magnesium and calcium concentrations. From this the total mass of calcium and magnesium on the catheter was calculated.

2.4 Microbiological Analysis

2.4.1 Urinary Culture

Standard calibrated loopfulls of urine were streaked onto Cysteine Lactose Electrolyte Deficient (CLED) agar, Tryptone Soya Agar (TSA), and chromogenic UTI agar (all Oxoid Ltd, Basingstoke, UK). Plates were incubated for 24 hours at 37°C. In the majority of cases multiple colony types were identified. Colony types were differentiated by the varying morphologies of the colony forming units and the reactions of the indicator substances in the agar media giving different groups of species a distinct colour. Each colony type was subcultured onto CLED agar, and after a further 24 hours incubation at 37°C these pure colonies were Gram stained. Further identification of the colony to species level is traditionally achieved by performing a variety of biochemical tests for reactions known to differentiate between species. For example, inoculating the colony to a mixture of urea and a buffered pH indicator medium will differentiate urease positive from urease negative species. Once the possible identities of a colony type have been limited by its source, morphology, reactions with the agar culture media, and Gram stain, the order to perform these tests most efficiently is obtained from flowcharts available in bacteriology laboratory manuals. However, in common with many modern laboratories, the colonies in this study were tested in commercially available pre-prepared test kits in which the sample is inoculated to an array of small wells in a plastic tray. Each well contains a different biochemical test which can then all be performed simultaneously. From the pattern of positive tests, the species can be identified. The Gram-Positive and Enteric BBL Crystal Identification Kits were used (Becton Dickinson Europe, Meylan, France).

2.4.2 Catheter Biofilm Culture

The tips of the catheters were removed to a level just including the eyeholes. These were immersed in 5 ml sterile deionised water in universal containers, sonicated for 5 minutes in a Transsonic T310 cleaning bath (CamLab Ltd Cambridge, UK) and vortex mixed for 30 seconds to shake off the biofilm organisms. Removal of the biofilm was confirmed visually. The resulting suspension was cultured and identified as detailed in section 2.4.1.

2.4.3 Faecal Sample Culture

Faecal samples were swabbed onto agar plates, and cultured and identified as detailed in section 2.4.1. Any *Proteus* spp present was isolated and stored for comparison with the *Proteus* isolates from urine samples collected at the same time. Typing methods are detailed in section 2.4.7.

2.4.4 Storage of Bacterial Specimens

Isolated strains of bacteria were stored at -80°C on Microbank beads (Pro-Lab diagnostics, Neston, UK).

2.4.5 Antibiotic Sensitivities

The minimum inhibitory concentrations (MIC) of a panel of antibiotics were tested against the main encrusting organisms in the participant's urine culture. E-test strips (AB Biodisk, Solna, Sweden) were used to determine MICs.

The E-test is a non-porous plastic strip calibrated with MIC values covering 15 two-fold dilutions. The strip contains an antibiotic with a defined concentration gradient along its length. When placed in agar, the antibiotic concentration to which the cultured organisms are exposed varies along the strip, and the concentration below which growth is not inhibited can be read from the calibrated scale on the reverse of the E-test strip [207].

Individual bacterial colonies from a pure overnight culture on CLED agar (Oxoid) were suspended in 0.85% saline to achieve a turbidity of 0.5 McFarland standard. A sterile swab was used to coat Iso-Sensitest Agar (Oxoid) plates with the inoculum. Once dry, Etest strips were applied to the inoculated plates. The plates were cultured, inverted, at 37°C for 24 hours. The MIC was read from the E-test strip following the guidelines in the product literature. In particular, areas of *Proteus* swarming on the plate were ignored.

Antibiotics tested were ciprofloxacin, trimethoprim, gentamicin, amoxicillin, co-amoxiclav, cefalothin and nalidixic acid. Breakpoint values for interpreting the MIC to determine whether the organism was resistant or sensitive to an antibiotic were obtained from the British Society for Antimicrobial Chemotherapy guidelines for Urinary Tract Infections with Gram-negative rods [208].

2.4.6 Urease Assay

The 'urease activity' of the *Proteus mirabilis* isolates was assayed using the modified Berthelot colorimetric reaction method of Creno *et al* [209]. Urease activity is defined as the rate of hydrolysis of urea per unit mass of protein, and is usually expressed in units of $\mu\text{mol urea hydrolysed} / \text{minute} / \text{mg protein}$. The procedure for measurement involves producing a protein extract from the cultured bacterial cells. The protein

concentration of this extract is determined. A known volume, and hence known mass, of protein is incubated in a urea solution for a set time, and the amount of ammonia produced is calculated with reference to a set of ammonium chloride standards. The rate of hydrolysis of urea can be determined by the stoichiometry of the reaction:



It can be seen that each two moles of ammonia derives from the hydrolysis of one mole of urea.

(i) Protein extraction

Bacterial strains were incubated in Luria broth supplemented with 0.1% (w/v) urea for 4 hours at 37°C in a shaking incubator. These cultures were then centrifuged at 4000 rpm for 4 minutes, the supernatant discarded, and the cells re-suspended in 25 ml ice cold 0.1 M sodium phosphate buffer (pH 7.3) with 0.01 M ethylenediaminetetraacetic acid (EDTA) to form a protein extract.

(ii) Protein concentration assay

A commercially available assay kit was used (Total Protein Kit, Micro Pyrogallol Red Method - manufactured by Sigma).

20 µl of protein extract was added to 1 ml of Total Protein Reagent (0.05 mM pyrogallol red, 0.16 mM sodium molybdate with chelating agent, stabiliser, surfactant and preservative) pre-warmed to 37°C. A blank was prepared with 20 µl distilled water and a standard with 20 µl of the supplied Protein Standard Solution (0.5 mg/ml

human serum albumin in saline with 0.1% sodium azide preservative) instead of a test protein extract. Samples were incubated at 37°C for 3 minutes.

Absorbance (A) was read in a spectrophotometer at 600 nm using a distilled water reference. The protein concentration of the sample was calculated as:

$$(A_{\text{test}} - A_{\text{blank}}) / (A_{\text{standard}} - A_{\text{blank}}) * \text{concentration of Standard (mg/ml)}$$

(iii) Ammonia production assay

A set of ammonium chloride standards was produced to calibrate the assay.

Standards were produced from 0.001 M to 0.009 M in 0.001 M increments, and from 0.01 M to 0.1 M in 0.01 M increments.

1 ml reaction mixtures were produced containing 50 mM urea, 100 mM sodium phosphate buffer and 0.2 ml of protein extract, ammonium chloride standard or a distilled water blank. The mixtures were incubated at 37°C for 10 minutes and the reaction terminated with 2 ml of 0.5% phenol and 0.0025% sodium nitroprusside solution.

Once the reaction was terminated, 2 ml of 0.25% sodium hydroxide and 0.2% sodium hypochlorite solution was added and colour development initiated by incubation at 56°C for 6 minutes.

Absorbance was read in a spectrophotometer at 600 nm using the distilled water blank as a reference. A calibration curve was plotted using the ammonium chloride standards, and the amount of ammonium in the incubated culture extracts determined from this.

(iv) Urease activity calculation

As 2 mol of ammonia is derived from each mol of urea, and the assay is run for 10 minutes, the urease activity of a sample is derived as:

$$[\text{ammonia}] / 2 / 10 / [\text{protein}] \text{ (}\mu\text{mol/min/mg)}$$

Three replicates were run for each bacterial strain. Urease activity was expressed as μmol urea hydrolysed / minute / mg protein.

2.4.7 Comparison of Urine and Stool Cultures

Where *Pr. mirabilis* was isolated from both the stool and urine of an individual participant on a single day, these isolates were compared by first Dienes typing and then Pulse Field Gel Electrophoresis (PFGE).

(i) Dienes typing

Stored isolates of *Pr. mirabilis* were plated to achieve single colonies and cultured overnight at 37°C on CLED agar. Single colonies of the isolates from the faecal and urine samples of each subject were inoculated as macrocolonies on TSA agar with up to four isolates on each plate. Each isolate was tested against every other isolate. Typing is based on the mutual inhibition of different strains causing the swarming colonies to repel each other. After overnight incubation at 37°C, those isolates showing a clear band, or Dienes demarcation line, between each other were designated as different strains. Those with no such demarcation line were regarded

as the same Dienes type and were then subjected to PFGE to further investigate their similarity.

(ii) Pulsed Field Gel Electrophoresis

This was performed using the method developed by Sabbuba *et al* [49].

Bacterial strains were incubated in Tryptone Soya Broth (Oxoid) overnight at 37°C in a shaking incubator. Bacteria were harvested by centrifuging the cultures for 10 mins at 3000 rpm, and the cells re-suspended in 8 ml of SE buffer, comprised of 75 mM sodium chloride (Fisher Scientific Ltd, Loughborough, UK) and 25 mM EDTA (Sigma Chemical Co, St Louis MI, USA). Suspensions were warmed to 45°C and mixed with equal volumes of molten 2% low gelling temperature agarose (Type VII, Sigma) at the same temperature. This mixture was pipetted into 80 µl disposable plug moulds (BioRad Laboratories, Hercules CA, USA). Four plugs were made per strain and chilled at 4°C for 15 mins. The plugs from each strain were placed in sterile tubes with 10 ml of PEN buffer, containing 0.5 M EDTA and 1% N-lauryl sarcosine (MP Biomedicals GmbH, Eschwege, Germany). Pronase (Roche Diagnostics, Indianapolis IN, USA) was added to each tube at 1 mg/ml and incubated overnight at 37°C in a shaking incubator. Plugs were then washed with five volume changes of TE buffer, containing 10 mM tris and 1 mM EDTA acidified to pH 8 with hydrochloric acid. Plugs were gently agitated on a rocking platform for 30 min between each volume change.

2 mm slices were cut from the plugs in a sterile manner and subjected to overnight restriction enzyme digestion at 37°C in individual sterile tubes. The digestion mixture contained 5 units of *Not* I restriction enzyme (Promega Corporation, Madison WI, USA) per tube along with 15 µl of 10X Bovine serum albumin and 15 µl of 10X Buffer (supplied with the enzyme) in sterile deionised water.

For the PFGE, the plugs were loaded into gels made with 1.2% pulsed field certified agarose (BioRad) in 1X TBE buffer, containing 5.4 g/l tris base, 2.75 g/l boric acid and 2 ml/l 0.5 M EDTA. The loaded gels were sealed with 1.2% low gelling temperature agarose. Bacterial DNA fragments were separated by electrophoresis at 14°C at 6 V/cm for 8 hours with a switch time of 1 to 30 s, followed by 16 hours with a switch time of 30 to 70 s. A CHEF-DR II apparatus was used (BioRad).

Bacteriophage λ DNA ladders (BioRad) were included as size standards. Gels were stained using ethidium bromide for approximately one hour, and de-stained under running water for 10 min. Gels were viewed and photographs taken under ultraviolet light.

The epidemiological relationship of the strains was determined using the criteria described by Tenover *et al* [210].

2.5 Statistical Analysis

Descriptive statistics, t tests, and simple linear correlation and regression analysis were performed using the Data Analysis Toolpak for Microsoft Excel 2003 (Microsoft Corporation, Redmond WA, USA). Tests of normality, non-parametric tests and frequency analysis was performed using the Analyse-it add in for Microsoft Excel (Analyse-It Software Ltd, Leeds UK).

Data was compared using either parametric or non-parametric tests dependent on an analysis of the normality of the data using the Shapiro-Wilk *W* test.

Multiple linear regression analysis was performed using SPSS for Windows (SPSS Inc, Chicago IL, USA).

Section 3 - Results

3.1 The Prospective Study

Overview of each study participant and summary of times to catheter blockage

3.2 Urinary Constituents and Encrustation

Variability in urine composition and examination of the 'blocking pH' hypothesis

3.3 Factors Influencing Nucleation pH

The effects of urinary composition on crystal precipitation

3.4 Urease Activity

The effect of urease production on encrustation

3.5 Behaviour of Bacterial Populations

Persistence and co-infection with other species

3.6 Antibiotic Sensitivities

Comparison of catheter colonising and normal pathogenic isolates

3.7 Comparison of Urine and Stool Cultures

Strain typing to determine the origin of urinary organisms

3.1 The Prospective Study

Subjects with long term urinary catheters and *Proteus* urinary tract colonisation were recruited. Samples of bladder urine were collected at the same time each week for approximately three months and analysed for voided pH (pH_v), nucleation pH (pH_n), calcium and magnesium concentration, and cultured to isolate urinary flora. Any catheters which were removed during the study were collected. The reason for removal and any visible encrustation was noted, and they were analysed for calcium and magnesium content and cultured to isolate biofilm flora. The data are described for each participant in section 3.1.1, and summarised graphically in figures 3.2 to 3.22.

Study participants were expected to be catheter blockers given the presence of urease positive organisms in their urine. The traditional division into 'blockers', whose catheters on average need changing more frequently than every six weeks, and 'non-blockers', whose catheters do not, was not thought appropriate [46]. They were therefore divided into two different groups for the purposes of analysis: those whose catheters lasted a mean of less than 28 days, and those whose catheters lasted a mean of greater than this time. As with the 'blocker' and 'non-blocker' definition this is essentially an arbitrary division designed to allow analysis of the data in similar ways to previously published studies. These groups represent the heaviest (*catheter* < 28 days group) and lightest (*catheter* > 28 days group) catheter encrusters. Regression analysis was also used as the variables appeared to have a continuous distribution, and this was therefore felt to be more appropriate. Catheters which were expelled, or removed when clearly not blocked, were not included in the analysis.

3.1.1 Summary of Data for each Study Participant

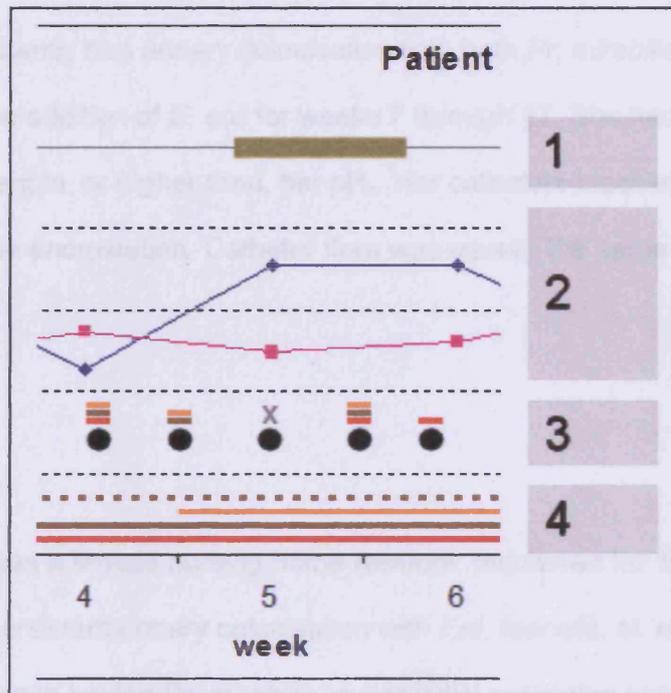
Table 3.1 summarises the demographic details of those catheter users participating in the trial. As expected, there is a large representation of older subjects and residents of long term care facilities.

Table 3.1: demographic details, catheter details and length of involvement in the trial of the main study participants

<i>participant</i>	<i>age</i>	<i>sex</i>	<i>residence</i>	<i>catheter</i>	<i>size</i>	<i>weeks in study</i>
02	78	F	nursing home	urethral	16	19
03	69	F	nursing home	urethral	14	19
04	73	F	nursing home	urethral	14	19
05	90	M	nursing home	urethral	16	19
06	102	F	nursing home	urethral	14	17
07	83	F	own home	urethral	14	15
08	85	F	nursing home	suprapubic	14	15
09	76	F	sheltered accommodation	suprapubic	16	14
10	73	M	nursing home	urethral	16	5
11	81	M	own home	urethral	16	9
12	85	M	nursing home	suprapubic	16	5
13	74	F	nursing home	urethral	12	13
14	86	F	nursing home	urethral	14	14
15	88	F	own home	urethral	14	9
16	80	M	own home	suprapubic	20	14
17	43	F	own home	suprapubic	18	14
18	53	F	own home	urethral	18	13
19	86	M	own home	urethral	14	13
20	81	F	nursing home	suprapubic	18	13
21	58	M	own home	urethral	16	16
22	49	F	own home	suprapubic	18	12
23	71	F	nursing home	urethral	14	genotyping only
24	84	F	own home	suprapubic	16	genotyping only
25	60	F	own home	urethral	14	genotyping only
26	81	M	own home	urethral	16	genotyping only

The data shown in each summary graph (figures 3.2 to 3.22) is divided into four main sections as shown in figure 3.1 and described below.

Figure 3.1: Example of a segment from a participant summary graph, with data grouped into four sections as shown by the numbered grey boxes on the right.



Section 1 contains data on antibiotic and catheter maintenance solution treatments given during the trial. Section 2 shows the graphs of pH_v and pH_n as measured on the y-axis scale. Section 3 has circles representing the catheters removed during the study, with the short coloured bars above each catheter circle detailing the bacteria isolated from it. A grey X indicates that no microbiological analysis was performed, either because the catheter was lost or was unsuitable for analysis. Catheters are marked as being changed when blocked, undergoing a scheduled routine change before being blocked, or undergoing an unscheduled change when not blocked. Section 4 contains long coloured bars indicating the bacteria isolated from the urine as measured by the weekly urine samples.

A key is provided below each figure.

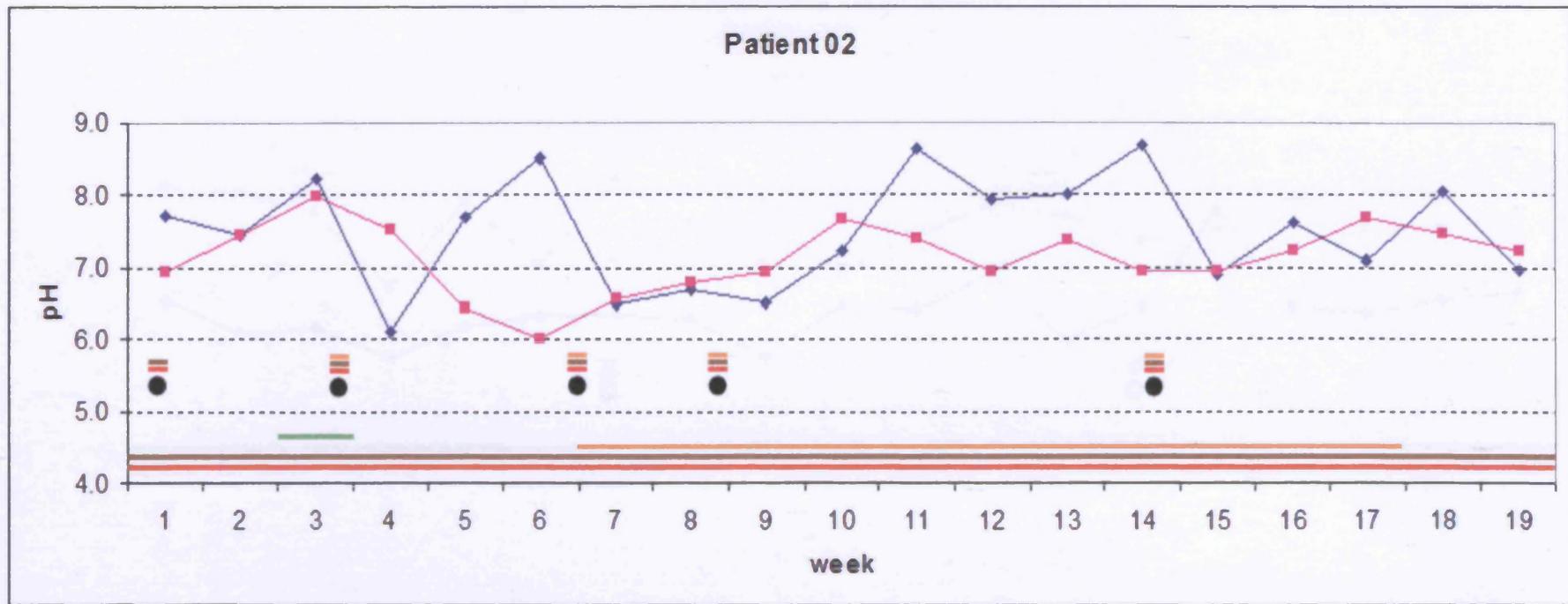
Participant 02

Participant 02 was a female nursing home resident, monitored for 19 weeks (figure 3.2). She persistently had urinary colonisation with both *Pr. mirabilis* and *Ent. faecalis*, with the addition of *E. coli* for weeks 7 through 17. She had a pH_v which was usually equivalent to, or higher than, her pH_n . Her catheters blocked frequently with significant visible encrustation. Catheter flora was usually the same as that isolated from the urine.

Participant 03

Participant 03 was a female nursing home resident, monitored for 19 weeks (figure 3.3). She had persistent urinary colonisation with *Ent. faecalis*, *M. morganii*, and *Ps. aeruginosa*. Despite having *Pr. mirabilis* on her initial screening sample, this organism was not isolated again during the study. The urinary pH_v was almost always considerably lower than its pH_n . Her catheters were changed infrequently, and showed minimal encrustation. Catheter flora species were the same as those isolated from the urine.

Figure 3.2: Participant 02 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

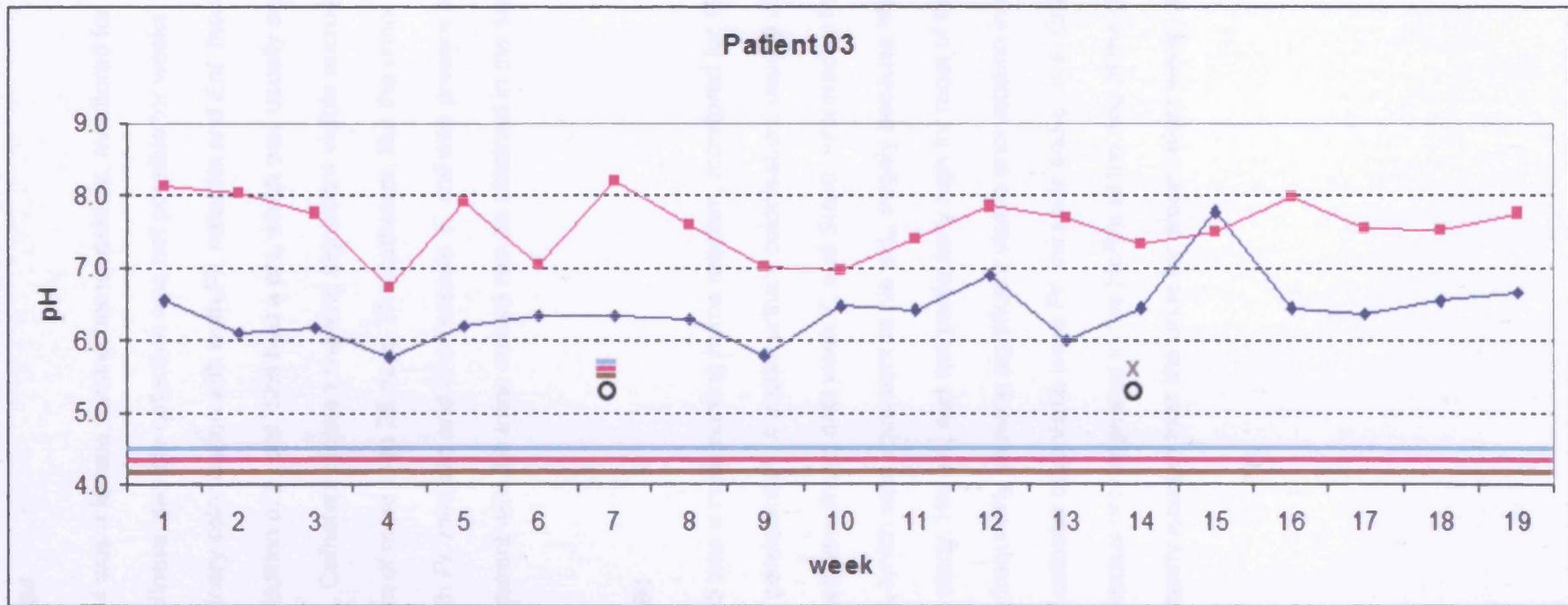
Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Citrobacter* spp
- *E. coli*
- *Ent. faecalis*
- *Pr. mirabilis*

Figure 3.3: Participant 03 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values	Catheters	Bacteria
■ Nucleation pH	● Blocked	— <i>M. morganii</i>
◆ Voided pH	○ Routine change	— <i>Ps. aeruginosa</i>
	○ Not blocked	— <i>Ent. faecalis</i>

Participant 04

Participant 04 was a female nursing home resident, monitored for 19 weeks (figure 3.4). Due to illness, sample collection was not possible for weeks 17 and 18. She had persistent urinary colonisation with both *Pr. mirabilis* and *Ent. faecalis*, with the occasional addition of *E. coli*. She had a pH_v, which was usually equivalent to or lower than her pH_n. Catheters blocked showing significant visible encrustation, but with a mean lifespan of more than 28 days. The catheters, like the urine, were consistently colonised with *Pr. mirabilis* and *Ent. faecalis*. *E. coli* was present occasionally, but was not consistent with the urine culture results obtained in the same week.

Participant 05

Participant 05 was a male nursing home resident, monitored for 19 weeks (figure 3.5). He had persistent *Pr. mirabilis* urinary colonisation, usually with *Ent. faecalis* present. *E. coli* were found until week 7, and *Staph. intermedius* from week 11 to 18. He had a pH_v, which was equivalent to his pH_n, largely because his mean pH_n was the lowest in the study. His pH_v was not particularly high for most of the study. Catheters blocked very frequently showing significant visible encrustation in each case. The predominant catheter organisms were *Pr. mirabilis* and *E. coli*. *Ent. faecalis* and *Staph. intermedius* only appeared in the biofilm at the end of the study period, despite being consistently isolated from the urine for around eight weeks beforehand in each case.

Figure 3.4: Participant 04 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.

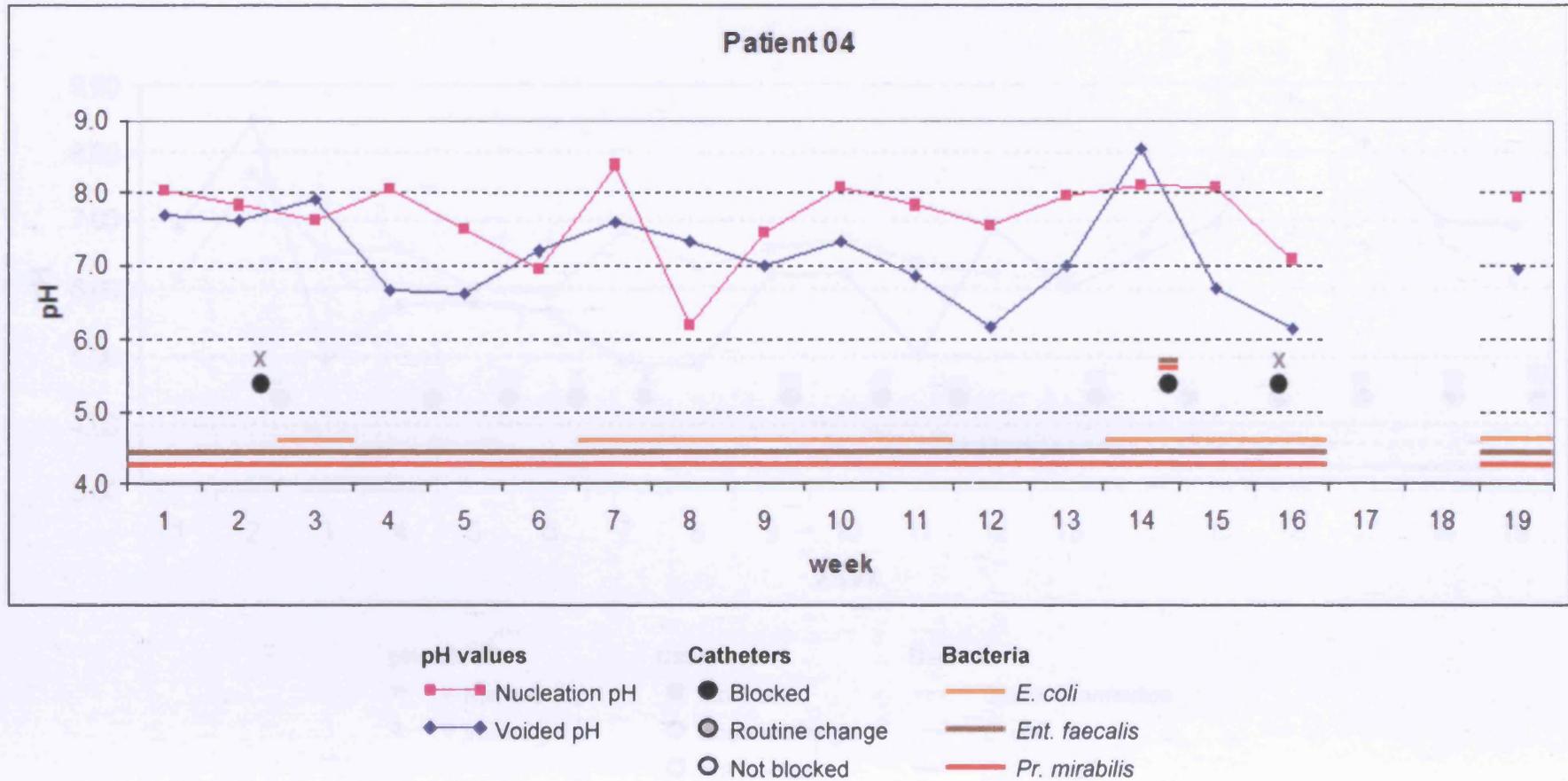
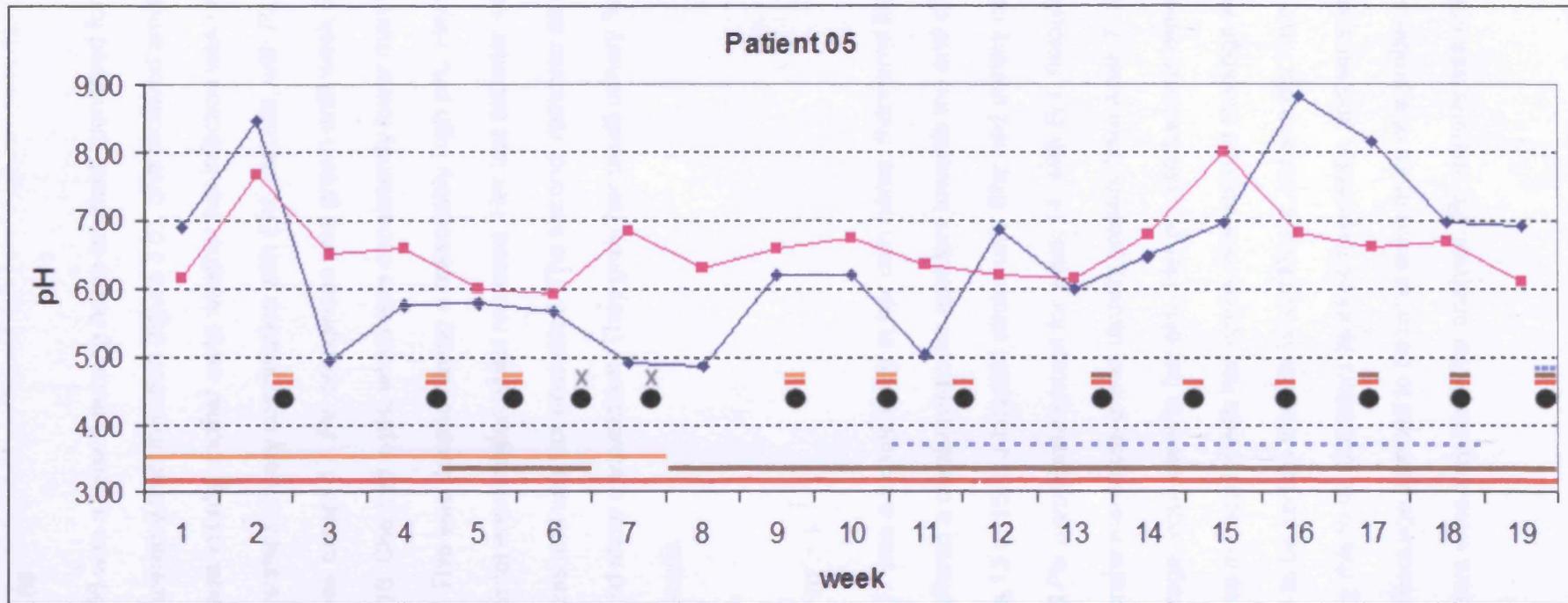


Figure 3.5: Participant 05 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- ⋯ Staph. intermedius
- E. coli
- Ent. faecalis
- Pr. mirabilis

Participant 06

Participant 06 was a female nursing home resident, monitored for 17 weeks until she died of a respiratory tract infection (figure 3.6). She received amoxicillin from week 16, when it was initially unclear as to whether her infection was urinary or respiratory. She had persistent urinary colonisation with *Ent. faecalis*, with *Pr. mirabilis* present for every week except 17. *Ps. aeruginosa* was grown until week 5, and *E. coli* from week 14 to 16. She had a pH_v which was consistently lower than her pH_n by at least one pH unit. This was despite having a reasonably high pH_v . Her first catheter showed minimal encrustation when removed. Her last catheter was removed during her final illness and was not encrusted. The second, removed as an adjunct to her antibiotics, did show encrustation. They grew her usual urinary flora of *Pr. mirabilis* with *Ent. faecalis*.

Participant 07

Participant 07 was a female living in her own home, monitored for 15 weeks (figure 3.7). She suffered a cerebrovascular accident towards the end of this period and so spent weeks 13 to 15 in a hospital stroke unit. She had urinary colonisation with *Pr. vulgaris* and *Ps. aeruginosa* except for week 14, with *Ent. faecalis* persistent from week 3. *Serratia marcescens* was usually present from week 7. She had a pH_v which was on average, equivalent to her pH_n . Her pH_v did become elevated above her pH_n while she was in hospital with her stroke. Not enough readings were taken during this short period to be certain that this is significant, but she did have problems with fluid intake during this time. Catheters blocked frequently, and were significantly encrusted. Blockage seemed to become even more of a problem in hospital, although these catheters were unsuitable for analysis. *Pr. vulgaris* was not isolated from the

catheters. They were colonised with *Ps. aeruginosa* and *Ent. faecalis*, and with *Serratia marcescens* even before it was isolated from the urine.

Figure 3.6: Participant 06 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.

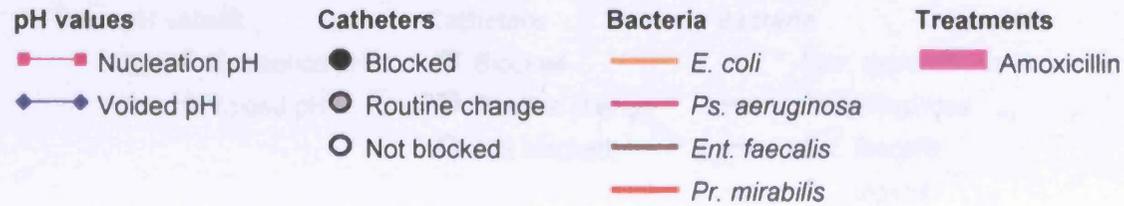
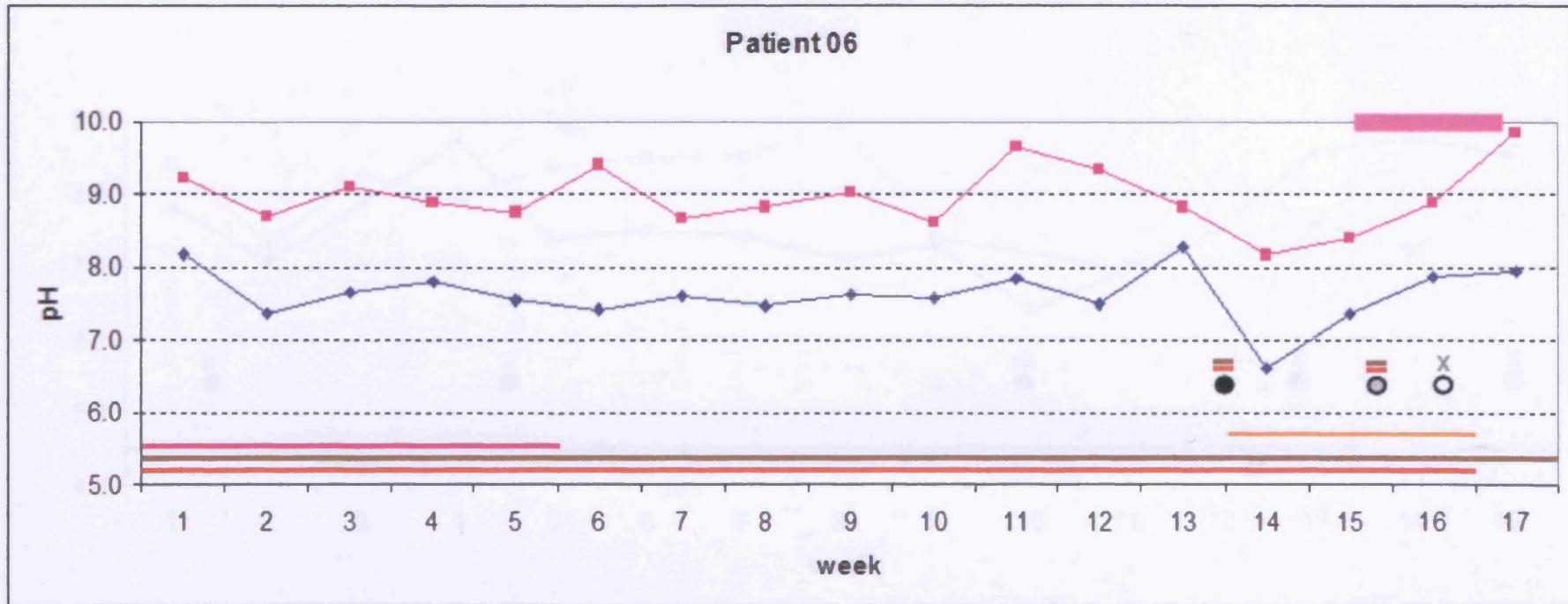
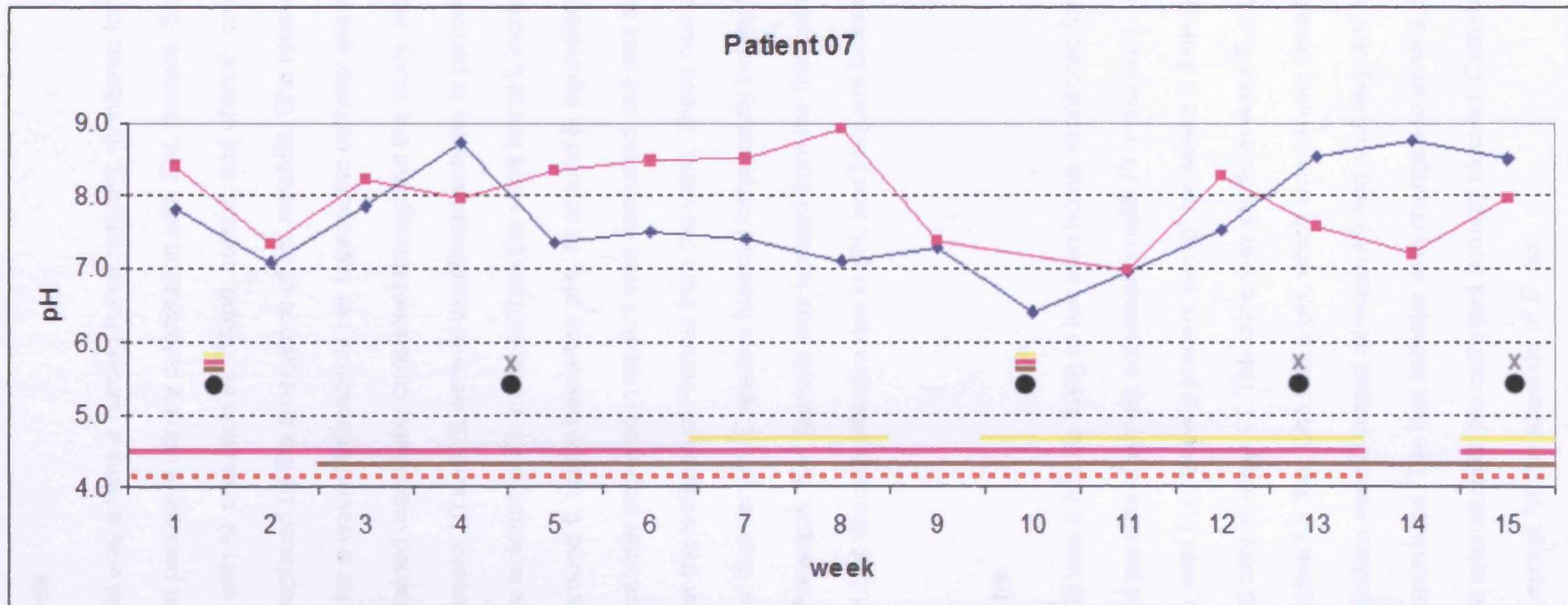


Figure 3.7: Participant 07 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Serr. marcescens*
- *Ps. aeruginosa*
- *Ent. faecalis*
- ... *Pr. vulgaris*

Participant 08

Participant 08 was a female nursing home resident, monitored for 15 weeks (figure 3.8). She had persistent urinary colonisation with *Ent. faecalis*. She also had colonisation with *Kl. pneumoniae*, *Staph. aureus*, and often *E. coli* until week 7 when these were replaced by the emergence of *Pr. mirabilis*. She received a course of minocycline for a wound infection at her suprapubic catheter site in weeks 5 and 6. Her pH_n remained reasonably consistent throughout the study, although urine samples in weeks 10 and 12 were of insufficient volume to perform pH_n analysis. Following the isolation of *Pr. mirabilis* from the urine her pH_v increased from an average of around 5, safely below the pH_n, to around 8, equivalent to or higher than it. The first catheter removed in week 1 was encrusted, but less so than those removed after the isolation of *Proteus* from the urine. These had crystals present along more of their length. Catheters blocked infrequently before *Proteus*, but very frequently afterwards. *Ent. faecalis* was isolated from the first catheter, and *Pr. mirabilis* and *Ent. faecalis* after the rise in pH_v and frequent blockage started.

Participant 09

Participant 09 was a female living in her own home, monitored for 14 weeks (figure 3.9). She had persistent urinary colonisation with *Pr. mirabilis*, *E. coli* and *Kl. pneumoniae*, with *Ent. faecalis* present except for weeks 5 through 9, and *Staph. aureus* found only in week 11. The urine was sterile in week 6, although there is no clear explanation for this. She had a pH_v which was usually considerably lower than her pH_n. Catheters were changed infrequently and routinely, and were only moderately encrusted. The last catheter was changed to allow a diagnostic test and had very little encrustation. No catheters actually blocked. Catheter flora reflected urinary flora except for the absence of *E. coli*

Figure 3.8: Participant 08 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.

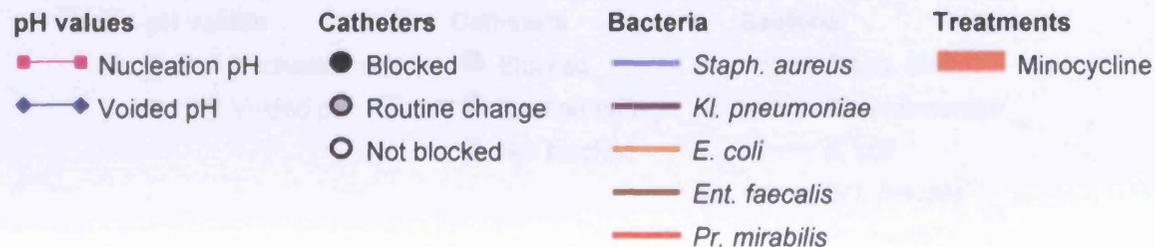
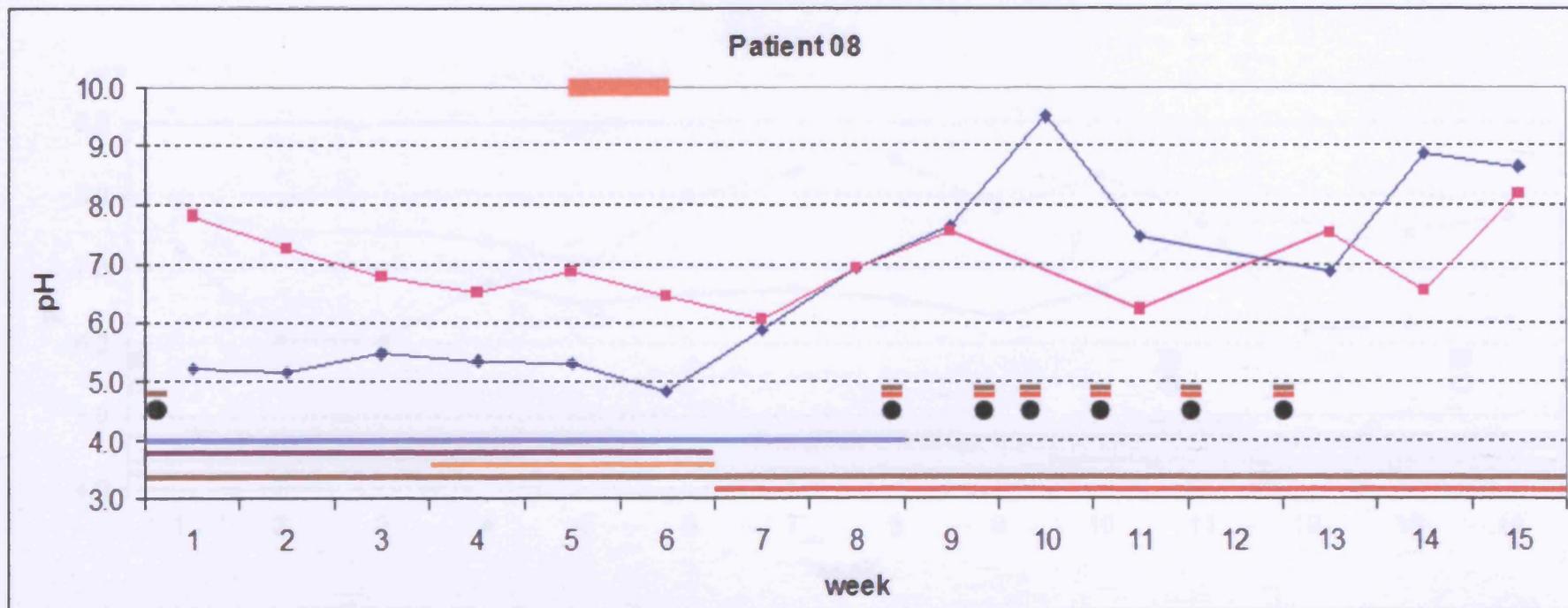
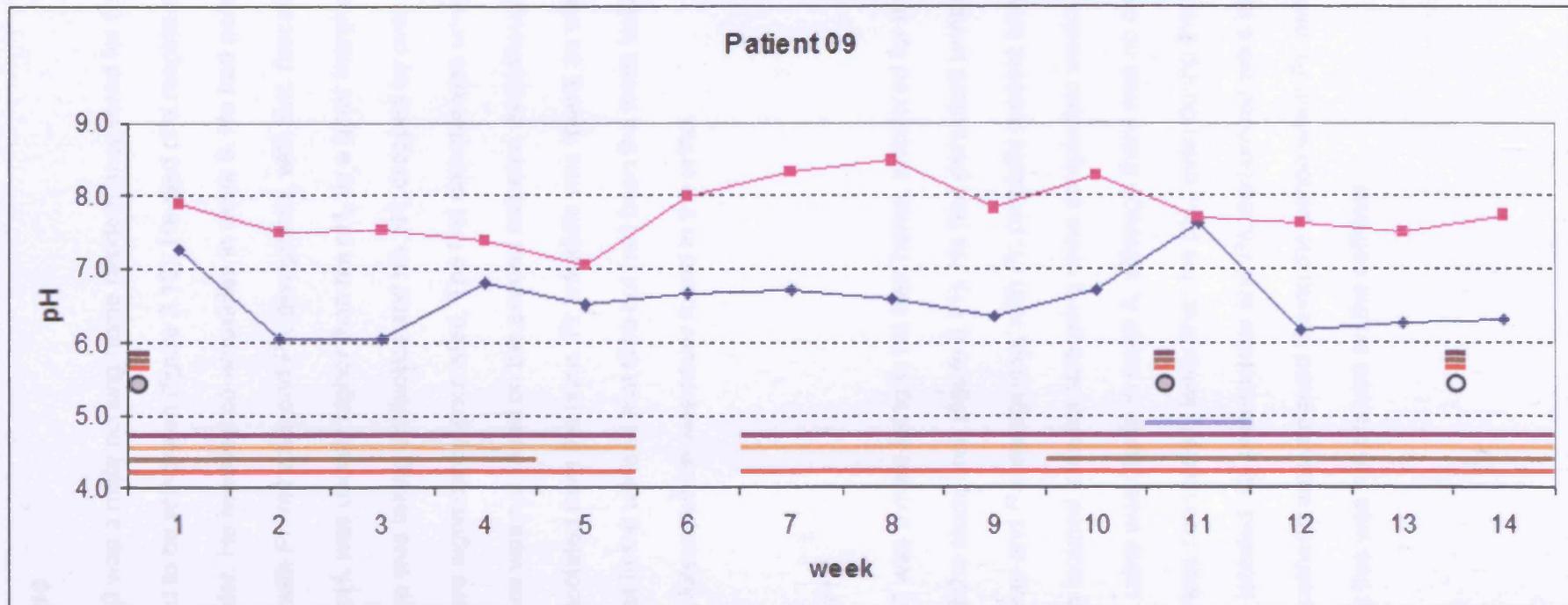


Figure 3.9: Participant 09 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Staph. aureus*
- *Kl. pneumoniae*
- *E. coli*
- *Ent. faecalis*
- *Pr. mirabilis*

Participant 10

Participant 10 was a male nursing home resident, monitored for 5 weeks until illness meant he had to be withdrawn (figure 3.10). He died of a respiratory tract infection two weeks later. He received co-amoxiclav in week 5. He had persistent urinary colonisation with *Pr. mirabilis* and *Ps. aeruginosa*, with *Ent. faecalis* present only in week 3. His pH_v was usually higher than his pH_n by a good margin, except for the final week when he was taking antibiotics and his pH_v dropped by over 2 pH units. Catheters were significantly encrusted. The first catheter was unsuitable for culture. *Ps. aeruginosa* was not found on the second catheter, even though it was consistently isolated from the urine. *Pr. mirabilis* was found, as was *Ent. faecalis*, which was not in the urine at that time but had been the week before, and *Micrococcus luteus*, which was never found in the urine.

Participant 11

Participant 11 was a male living in his own home, monitored for 9 weeks until he moved out of the study area (figure 3.11). He had persistent urinary colonisation with *Kl. pneumoniae* and *Ps. aeruginosa*, with *Pr. mirabilis* present only in weeks 5 and 6, despite being isolated from his screening urine sample two weeks before the start of the trial. The urine was sterile in week 8, although there was no clear explanation for this. His pH_v was consistently lower than his pH_n , even during the weeks when *Proteus* was isolated. *Kl. pneumoniae* and *Ps. aeruginosa* were isolated from both. The second catheter was removed during the period when *Pr. mirabilis* was found in the urine, but this was not present on the catheter.

Figure 3.10: Participant 10 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.

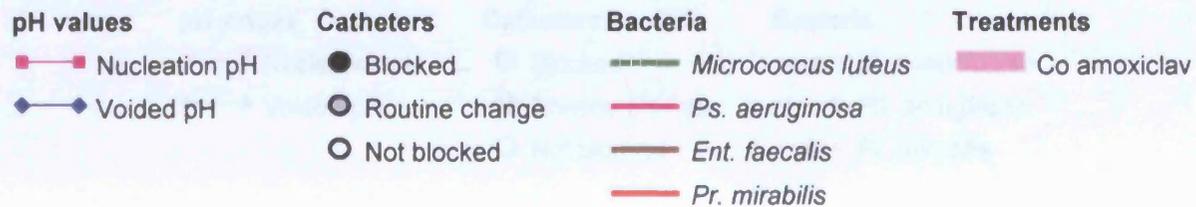
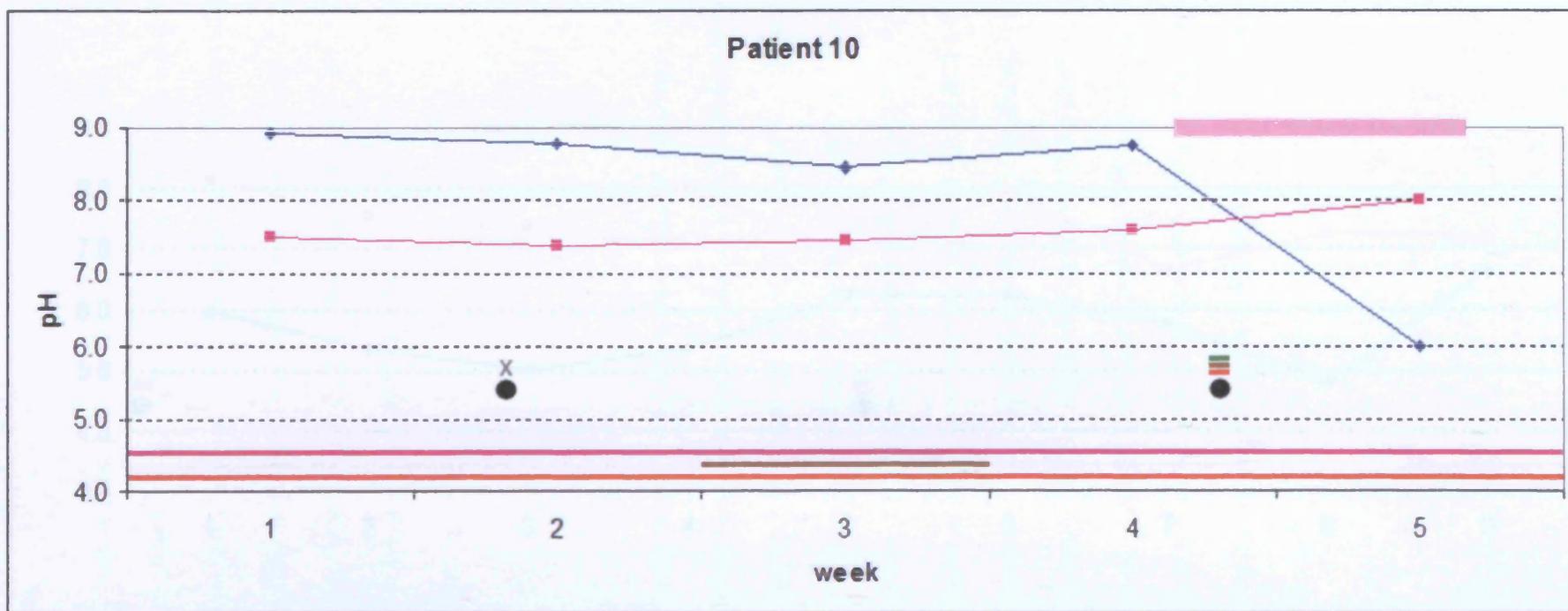
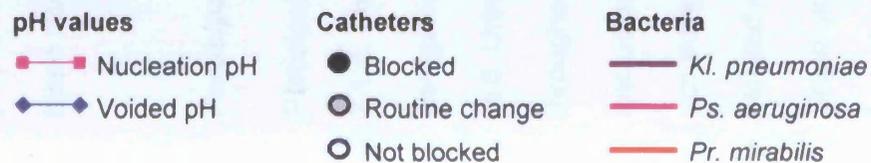
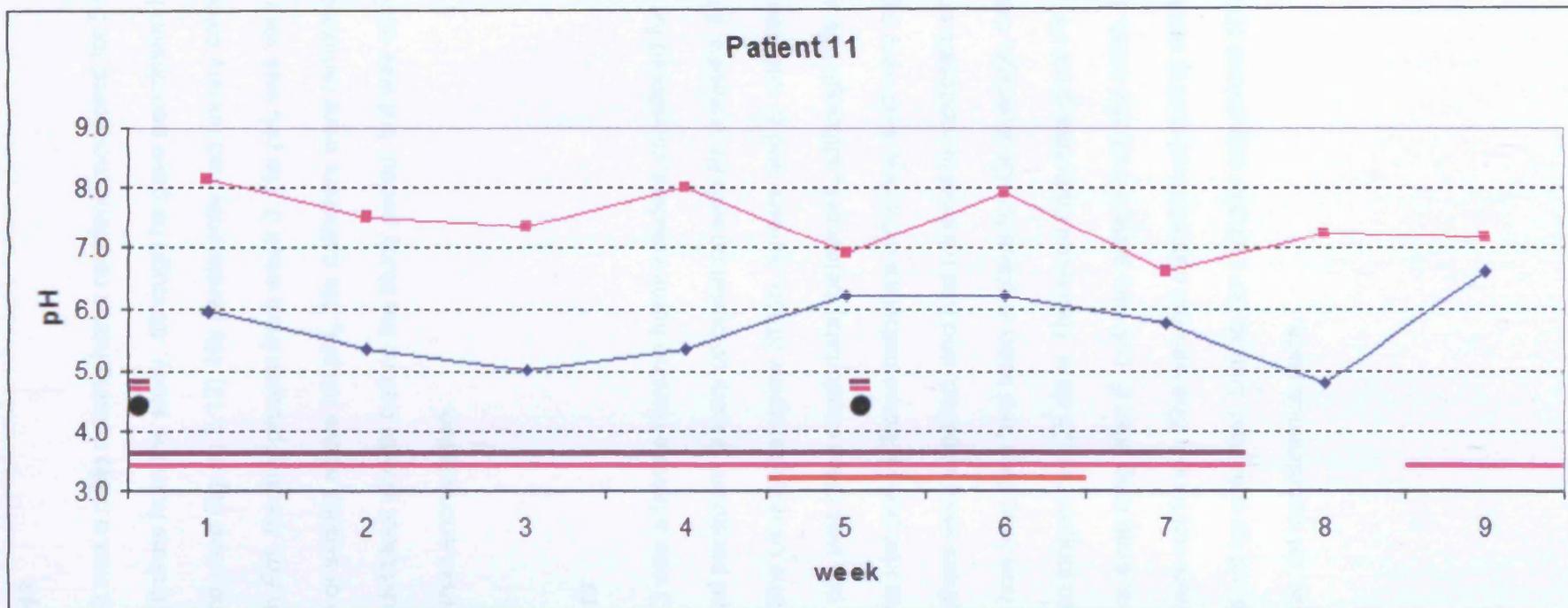


Figure 3.11: Participant 11 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



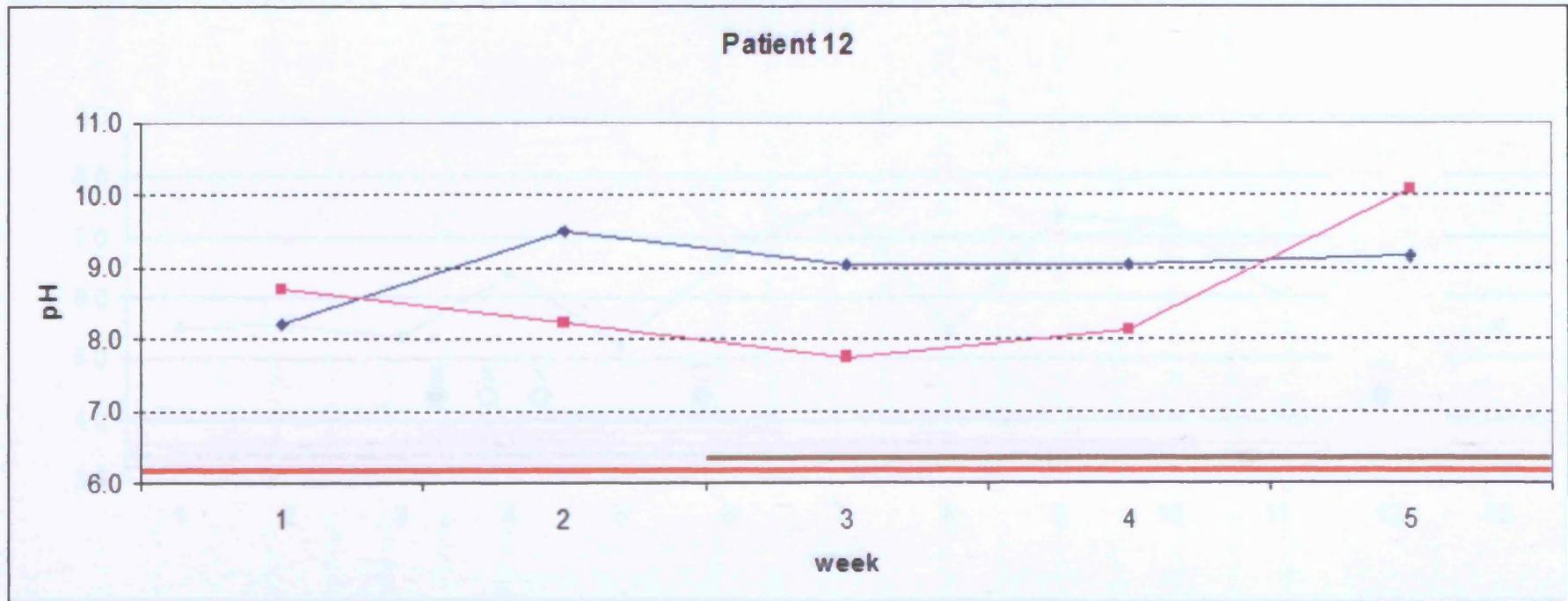
Participant 12

Participant 12 was a male nursing home resident, monitored for 5 weeks until he voluntarily withdrew from the study, although he gave permission for data already collected to be used (figure 3.12). He persistently had urinary colonisation with *Pr. mirabilis*, with *Ent. faecalis* present from week 3. His pH_v was very high, and was equivalent to or slightly above his pH_n. No catheters were removed during the study period. One did block shortly before the study period, but was occluded with clotted blood rather than encrustation.

Participant 13

Participant 13 was a female nursing home resident, monitored for 13 weeks (figure 3.13). She had persistent urinary colonisation with *Pr. mirabilis*, *Ent. faecalis* and *Ps. aeruginosa*. She usually also grew *Staph. aureus*, and *E. coli* was found in weeks 4 to 6. Urinary pH_v was often comfortably below pH_n, although this was not consistent throughout the trial period. Some catheters did block, and were significantly encrusted. Others were expelled, and had minimal to moderate encrustation according to how long they had been in place for. On average, catheters which blocked lasted longer than 28 days. Retrieved catheters grew *Pr. mirabilis*, *E. coli* and *Staph. aureus*, even long after *E. coli* was absent from the urine. *Ent. faecalis* and *Ps. aeruginosa* were not found. She was not catheterised during week 12, so a urine sample could not be collected. Due to her catheter expulsions she was being trialled, unsuccessfully, on incontinence pads.

Figure 3.12: Participant 12 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

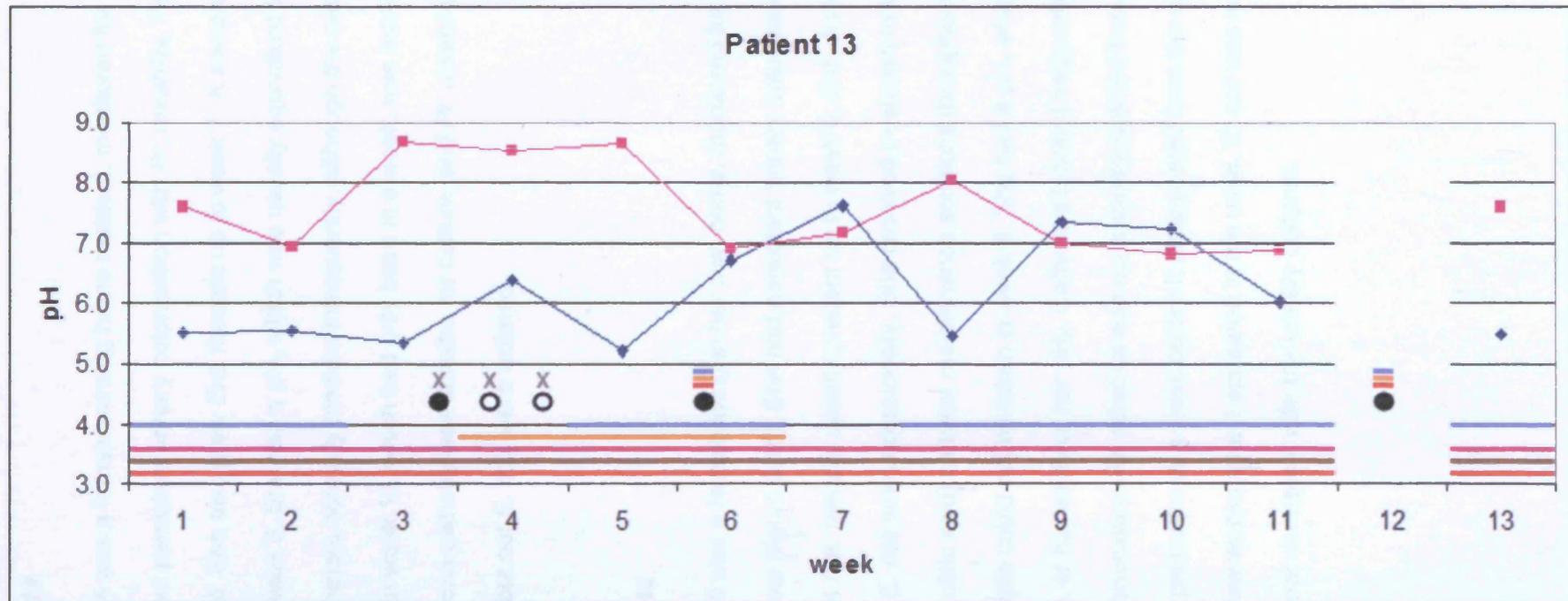
Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Ent. faecalis*
- *Pr. mirabilis*

Figure 3.13: Participant 13 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Staph. aureus*
- *E. coli*
- *Ps. aeruginosa*
- *Ent. faecalis*
- *Pr. mirabilis*

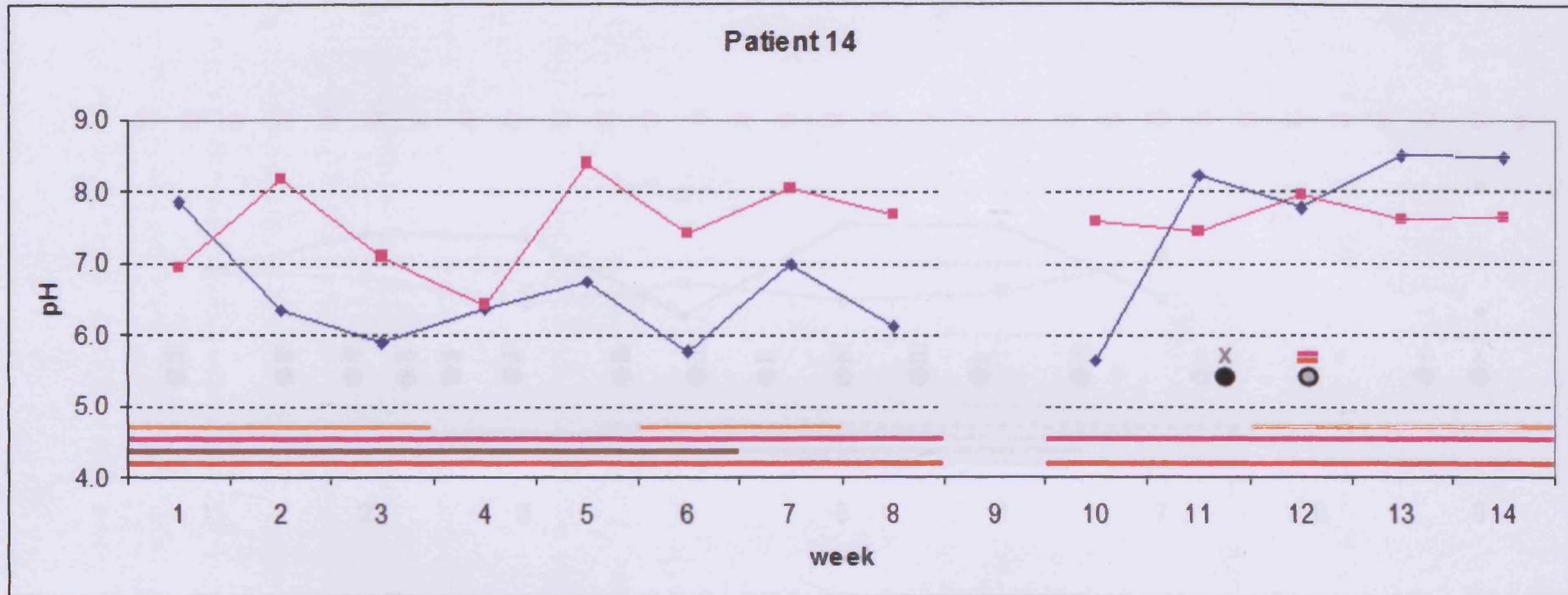
Participant 14

Participant 14 was a female nursing home resident, monitored for 14 weeks (figure 3.14). She had persistent urinary colonisation with *Pr. mirabilis*, *Ps. aeruginosa*, and usually *E. coli*. She also grew *Ent. faecalis* up to week 7. A sample could not be collected in week 9. She had a pH_v which was usually equivalent to, or lower than, her pH_n . Catheters normally blocked infrequently, although the catheter removed accidentally in week 12, which had only been in a week, was markedly encrusted. Only the second catheter was suitable for culture, and *Pr. mirabilis* and *Ps. aeruginosa*, but not *E. coli*, were isolated.

Participant 15

Participant 15 was a female living in her own home, monitored for 9 weeks until she died of a stroke (figure 3.15). She had persistent urinary colonisation with *Pr. mirabilis*, with *Ent. faecalis* usually present up to week 6, *Ent. durans* present from week 4, and *E. coli* seen occasionally. She received treatment twice weekly with Suby G (3% citric acid) catheter maintenance solutions throughout the course of the study. A sample could not be taken in week 8. She had a pH_v which was usually equivalent to, or higher than, her pH_n . Catheters blocked frequently and were significantly encrusted. *Pr. mirabilis* was consistently isolated from the catheters. *Ent. faecalis* was present until a week before it disappeared from the urine. *Ent. durans* was seen a few weeks after it appeared in the urine. *E. coli* was isolated frequently, but this was not consistent with the urinary cultures.

Figure 3.14: Participant 14 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

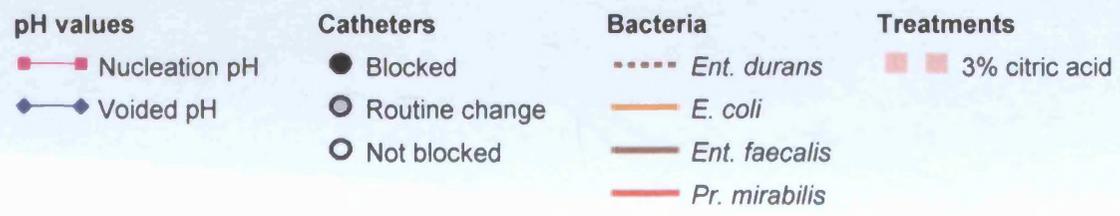
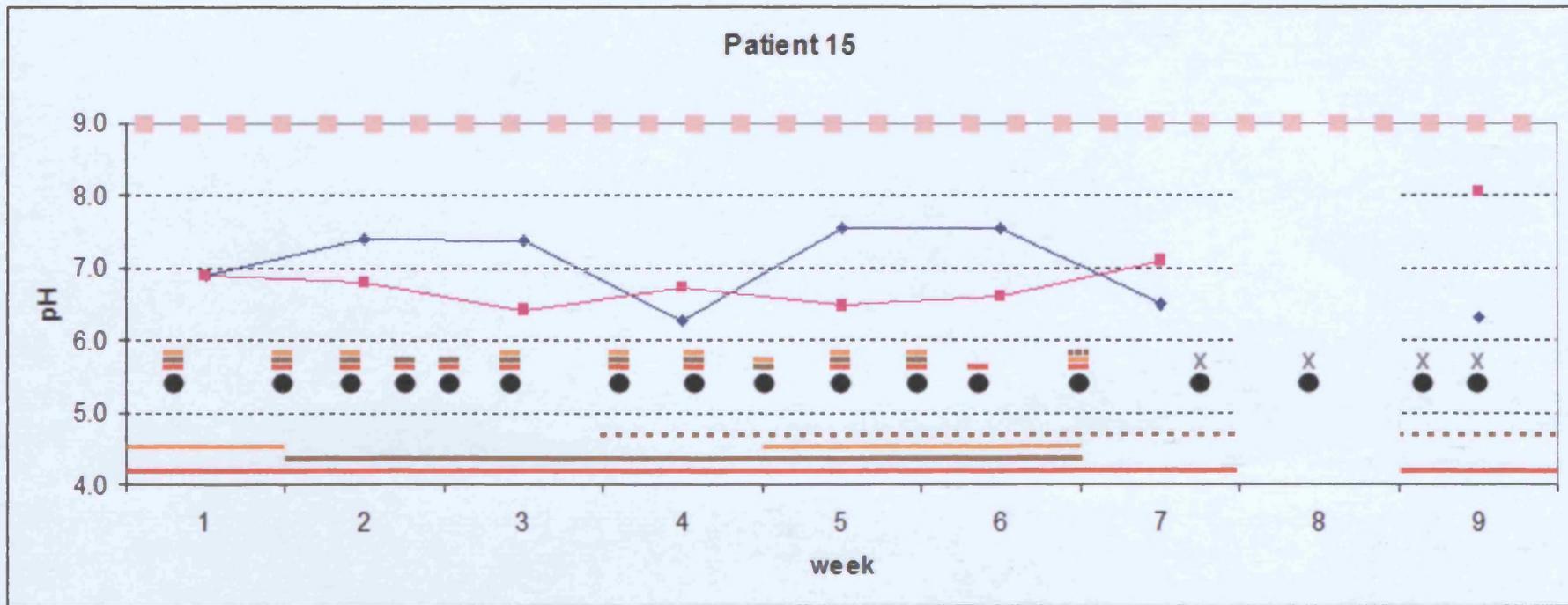
Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *E. coli*
- *Ps. aeruginosa*
- *Ent. faecalis*
- *Pr. mirabilis*

Figure 3.15: Participant 15 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



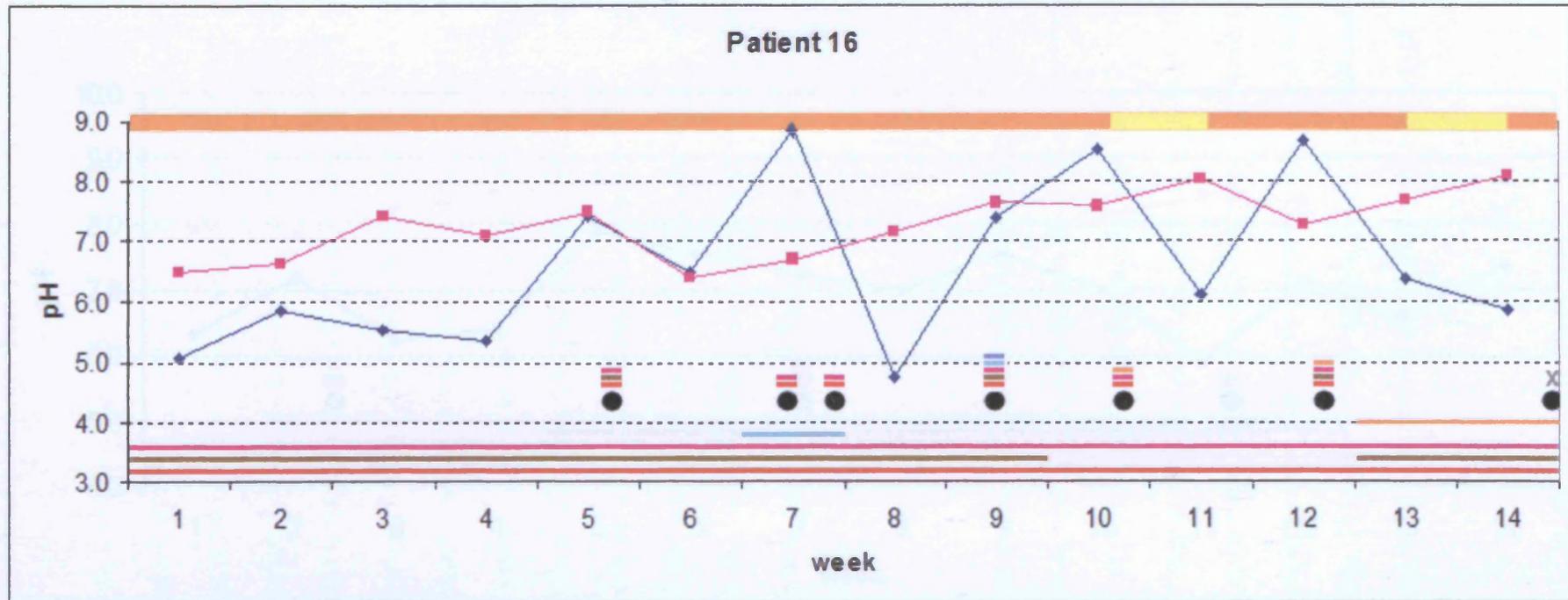
Participant 16

Participant 16 was a male living in his own home, monitored for 14 weeks (figure 3.16). He persistently had urinary colonisation with *Pr. mirabilis* and *Ps. aeruginosa*, with *Ent. faecalis* present except for weeks 10 through 12. There was occasional growth of *E. coli* and *M. morganii*. He was taking low dose nitrofurantoin throughout the study, and therapeutic doses of ciprofloxacin in weeks 11 and 14. Although pH_v was, on average, slightly lower than pH_n , it varied considerably around it. Catheters blocked frequently and were heavily encrusted down much of their length. *Pr. mirabilis* and *Ps. aeruginosa* were consistently isolated. *E. coli* was seen on the catheters before appearing in the urine, but *Ent. faecalis* was inconsistent. *Morganella* was not present on the catheters when present in the urine, but was isolated from the next catheter in week 9.

Participant 17

Participant 17 was a female living in her own home, monitored for 14 weeks (figure 3.17). She persistently had urinary colonisation with *Pr. mirabilis*, *Ps. aeruginosa* and *Ent. faecalis*, with *Ent. durans* and a non-swarming strain of *Pr. mirabilis* present during the middle part of the study, and *E. coli* appearing in the final week. She had a pH_v which was consistently lower than her pH_n by a comfortable margin. Catheters were changed routinely every month, and were moderately encrusted. *Pr. mirabilis* and *Ent. faecalis* were isolated from all catheters. *Ps. aeruginosa* was absent from the first catheter. *Ent. durans* was isolated from the catheter removed in week 7, but not in week 11, shortly before it disappeared from the urine.

Figure 3.16: Participant 16 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

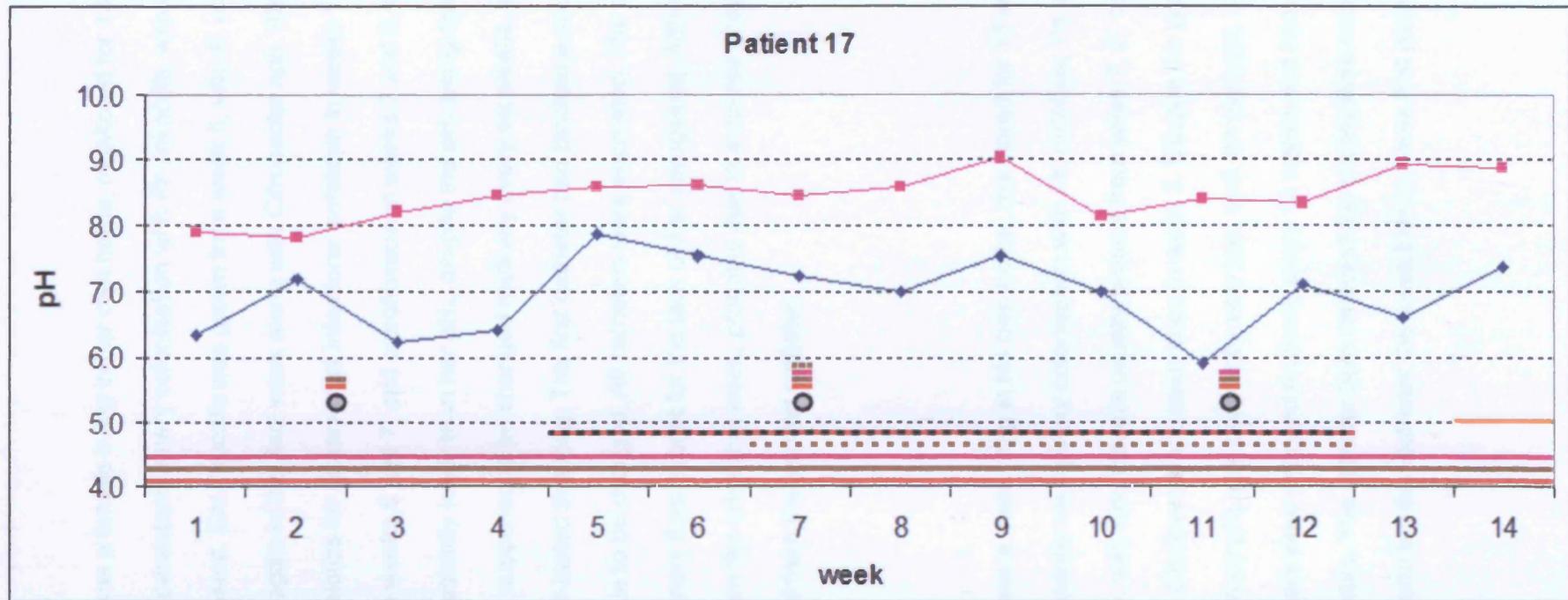
Bacteria

- *Staph. aureus*
- *E. coli*
- *M. morgani*
- *Ps. aeruginosa*
- *Ent. faecalis*
- *Pr. mirabilis*

Treatments

- Nitrofurantoin
- Ciprofloxacin

Figure 3.17: Participant 17 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *E. coli*
- .- *non swarming Pr. mirabilis*
- ... *Ent. durans*
- *Ps. aeruginosa*
- *Ent. faecalis*
- *Pr. mirabilis*

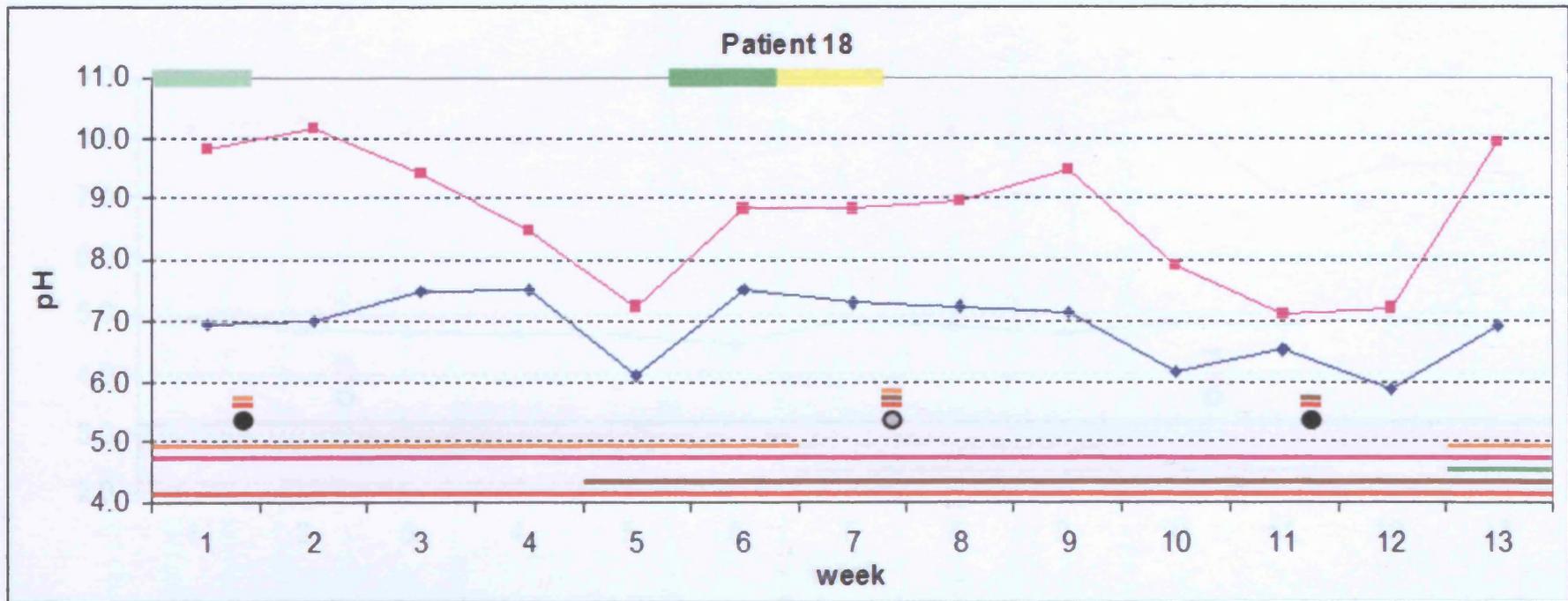
Participant 18

Participant 18 was a female living in her own home, monitored for 13 weeks (figure 3.18). She had persistent urinary colonisation with *Pr. mirabilis*, with *Ps. aeruginosa* usually also present. *Ent. faecalis* was grown from week 5, with *E. coli* being seen up to week 6, and again in the final week along with *Citrobacter spp.* She had several courses of antibiotics for urinary tract infections: cefalexin in weeks 1 and 2, clarithromycin in weeks 6 and 7, and ciprofloxacin in weeks 7 and 8. She had a pH_v which was consistently lower than her pH_n , despite the pH_n being quite variable. Catheters were supposed to be changed routinely every six weeks, although only the second catheter lasted this long. The first catheter had blocked a couple of days before it was due to be changed. All catheters were encrusted. The catheter flora reflected the urinary flora except for the lack of *Ps. aeruginosa*. Although it had disappeared from the urine that week, probably due to a course of antibiotics, *E. coli* was still isolated from the second catheter.

Participant 19

Participant 19 was a male living in his own home, monitored for 13 weeks (figure 3.19). He persistently had urinary colonisation with *Pr. mirabilis*, *Ps. aeruginosa* and *Kl. pneumoniae*, with *Ent. faecalis* usually present from week 7, *E. coli* appearing commonly, and *Citrobacter spp.* seen once in week 3. Despite the presence of *Pr. mirabilis* the urinary pH_v was constantly very low, and, on average, 3 pH points below the pH_n . Catheters were changed routinely every 12 weeks and had almost no encrustation visible. The catheter flora reflected the urinary flora except for *E. coli* being isolated from the first catheter the week before it was first isolated from the urine.

Figure 3.18: Participant 18 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

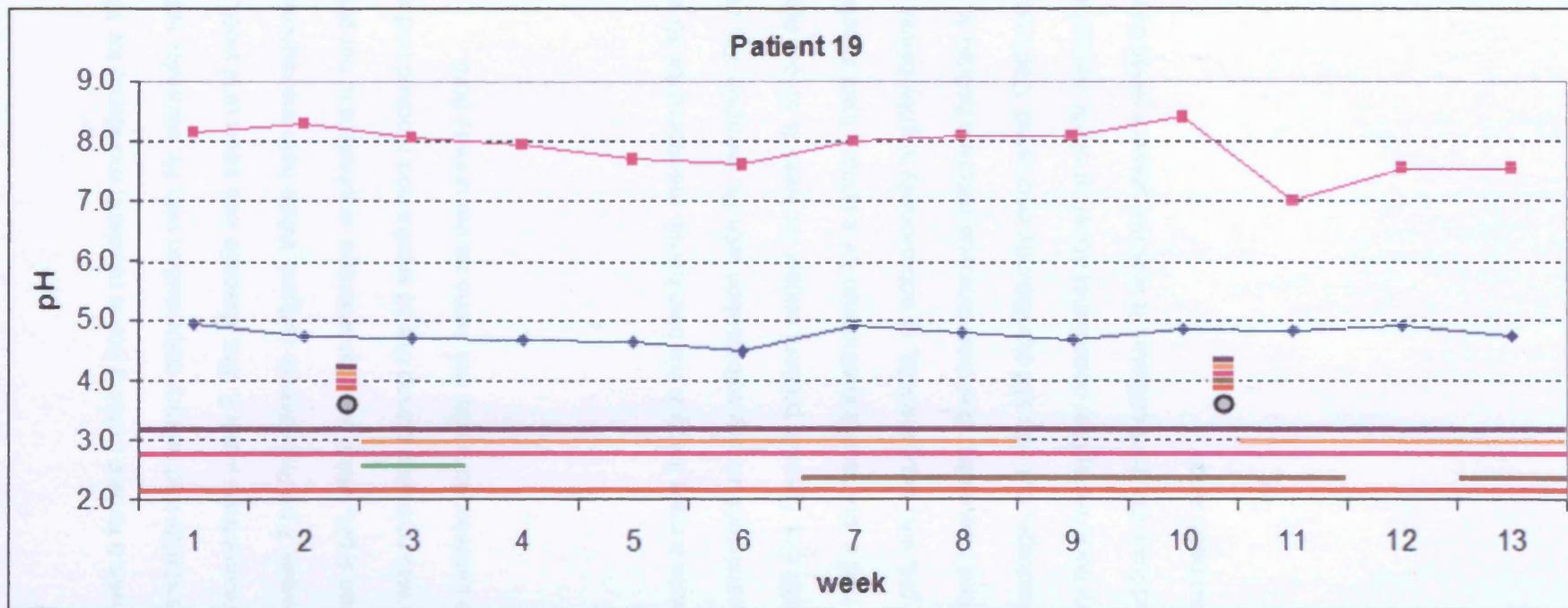
Bacteria

- *E. coli*
- *Ps. aeruginosa*
- *Citrobacter spp*
- *Ent. faecalis*
- *Pr. mirabilis*

Treatments

- Cefalexin
- Clarithromycin
- Ciprofloxacin

Figure 3.19: Participant 19 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



pH values

- Nucleation pH
- ◆ Voided pH

Catheters

- Blocked
- Routine change
- Not blocked

Bacteria

- *Kl. pneumoniae*
- *E. coli*
- *Ps. aeruginosa*
- *Citrobacter spp*
- *Ent. faecalis*
- *Pr. mirabilis*

Participant 20

Participant 20 was a female nursing home resident, monitored for 13 weeks (figure 3.20). She persistently had urinary colonisation with *Pr. mirabilis*, with *Kl. pneumoniae* present from week 2. *Ent. faecalis* was seen until week 6, being replaced from week 7 by *Providencia rettgeri*. While both measurements were quite variable, she had a pH_v which was, on average, equivalent to her pH_n . The only catheter which was removed during the 13 weeks had blocked and was heavily encrusted. The catheter flora was the same as the urinary flora.

Participant 21

Participant 21 was a male living in his own home, monitored for 16 weeks (figure 3.21). He had persistent urinary colonisation with *Pr. mirabilis*, *Ps. aeruginosa* and *Kl. pneumoniae*, with *Ent. faecalis* present except for week 8. *E. coli* appeared in the final week. He had a course of trimethoprim for a urinary tract infection in weeks 12 to 13. His urinary pH_v was equivalent to, or occasionally slightly below, his pH_n . Catheters blocked frequently. One was removed before a holiday to prevent an inconvenient blockage, but was still significantly encrusted. Catheter flora usually reflected urinary flora, except for occasional absence of *Ps. aeruginosa*. *Ent. faecalis* was not isolated from the third catheter. It was not present when this catheter was inserted, but had been since.

Figure 3.20: Participant 20 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.

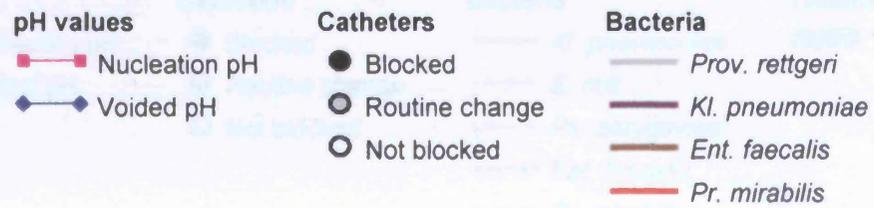
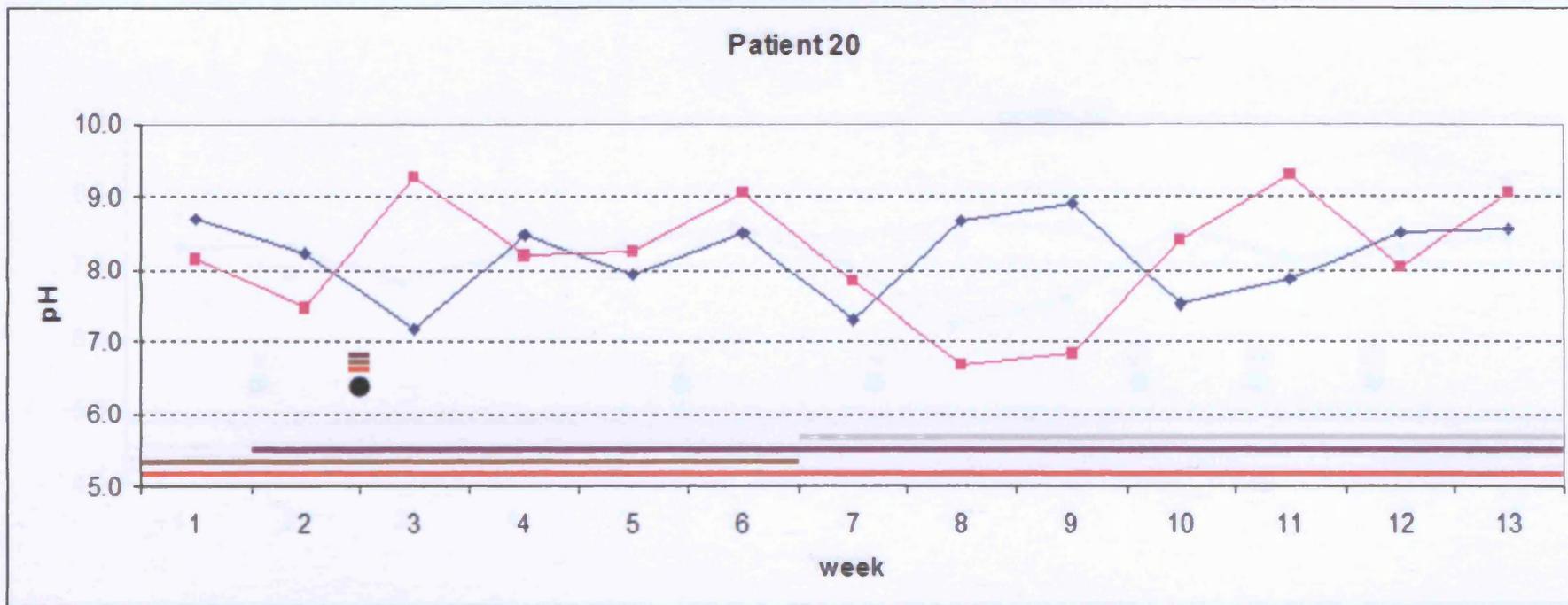
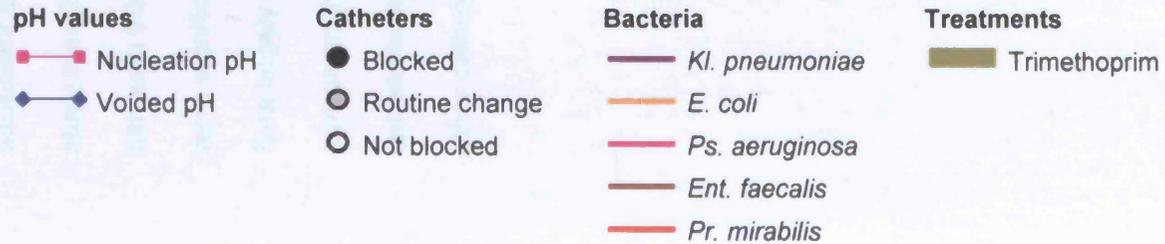
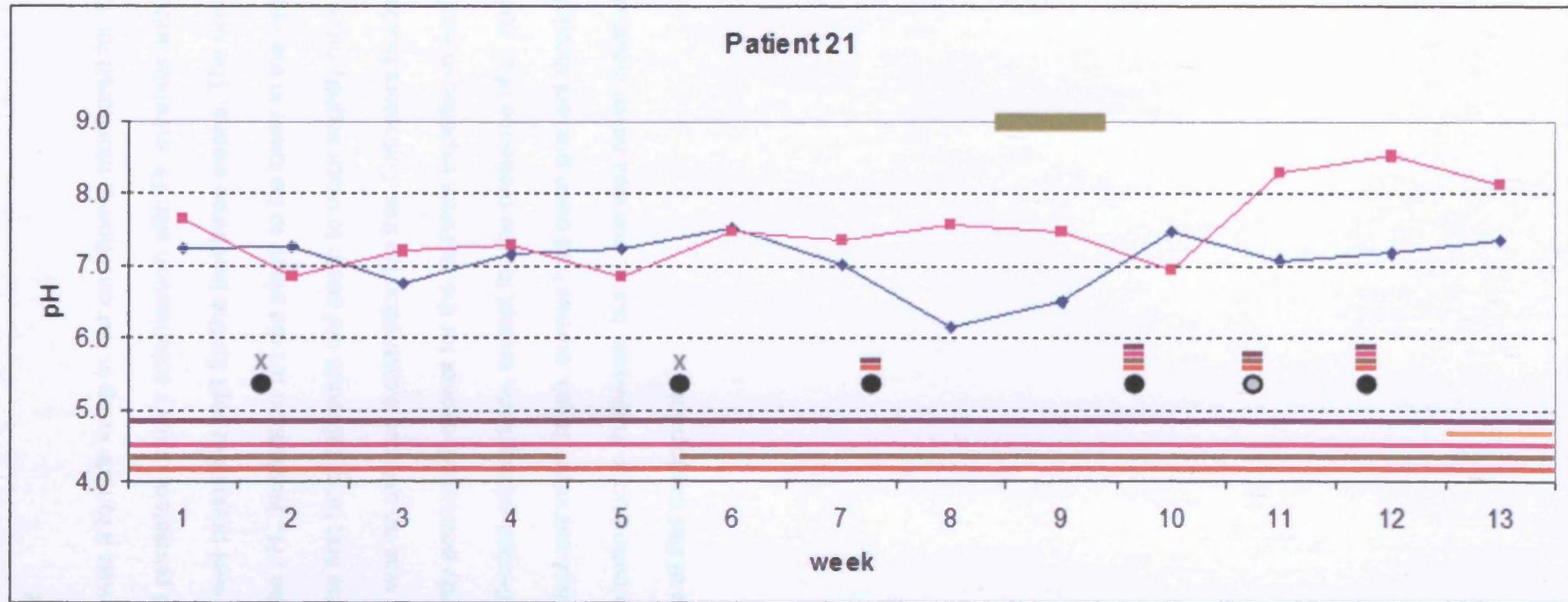


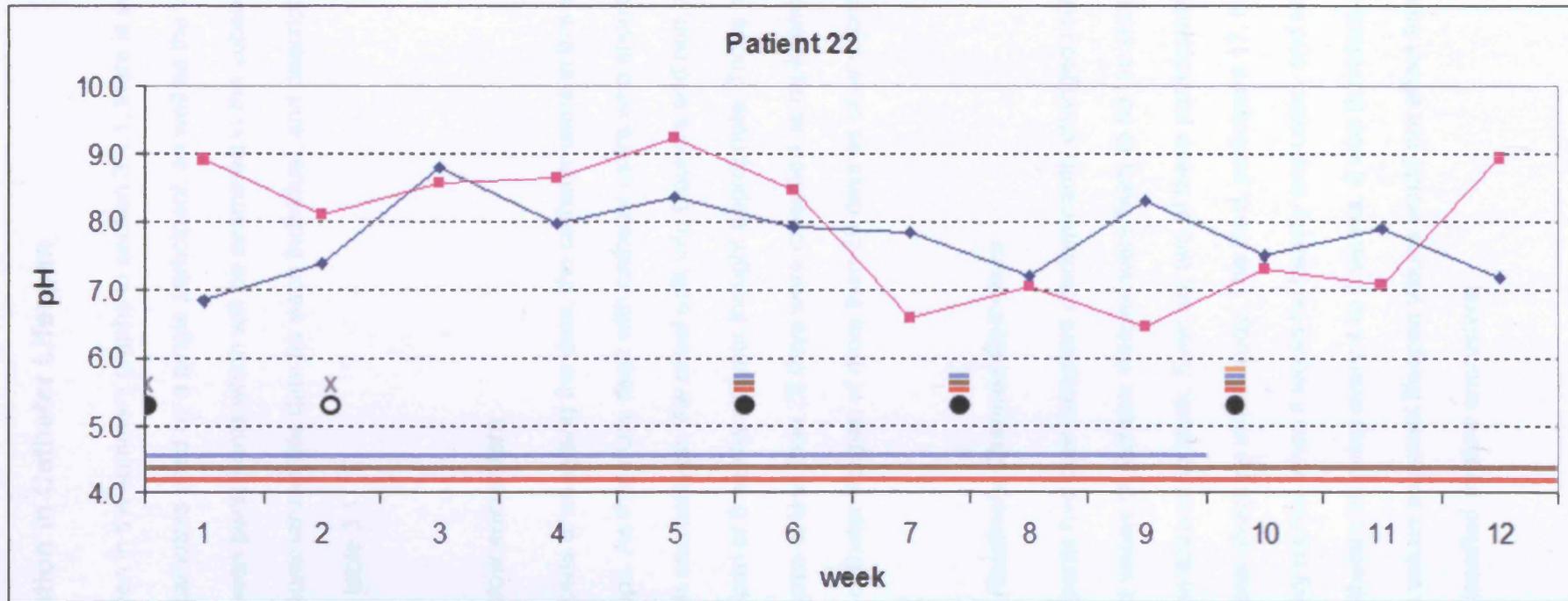
Figure 3.21: Participant 21 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



Participant 22

Participant 22 was a female living in her own home, monitored for 12 weeks (figure 3.22). She had persistent urinary colonisation with *Pr. mirabilis* and *Ent. faecalis*. *Staph. aureus* was present except for the last three weeks. The urinary pH_v was equivalent to the pH_n. Nucleation pH did seem to be lower in the second half of the study than in the first (and catheters did seem to block slightly more frequently) although there was no obvious explanation for this. Catheters blocked frequently and were significantly encrusted, except for the catheter expelled in week 2. Catheter flora largely reflected urinary flora, except for the presence of *E. coli* and *Staph. aureus* on the last catheter. *Staph. aureus* had been present throughout the catheterisation period up to that week, but *E. coli* was never isolated from the urine or other catheters of this participant.

Figure 3.22: Participant 22 - treatments, nucleation pH, voided pH, catheter changes and bacterial isolates over the course of the study period.



3.1.2 Variation in Catheter Lifespan

As can be seen in the summary graphs in section 3.1.1, there is some variation in the lifespans of catheters used by a single participant, as well as the variation in catheter lifespan between participants which will be examined in the subsequent section.

Figure 3.23 summarises this data for each individual, and descriptive statistics are presented in table 3.1.

Rapid and slow encrusters

For the purposes of analysing the data, the catheter users in this study were divided into two groups. As this study dealt with catheter users who should all have had problems with encrustation, the usual split into blockers and non-blockers based on a catheter lifespan of 6 weeks was not thought appropriate. Those having a mean catheter lifespan of less than 28 days were classified as *rapid encrusters*, and those with a mean catheter lifespan of more than 28 days as *slow encrusters*.

Analysis of Routinely Changed Catheters

Three participants had their catheters predominantly changed routinely, which meant that recorded values of catheter lifespan were likely to be too short compared to if they had been allowed to block. Even so, two of these participants had the two longest catheter lifespans in the study. The third, participant 17, had her catheter changed every month, when it would be heavily encrusted, and she had a historical pattern of catheters blocking every 4 to 5 weeks. It was therefore assumed that using the recorded values as actual lifespan values would not affect analysis of the data. All three were classified as slow encrusters.

Figure 3.23: Mean catheter lifespan for each study participant with error bars representing one standard deviation. Column coloured white shows an individual from whom only one suitable catheter was removed. Striped columns indicate those individuals whose catheters were usually changed routinely.

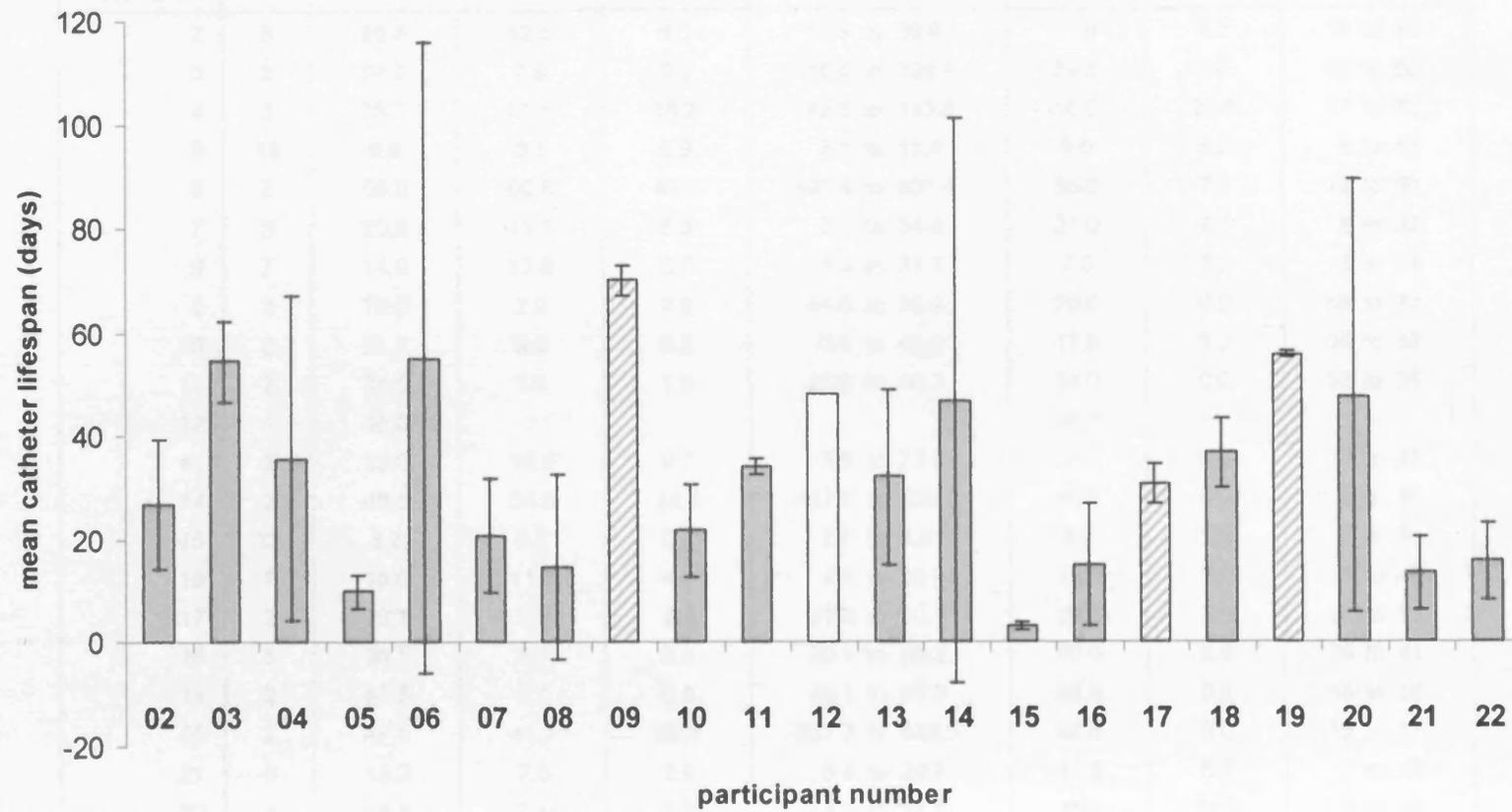


Table 3.2: Descriptive statistics representing the variability in catheter lifespan, measured in days, for each study participant (n - number of catheters, SD - standard deviation, SE - standard error, CI - confidence interval, IQR - interquartile range).

<i>participant</i>	<i>n</i>	<i>mean</i>	<i>SD</i>	<i>SE</i>	<i>95% CI of mean</i>	<i>median</i>	<i>IQR</i>	<i>range</i>
2	6	26.8	12.5	5.1	13.8 to 39.9	21.0	7.3	15 to 45
3	2	54.5	7.8	5.5	-15.4 to 124.4	54.5	0.0	49 to 60
4	3	35.7	31.5	18.2	-42.5 to 113.8	18.0	27.5	17 to 72
5	15	9.9	3.3	0.9	8.1 to 11.8	9.0	5.5	6 to 16
6	2	55.0	60.8	43.0	-491.4 to 601.4	55.0	0.0	12 to 98
7	5	20.8	11.1	5.0	7.0 to 34.6	21.0	7.0	8 to 38
8	7	14.6	17.8	6.7	-1.9 to 31.1	7.0	5.0	3 to 54
9	2	70.0	2.8	2.0	44.6 to 95.4	70.0	0.0	68 to 72
10	3	21.7	9.0	5.2	-0.6 to 43.9	17.0	8.0	16 to 32
11	2	34.0	1.4	1.0	21.3 to 46.7	34.0	0.0	33 to 35
12	1	48.0	-	-	-	48.0	-	-
13	3	32.0	16.8	9.7	-9.8 to 73.8	38.0	16.0	13 to 45
14	2	46.5	54.5	38.5	-442.7 to 535.7	46.5	0.0	8 to 85
15	13	3.2	0.7	0.2	2.7 to 3.6	3.0	1.0	2 to 4
16	7	15.0	11.8	4.4	4.1 to 25.9	12.0	7.0	5 to 40
17	3	30.7	3.8	2.2	21.3 to 40.1	29.0	3.5	28 to 35
18	3	36.7	6.7	3.8	20.1 to 53.2	40.0	6.0	29 to 41
19	2	55.5	0.7	0.5	49.1 to 61.9	55.5	0.0	55 to 56
20	2	47.5	41.7	29.5	-327.3 to 422.3	47.5	0.0	18 to 77
21	6	13.3	7.0	2.9	6.0 to 20.7	11.5	5.5	7 to 25
22	4	15.8	7.4	3.7	4.0 to 27.5	15.0	12.3	8 to 25

3.2 Urinary Constituents and Encrustation

3.2.1 Variability in voided pH and nucleation pH

The values of mean pH_v for each participant are normally distributed (Shapiro-Wilk test $P=0.868$) with values as shown in table 3.3. It can be seen that an infection with a urease producing organism does not inevitably produce a high urinary pH. Of the 21 study participants, 12 had a mean pH_v of less than 7.0, and 4 had a mean pH_v of less than 6.5. In 5 cases, the value of their mean pH_v plus one standard deviation was still only neutral, and 2 participants never recorded an alkaline pH_v value. It can be seen that voided pH is variable between individuals even with *Pr. mirabilis* infections.

The spread of mean values of pH_n also follows a normal distribution (Shapiro-Wilk test $P=0.860$) as shown in table 3.3. Again, the mean value for nucleation pH is clearly seen to vary between individuals, with some particularly low or high readings. Overall, the spread of values is less than for pH_v .

It is clear from a casual observation of the individual graphs for each study participant that there is considerable variability in the weekly readings of both pH_v and pH_n . Figure 3.25 graphically represents the spread of these variables in each study participant.

Table 3.3: Distribution of mean values of pH_v and pH_n of study participants (SD - standard deviation, SE - standard error, IQR - interquartile range)

	<i>mean</i>	<i>SD</i>	<i>SE</i>	<i>median</i>	<i>range</i>	<i>IQR</i>
<i>mean pH_v</i>	7.01	0.92	0.20	6.98	4.77 - 8.98	6.58 - 7.65
<i>mean pH_n</i>	7.73	0.61	0.13	7.59	6.57 - 8.96	7.44 - 7.97

Note on Statistics

It should also be noted that participant 03 did not have *Proteus* colonisation, while participant 11 only had *Proteus* for a short period during the trail. Excluding values for these individuals made no difference to the analysis of the study results, with the results remaining statistically significant or not with or without their data. The results shown here are using the data from all participants. A comparison of the statistical results with and without participants 03 and 11 included can be found in Appendix 2.

Differences in pH_v and pH_n

The independent samples t test was used to see if there was a difference in mean pH_v or pH_n between rapid and slow encrusters (tables 3.5 and 3.6). It was found that the rapid encrusters had a higher mean pH_v than the others (7.20 compared with 6.26), but this difference was not statistically significant (P=0.385). The difference was not significant even when the mean pH_v values pre and post *Pr. mirabilis* infection for participant 08 were treated separately. The lower pH_n in the rapid encrusters (7.32 compared with 8.03) was highly significant, however (P=0.004).

The safety margin

From figure 3.25 it can be seen that for some individuals the ranges of pH_v and pH_n overlap considerably, whereas for others there is a considerable difference between the means and little overlap of the one standard deviation distributions. It can be hypothesised that those in the first group, where their urinary pH remains close to the pH at which crystals will form, will suffer more severely than those in the second group whose pH_v usually remains below their pH_n . The difference between these values was calculated for each urine sample and termed the *safety margin*, such that:

$$\text{safety margin} = pH_n - pH_v$$

The mean safety margins for each participant are shown in figure 3.24. Theoretically, in those cases where crystal precipitation would be occurring, when pH_v is higher than pH_n , the safety margin would be negative. The more positive the value, the less likely crystallisation would be to occur. From observing the graphs of individual participants in section 3.1.1, and from previously published work [148], it seems that encrustation occurs when pH_v is close to pH_n , not just when it is above it. In other words, when the safety margin is small, as well as when it is negative.

This is likely to represent variability of pH_n and pH_v around the values recorded, so that safety margins will become negative at some time, allowing crystal precipitation, with a frequency inversely proportional to the value of the mean safety margin.

The mean safety margins of those study participants who on average block their catheters within 28 days, and those with slower encrustation whose catheters on average lasted more than 28 days, were compared using the independent samples t test (table 3.4). Those catheter users who have the most rapid encrustation, the 28

day blockers, have a safety margin significantly lower than those who block their catheters less frequently ($P=0.003$).

This can be seen in figures 3.25 and 3.24, where participants 05, 10 and 15 are examples of rapid catheter encrusters, and participants 06, 09 and 19 are examples of slow encrusters. Participant 12 is an exception to this pattern as, despite the results shown, he was not a rapid encruster. This is discussed in section 4.1.1.

Figure 3.24: Mean safety margin for each study participant.

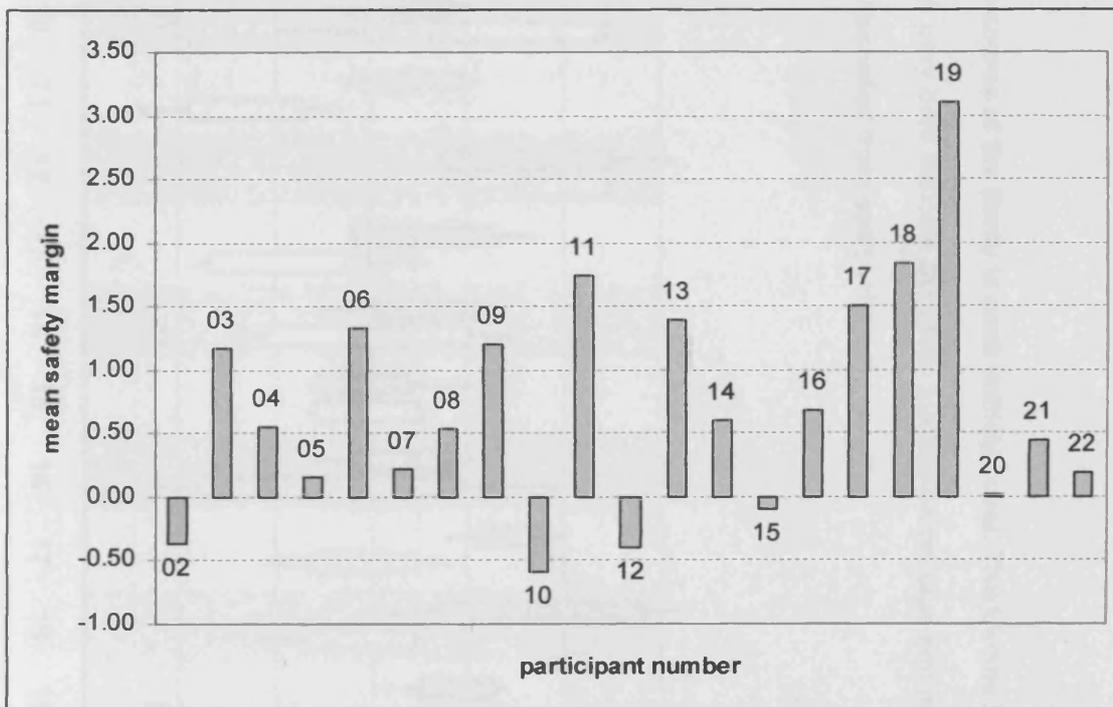
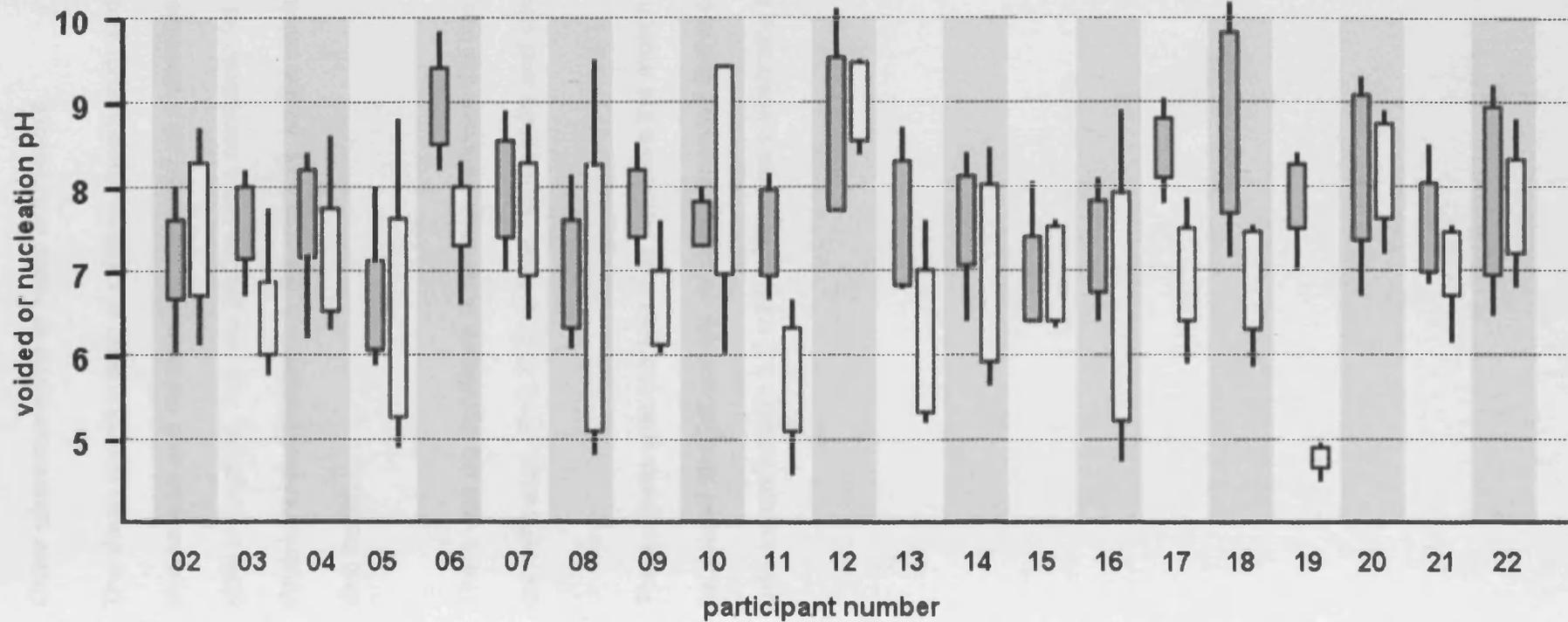


Figure 3.25: Variability of voided pH and nucleation pH over the course of the study in each catheter user. Thick white bars indicate the mean voided pH plus and minus one sample standard deviation. Thick grey bars indicate the mean nucleation pH plus and minus one sample standard deviation. The thin black bars indicate the total range of values recorded from each category.



Other characteristics of rapid encrusters

The mean concentration of calcium, magnesium and the summed concentration of magnesium and calcium was analysed as a possible difference between rapid and slow encrusters. The two groups were compared for these factors using the Mann-Whitney U test (tables 3.7, 3.8 and 3.9), as the calcium data was not normally distributed.

There was no significant difference between the two groups in terms of magnesium concentration ($P=0.464$) or total magnesium and calcium concentration ($P=0.129$).

The subjects who blocked their catheters the most frequently did, however, have an increased level of urinary calcium compared to the other participants (a mean of 7.6 mM compared with 3.9 mM) which was statistically significant ($P=0.007$).

Table 3.4: t test - comparison of mean safety margin (pH units) between those catheter users with a mean catheter lifespan greater than and less than 28 days

	<i>catheter < 28 days</i>	<i>catheter > 28 days</i>
Mean safety margin	0.13	1.17
Variance	0.17	0.84
Observations	9	12
t Stat	3.487	
2-tailed p	0.003	
t Critical	2.120	

Table 3.5: t test - comparison of mean voided pH between those catheter users with a mean catheter lifespan greater than and less than 28 days

	<i>catheter < 28 days</i>	<i>catheter > 28 days</i>
Mean pH _v	7.20	6.86
Variance	0.36	1.23
Observations	9	12
t Stat	0.890	
2-tailed p	0.385	
t Critical	2.101	

Table 3.6: t test - comparison of mean nucleation pH between those catheter users with a mean catheter lifespan greater than and less than 28 days

	<i>catheter < 28 days</i>	<i>catheter > 28 days</i>
Mean pH _n	7.32	8.03
Variance	0.23	0.28
Observations	9	12
t Stat	3.265	
2-tailed p	0.004	
t Critical	2.101	

Table 3.7: Mann-Whitney U test - comparison of mean urinary calcium concentration (mM) between those catheter users with a mean catheter lifespan greater than and less than 28 days

	n	21		
[Ca] by catheter lifespan	n	Rank sum	Mean rank	U
<i>catheter < 28 days</i>	9	136.0	15.11	17.0
<i>catheter > 28 days</i>	12	95.0	7.92	91.0
Difference between medians	3.09			
95.1% CI	0.79 to 5.94			
Mann-Whitney U statistic	17			
2-tailed p	0.007			

Table 3.8: Mann-Whitney U test - comparison of mean urinary magnesium concentration (mM) between those catheter users with a mean catheter lifespan greater than and less than 28 days

	n	21		
[Mg] by catheter lifespan	n	Rank sum	Mean rank	U
<i>catheter < 28 days</i>	9	110.0	12.22	43.0
<i>catheter > 28 days</i>	12	121.0	10.08	65.0
Difference between medians	1.40			
95.1% CI	-2.46 to 4.97			
Mann-Whitney U statistic	43			
2-tailed p	0.464			

Table 3.9: Mann-Whitney U test - comparison of mean total calcium and magnesium concentration (mM) between those catheter users with a mean catheter lifespan greater than and less than 28 days

n	21			
[Ca + Mg] by catheter lifespan	n	Rank sum	Mean rank	U
<i>catheter < 28 days</i>	9	121.0	13.44	32.0
<i>catheter > 28 days</i>	12	110.0	9.17	76.0
Difference between medians	3.88			
95.1% CI	-1.35 to 10.10			
Mann-Whitney U statistic	32			
2-tailed p	0.129			

Regression analysis

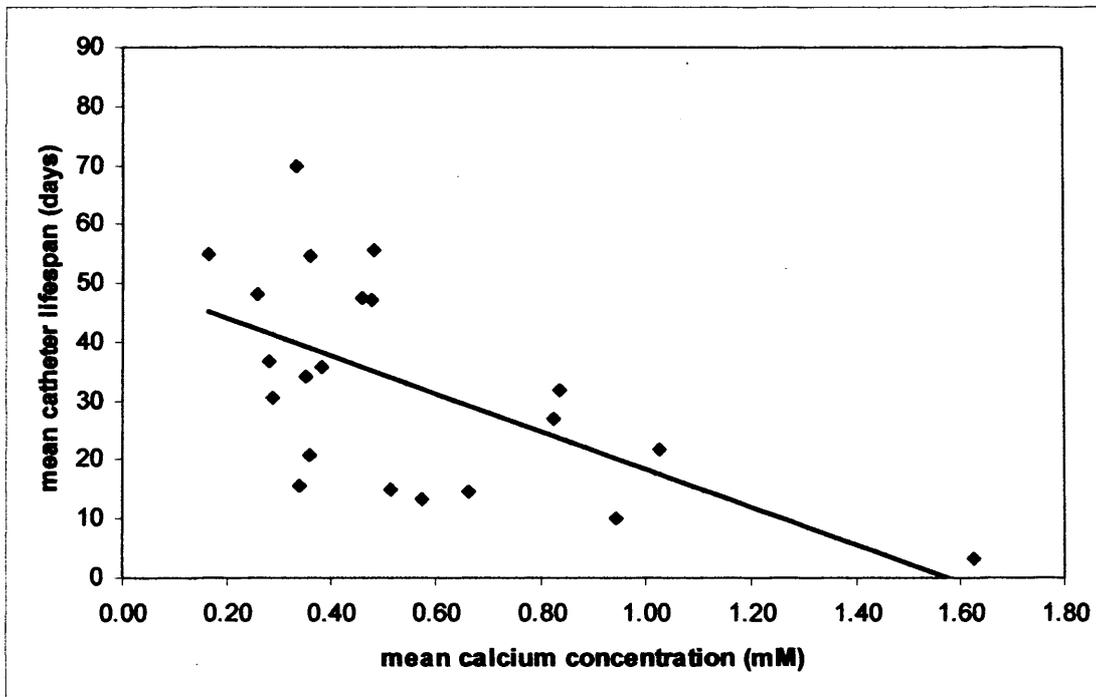
As well as differences between the two groups of rapid and slow encrusters, calculating the Pearson correlation coefficient r showed a significant negative correlation between mean catheter lifespan and mean urinary calcium ($r = -0.601$, $P=0.002$), and a significant positive correlation with mean nucleation pH ($r = 0.562$, $P=0.004$) and mean safety margin ($r = 0.465$, $P=0.017$). Scatter plots showing the calculated regression lines are seen in figures 3.26, 3.27 and 3.28.

Although these regression lines are certainly significant, calculating the coefficient of determination r^2 showed that there remains considerable variation in catheter lifespan not predicted by independent analysis of any one of these variables. As these variables can all, to some extent, be thought of as determining each other, it is not appropriate to calculate a predictive model for all of them using multiple linear regression.

Conclusion

The statistically significant characteristics of those catheter users who suffer the most from catheter encrustation, as defined by having a mean catheter lifespan of less than 28 days, are that they have a higher urinary calcium concentration, a lower nucleation pH, and a smaller gap between their nucleation and voided pH than those whose catheters last longer.

Figure 3.26: Linear regression to illustrate correlation between mean catheter lifespan and mean urinary calcium



Pearson correlation coefficient $r = -0.601$

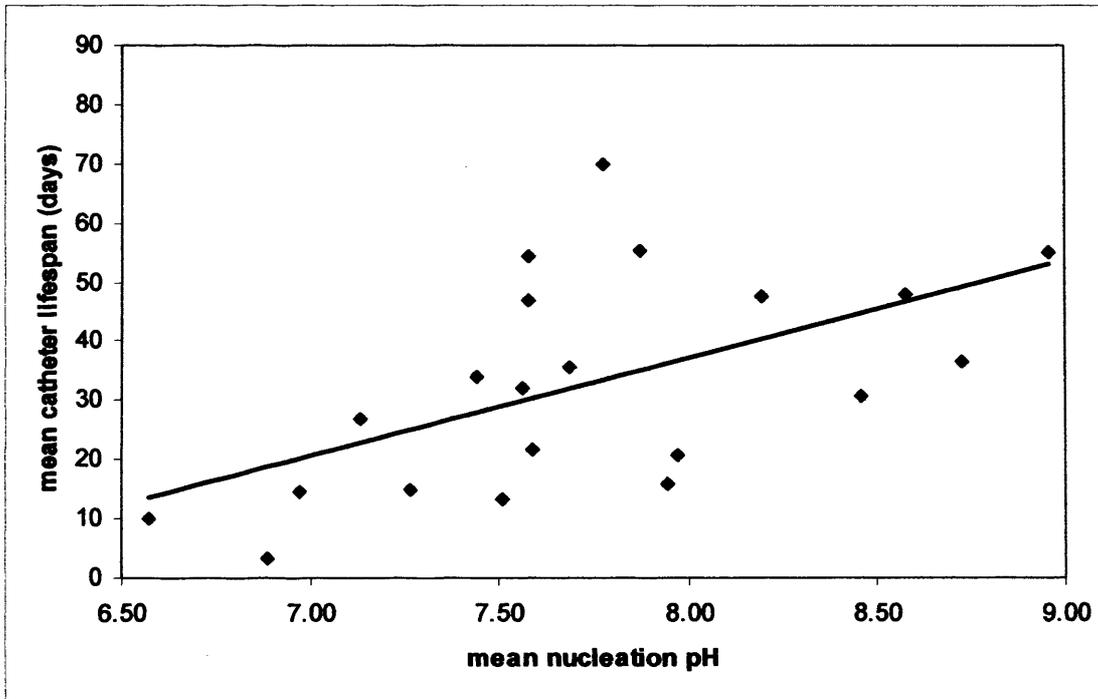
Coefficient of determination $r^2 = 0.361$

Regression equation: catheter lifespan = $50.2 - 31.8 [\text{Ca}]$

$P = 0.002$

Linear regression demonstrates a highly significant negative correlation between mean catheter lifespan and mean calcium concentration

Figure 3.27: Linear regression to illustrate correlation between mean catheter lifespan and mean urinary nucleation pH



Pearson correlation coefficient $r = 0.562$

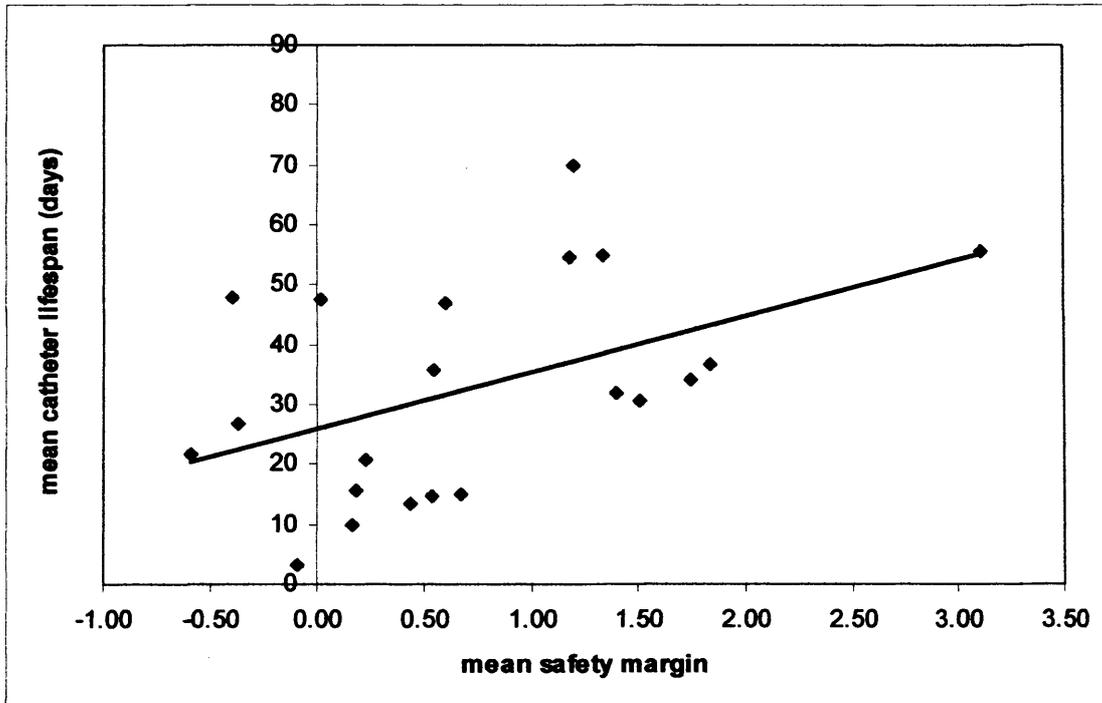
Coefficient of determination $r^2 = 0.315$

Regression equation: catheter lifespan = $16.6 \text{ pH}_n - 95.4$

$P = 0.004$

Linear regression demonstrates a highly significant positive correlation between mean catheter lifespan and mean urinary nucleation pH

Figure 3.28: Linear regression to illustrate correlation between mean catheter lifespan and mean urinary safety margin



Pearson correlation coefficient $r = 0.465$

Coefficient of determination $r^2 = 0.217$

Regression equation: catheter lifespan = 9.37 safety margin + 26.0

$P = 0.017$

Linear regression demonstrates a significant positive correlation between mean catheter lifespan and mean safety margin

3.2.2 Catheter Encrustation Rate

An attempt was made to measure the rate at which encrustation occurs on urinary catheters, as opposed to simply measuring the catheter lifespan. When a catheter was removed the amount of encrustation was calculated by measuring the mass of magnesium and calcium present in the urinary drainage channel of the catheter. To derive the encrustation rate, this was divided by the number of days the catheter had been in place. This would hopefully be able to give more useful data than simply the catheter lifespan, as some catheters were removed routinely before they could block, and others were removed accidentally or because of bladder spasm mimicking blockage. This would lead to an inaccurately short measured value of catheter lifespan. By calculating the encrustation rate in this way, catheters which had not blocked should still yield useful data on the severity of crystallisation.

The encrustation rate data for each participant is summarised in table 3.10.

Participant 12 is not included as their only catheter was lost. As can be seen from the figures in the table, there is considerable variability in the results for each participant. In all but an insignificant number of cases each participant continued to use the same catheter size, type, and manufacturer throughout the study, so this does not explain the occurrence of the variability.

The mean encrustation rate was used to differentiate rapid from slow encrusters as an alternative to mean catheter lifespan. An arbitrary cut off value of 0.7 mg/day was chosen, as this fell in a natural gap in the results and obtained a roughly equal split.

The participants falling into the category of rapid encruster under both cut off policies are detailed in table 3.11.

Table 3.10: Encrustation rate (mg calcium and magnesium deposited per day) of catheters in the study, shown as minimum, maximum, mean and sample standard deviation values for each study participant. If only the mean value is shown, there was only one catheter analysed.

<i>participant</i>	<i>minimum</i>	<i>maximum</i>	<i>mean</i>	<i>stand dev</i>
02	0.40	1.83	0.86	0.59
03	-	-	0.003	-
04	0.11	0.21	0.16	0.08
05	1.02	3.16	1.94	0.80
06	0.01	0.45	0.23	0.31
07	0.06	0.70	0.38	0.45
08	0.38	3.18	1.84	1.08
09	0.01	0.08	0.04	0.05
10	0.27	0.83	0.55	0.40
11	0.021	0.022	0.02	0.00
13	0.28	1.59	0.93	0.92
14	-	-	0.99	-
15	0.45	6.55	1.60	1.79
16	0.22	9.49	3.60	3.22
17	0.02	0.16	0.11	0.08
18	0.01	0.06	0.04	0.03
19	0.001	0.002	0.002	0.000
20	-	-	1.29	-
21	1.21	2.56	1.84	0.56
22	1.08	2.29	1.39	0.60

Table 3.11: List of those participants characterised as rapid encrusters using cut off values of mean catheter life < 28 days or mean encrustation rate > 0.7 mg/day

<i>mean catheter life < 28 days</i>	participants 02, 05, 07, 08, 10, 15, 16, 21, 22
<i>mean encrustation rate > 0.7 mg/day</i>	participants 02, 05, 08, 13, 14, 15, 16, 21, 22

The independent samples t tests and Mann-Whitney U tests detailed in section 3.2.1 were repeated to determine whether differences in mean safety margin, pH_v , pH_n , urinary calcium, magnesium, and summed calcium and magnesium concentrations were significant between the two groups divided by encrustation rate. In most cases, the same results were obtained as when using a mean catheter lifespan of 28 days to divide the participants into two groups. The only exception is the comparison of mean safety margin, where the difference is statistically significant using catheter lifespan ($P=0.003$) but not when using encrustation rate ($P=0.062$). These results are detailed in Appendix 3.

3.2.3 Catheter Specific Safety Margin

An attempt was made to increase the accuracy of the calculation of the safety margin, the difference between voided and nucleation pH. It has been shown that pH_v is variable within most individuals, and that pH_n is variable in some, thus causing a week to week variation in safety margin. This may lead to some catheters being exposed to a higher safety margin than others, even within one individual, and so may explain the variation in catheter lifespan seen. This is most obviously demonstrated by participant 08 (figure 3.8), whose pH_v increased following acquisition of *Pr. mirabilis*, leading to a reduction in safety margin and catheter lifespan, but it may be a factor in other participants.

The mean safety margin to which a catheter was exposed was calculated to determine a *catheter specific safety margin* (CSSM) for each catheter. This was then correlated against the lifespan of that catheter to give the results shown in figure 3.29. This demonstrates a highly significant positive correlation between CSSM and the lifespans of individual catheters. The value of r of 0.551 for this model is slightly

greater than for the model of mean safety margin predicting mean catheter lifespan ($r = 0.465$).

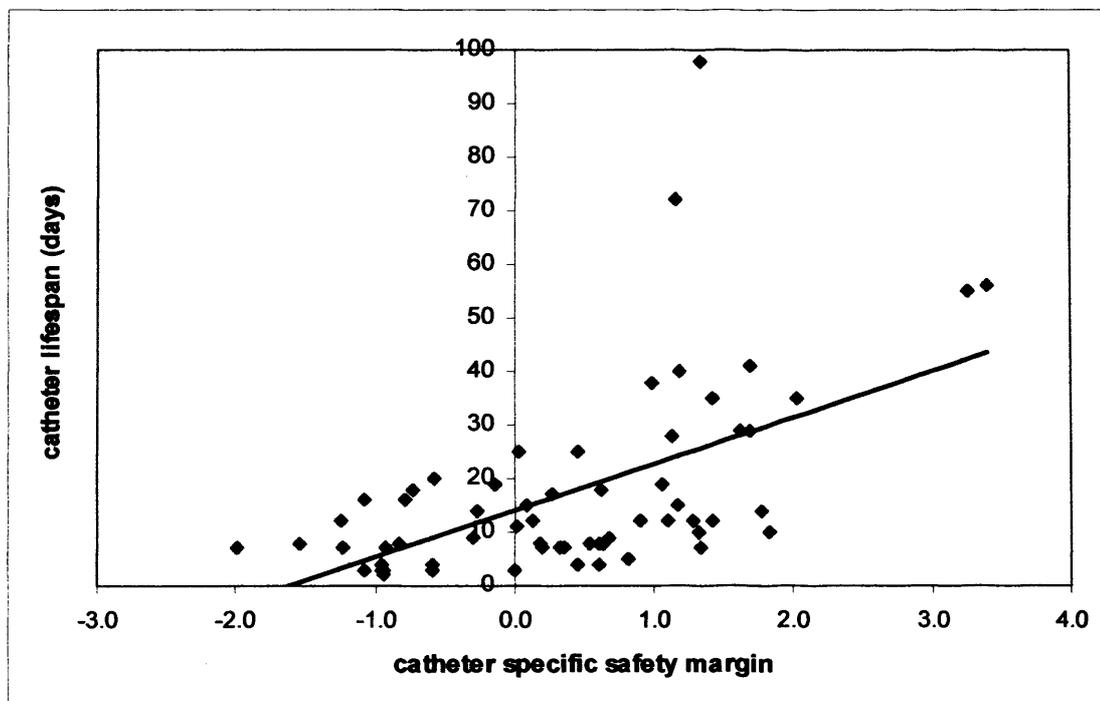
3.2.4 Investigating the 'Blocking pH'

The concept of the blocking pH of a catheter user is based on the idea that the pH of their voided urine will increase the longer the catheter is left in place, as the encrustation builds up. Once it reaches a certain pH, this is a sign that the catheter will block imminently. This can then be used clinically to predict catheter blockages, and to change the catheter before this happens. It is possible that each individual may have a different blocking pH.

Theoretically, a rise in pH_v as the catheter remains in place could be caused by the increasing numbers of urease producing organisms in a growing biofilm. Catheter blockage above a certain pH_v level could be explained if encrustation only occurs, or is much more rapid, once the biofilm generates this pH_v level in the urine. In this case, blocking pH would be related to nucleation pH. Another explanation is that crystallisation occurs steadily in a localised high pH environment in the biofilm, but only once the biofilm has grown to contain a certain number of urease positive organisms does it affect the pH of the voided urine as a whole. This may coincide with a crystalline biofilm of sufficient size to occlude the catheter lumen.

Although this theory has been raised in several discussions with District Nursing staff, and is apparently supported by enough anecdotal evidence for it to be implemented as a method of catheter management in parts of the Bristol region with what seems like some success, there is little evidence to support it.

Figure 3.29: Linear regression to illustrate correlation between catheter specific safety margin and individual catheter lifespans



Pearson correlation coefficient $r = 0.551$

Coefficient of determination $r^2 = 0.304$

Regression equation: individual catheter lifespan = $8.74 \text{ CSSM} + 14.1$

$P < 0.001$

Linear regression demonstrates a highly significant positive correlation between catheter specific safety margin and individual catheter lifespans.

Being able to accurately predict catheter blockages would be extremely useful. The collected data on pH variability was analysed for evidence of such a trend. Casual observation of the pH_v data for individual study participants in section 3.1.1 did not immediately suggest a trend of rising values throughout the life of the catheter. If the theory were true, the data would at least show higher pH_v at the end of each catheter's lifespan than at the start. For the theory to be applied in practice to an individual catheter user, the mean start pH_v value should be lower than their mean end pH_v value. There should also be a corresponding fall in pH_v after the blocked catheter has been changed. Data points at the start and end of each catheter life were therefore analysed.

Dates of catheter blockage and weekly voided pH readings were recorded. The pH_v readings at the start and end of each catheter's lifespan were taken, and the readings compared using the related samples t test. The same was done for those readings immediately before and after the date of each catheter blockage

Pairs of pH_v readings were only used for catheters which had definitely blocked, not those that had been changed routinely or for some other reason. Readings were also excluded if they could not be described as a true start and end samples. A reading had to be in the first or last third of a catheter life to be included. For example, if the pre blockage reading was taken 5 days before the catheter blocked, but that catheter had only lasted a week, it would more accurately be described as the post blockage pH_v for the preceding catheter. Similarly, pairs of readings were excluded if the post blockage pH_v reading happened to be taken towards the end of the life of the next catheter. For this reason, most pairs of readings from individuals with very short catheter lifespans were excluded.

The data from the catheters which fit these criteria shows no significant rise in pH_v between the start and end of a catheter's lifespan (34 catheters, $P=0.071$), or a significant lowering of pH_v after the blocked catheter is replaced (36 catheters, $P=0.072$). The results are shown in tables 3.12 and 3.14.

The results are significant, however, and of a greater magnitude, in those study participants with the heaviest levels of encrustation, the 28 day blockers. The data shows a significant difference in pH_v between the start and end of a catheter's lifespan (21 catheters, $P=0.002$), and a fall in pH_v when the catheter is replaced (23 catheters, $P=0.029$). The results are shown in tables 3.13 and 3.15.

In all cases, however, even when the changes are statistically significant, they are small in magnitude (table 3.16). Observation of the data for the individual catheter users shows that this is not due to a few individuals with no observable pH_v changes dragging down the mean scores to these low levels. There is no subset of study participants with a greater pH_v change.

Table 3.12: t test - comparison of pH_v at the start and end of a catheter's lifespan in all blocked catheters

	<i>pH_v start</i>	<i>pH_v end</i>
Mean	6.84	7.23
Variance	0.98	0.97
Observations	34	34
t Stat	1.868	
2-tailed p	0.071	
t Critical	2.035	

Table 3.13: t test - comparison of pH_v at the start and end of a catheter's lifespan in catheters from 28 day blockers

	<i>pH_v start</i>	<i>pH_v end</i>
Mean	6.73	7.54
Variance	0.99	0.94
Observations	21	21
t Stat	3.513	
2-tailed p	0.002	
t Critical	2.086	

Table 3.14: t test - comparison of pH_v before and after catheter blockage in all blocked catheters

	<i>pH_v before</i>	<i>pH_v after</i>
Mean	7.35	7.00
Variance	0.77	0.62
Observations	36	36
t Stat	1.856	
2-tailed p	0.072	
t Critical	2.030	

Table 3.15: t test - comparison of pH_v before and after catheter blockage in catheters from 28 day blockers

	<i>pH_v before</i>	<i>pH_v after</i>
Mean	7.44	6.91
Variance	0.83	0.63
Observations	23	23
t Stat	2.339	
2-tailed p	0.029	
t Critical	2.074	

Table 3.16: Mean magnitude of pH_v changes during a catheter lifespan

<i>Data set</i>	<i>mean pH_v change</i>	<i>standard deviation</i>
pH change during catheter lifespan for all catheters	0.39	1.12
pH change after catheter blockage for all catheters	-0.35	1.12
pH change during catheter lifespan for 28 day blockers	0.81	1.06
pH change after catheter blockage for 28 day blockers	-0.53	1.08

3.3 Factors Influencing Nucleation pH

It is clear that the nucleation pH is a major factor determining the extent of encrustation suffered by a catheter user with urinary tract colonisation by a urease positive organism. Urinary calcium and magnesium concentrations were measured and related to the values of the nucleation pH found in urine samples.

3.3.1 Simple Linear Regression

Calculating the Pearson correlation coefficient r showed a highly significant negative correlation between mean pH_n and mean urinary calcium ($r = -0.704$, $P < 0.01$), magnesium ($r = -0.517$, $P < 0.01$) and total calcium and magnesium ($r = -0.658$, $P < 0.01$).

Calculating the coefficient of determination r^2 for the correlation between calcium and pH_n showed that almost half ($r^2 = 0.495$) of the variability in pH_n can be explained by changes in urinary calcium concentration alone, more than for magnesium or summed calcium and magnesium concentrations when considered alone.

The degree to which changes in calcium or magnesium affect pH_n is shown by the regression equation, which follows the pattern for an equation of a straight line:

$$y = c + mx$$

Where y is the dependent variable on the y axis, x is the independent variable on the x axis, c is the intercept of the straight line on the y axis, and m is the gradient of the line. In this case y is pH_n , and x is $[\text{Ca}]$, $[\text{Mg}]$ or $[\text{Ca} + \text{Mg}]$. In statistical terms, the

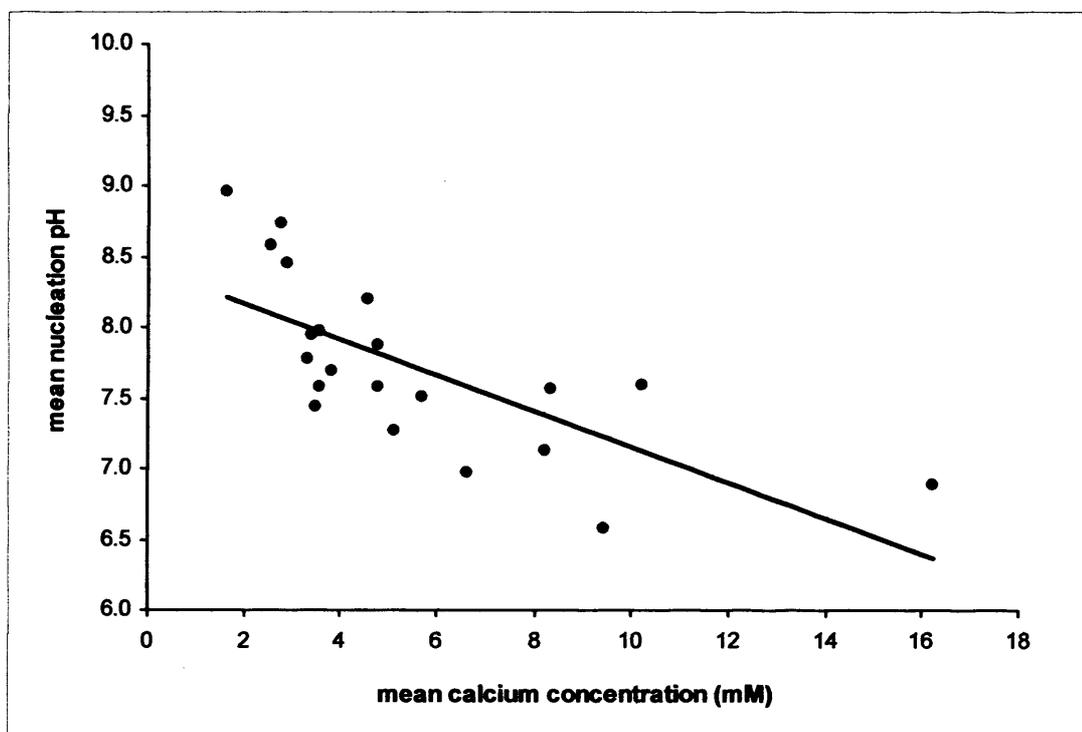
intercept is known as the *constant*. The gradient term represents the magnitude of the effect of the independent variable on pH_n , and is known as the *B coefficient*.

The values of the B coefficient predicted by the regressions equations are -1.26 for calcium and -0.85 for magnesium, showing calcium having a greater individual effect.

Scatter plots showing the regression are seen in figures 3.30, 3.31 and 3.32.

In summary, both mean urinary calcium and magnesium concentrations are significant individual predictors of pH_n , with changes in calcium producing more marked changes in pH_n than changes in magnesium.

Figure 3.30: Linear regression to illustrate correlation between mean urinary calcium concentration and mean urinary nucleation pH



Pearson correlation coefficient $r = -0.704$

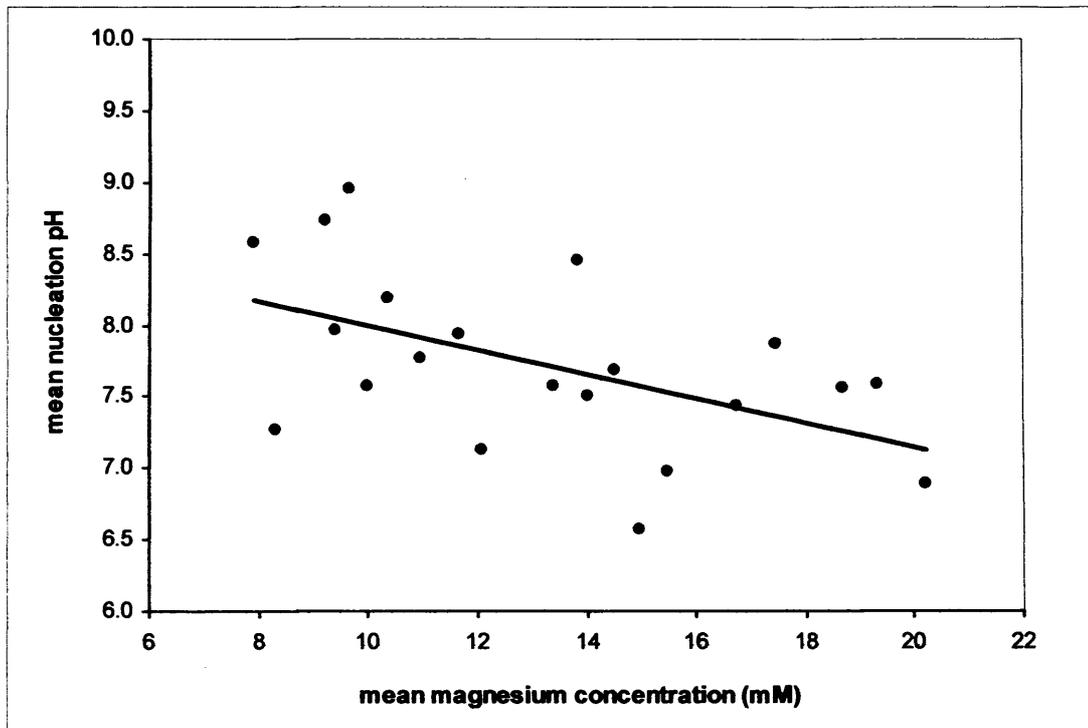
Coefficient of determination $r^2 = 0.495$

Regression equation: $\text{pH}_n = 8.42 - 0.126 [\text{Ca}]$

$P = 0.000$

Linear regression demonstrates a highly significant negative correlation between mean calcium concentration and mean pH_n

Figure 3.31: Linear regression to illustrate correlation between mean urinary magnesium concentration and mean urinary nucleation pH



Pearson correlation coefficient $r = -0.517$

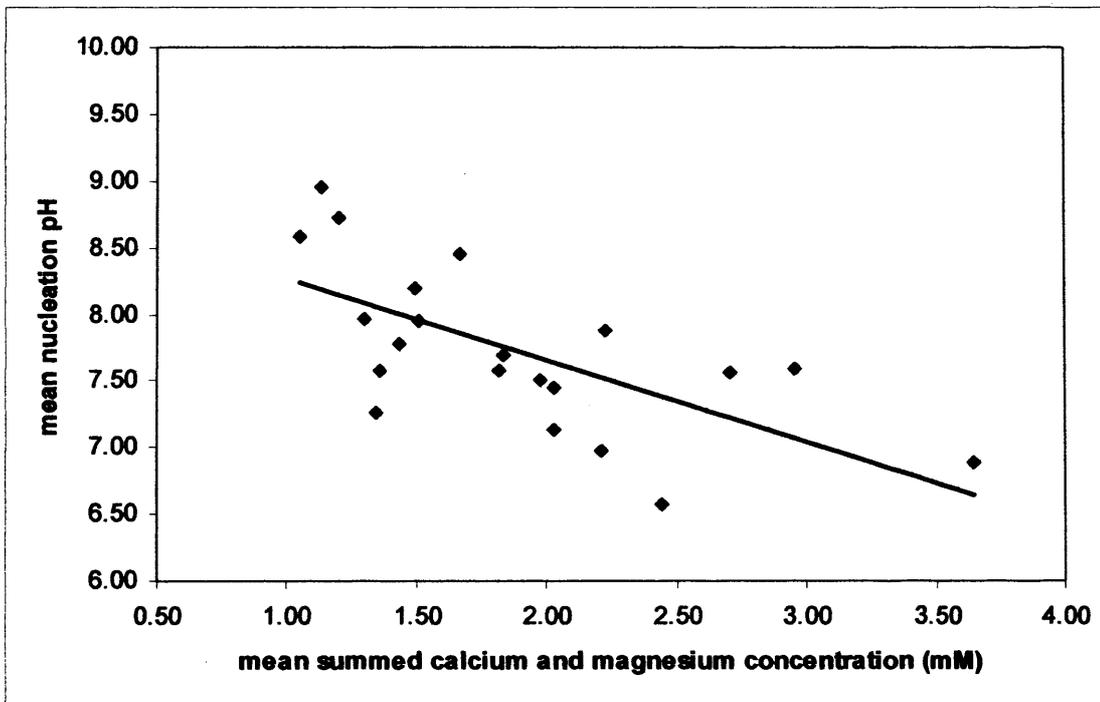
Coefficient of determination $r^2 = 0.267$

Regression equation: $\text{pH}_n = 8.85 - 0.085 [\text{Mg}]$

$P = 0.008$

Linear regression demonstrates a highly significant negative correlation between mean magnesium concentration and mean pH_n

Figure 3.32: Linear regression to illustrate correlation between the sum of mean urinary calcium and magnesium concentrations and mean urinary nucleation pH



Pearson correlation coefficient $r = -0.658$

Coefficient of determination $r^2 = 0.432$

Regression equation: $pH_n = 8.87 - 0.61 [Ca + Mg]$

$P = 0.001$

Linear regression demonstrates a highly significant negative correlation between the sum of mean calcium and magnesium concentrations and mean pH_n

3.3.2 Multiple Linear Regression

In order to improve on the predictive power of mean urinary calcium concentration alone, a multiple linear regression model was computed to determine the combined effects of both calcium and magnesium concentration on pH_n . These models produce equations of the form:

$$y = c + m_1x_1 + m_2x_2 \dots m_nx_n$$

Changes in multiple independent variables ($x_1, x_2 \dots x_n$) contribute in differing amounts ($m_1, m_2 \dots m_n$) to changes in the dependent variable (y). Obviously, equations of this complexity cannot be plotted graphically.

Additive Multiple Regression Model

A model was constructed in the above form using both mean urinary calcium [Ca] and magnesium [Mg] concentrations to predict values of pH_n (table 3.17)

[Mg] was not found to be a significant predictor of pH_n , allowing for the explanation of the variation in pH_n already predicted by the [Ca] variable. In other words, the P value of 0.831 for the [Mg] term indicates that there is no significant evidence that [Mg] should be included in this model as a predictor for pH_n , when [Ca] is also included as a predictor.

The conclusion is that the most appropriate model of those considered so far is the model with just [Ca] as a predictor, as used in Section 3.3.1.

Multiple Regression Model with Interaction

Additive models assume that the effects of changes in one variable are unaffected by the absolute level of the other variable. In other words, changes in calcium concentration would produce the same change in pH_n irrespective of the value of magnesium concentration. This is a good model of most systems.

To examine the theory that the effect of one variable might change depending on the value of the other, an interaction term $[\text{Ca}] * [\text{Mg}]$ was calculated, as the product of calcium and magnesium concentration (table 3.18)

In this model, all of the terms are highly significant: for the variable Ca, $P < 0.001$, for the variable Mg, $P = 0.003$, and for the interaction term $[\text{Ca}] * [\text{Mg}]$, $P < 0.001$.

The linear regression prediction equation is:

$$\text{pH}_n = 10.55 - 0.606 [\text{Ca}] - 0.130 [\text{Mg}] + 0.027 [\text{Ca}] * [\text{Mg}]$$

For the model with interaction $r = 0.875$, and so is higher than the r of 0.704 for the model with just $[\text{Ca}]$ as a predictor (section 3.3.1), indicating that the model with interaction provides the best fit for the data.

This model shows that changing the value of either calcium or magnesium concentration has less effect if the value of the other variable is already high.

Table 3.17: Multiple linear regression - a model of the effect of mean urinary calcium and magnesium concentrations on nucleation pH

R	0.705
R ²	0.496
Adjusted R ²	0.440
SE	0.4588

Term	B coefficient	SE	p	95% CI of Coefficient
<i>Intercept</i>	8.4922	0.3962	<0.0001	7.6599 to 9.3246
<i>[Ca]</i>	-0.1199	0.0419	0.0104	-0.2079 to -0.0319
<i>[Mg]</i>	-0.0083	0.0382	0.8314	-0.0886 to 0.0721

Table 3.18: Multiple linear regression - a model of the effect of mean urinary calcium and magnesium concentrations on nucleation pH with an interaction term ([Ca]*[Mg]) included

R	0.875
R ²	0.766
Adjusted R ²	0.725
SE	0.3216

Term	B coefficient	SE	p	95% CI of Coefficient
<i>Intercept</i>	10.5482	0.5408	<0.0001	9.4073 to 11.6891
<i>[Ca]</i>	-0.6064	0.1137	<0.0001	-0.8462 to -0.3666
<i>[Mg]</i>	-0.1303	0.0384	0.0035	-0.2113 to -0.0492
<i>[Ca]*[Mg]</i>	0.0275	0.0062	0.0004	0.0144 to 0.0405

3.4 Urease Activity

The ability of the urease enzyme to hydrolyse urea to produce ammonia is the driving force behind catheter encrustation. This reaction produces both the ammonium ions necessary to form struvite, and the required high pH. It was decided to investigate whether differences in urease activity between strains of *Proteus* isolated from different catheter users were significant, and if so, whether they were responsible for the differing levels of encrustation seen in catheter users.

Table 3.19: Mean urease activity of isolated urinary *Proteus* strains from three replicated assays.

<i>Participant</i>	<i>Strain number</i>	<i>Urease Activity ($\mu\text{mol urea hydrolysed / mg protein / minute}$)</i>
02	SWB001	0.38
04	SWB016	0.44
05	SWB017	0.35
06	SWB037	0.60
07	SWB023	1.04
09	SWB041	0.39
10	SWB046	0.74
11	SWB053	0.30
12	SWB056	0.51
13	SWB049	0.43
14	SWB083	0.07
15	SWB085	0.34
16	SWB089	0.28
17	SWB093	0.33
18	SWB098	0.30
19	SWB102	0.36
20	SWB108	0.36
21	SWB112	0.42
control	urease +ve	0.46
control	urease -ve	0.06

Table 3.19 shows the mean urease activities of the various *Proteus* strains isolated from the trial participants, as compared to a wild type urease positive *Pr. mirabilis* and a urease negative mutant as controls. Strains isolated from participants 08 and 22 were unavailable to test as they had been inadequately stored, and *Proteus* was not isolated from participant 03.

Two values stand out in this list. Strain SWB083 from participant 14 with an activity of 0.07 μmol urea hydrolysed / mg protein / minute is effectively urease negative.

Although this participant had a long catheter lifespan, their mean pH_v was 6.98, only slightly below average for the study. Strain SWB023, a *Pr. vulgaris* from participant 07, has a mean urease activity of 1.04 μmol urea hydrolysed / mg protein / minute which is considerably above average (3 standard deviations). This participant was a regular catheter blocker, but although they had an above average pH_v of 7.66 this was only the fifth highest in the sample group. Extremes of urease activity do not seem to be associated with extremes of pH_v as might be expected, and for this reason it does not seem reasonable to use this as a simple explanation for the behaviour of their catheters.

There is no significant difference ($P=0.659$) between the distribution of urease activities of those strains from catheter users with a mean catheter lifespan of less than 28 days and those whose catheters last longer when compared using the Mann-Whitney U test (table 3.20). The highest urease activities are not found significantly more often in the most rapid encrusters. In fact, without the outlying values of strains SWB023 and SWB083, the mean urease activities of the *catheter < 28 days* and *catheter > 28 days* groups are almost identical at 0.42 and 0.40 μmol urea hydrolysed / mg protein / minute respectively.

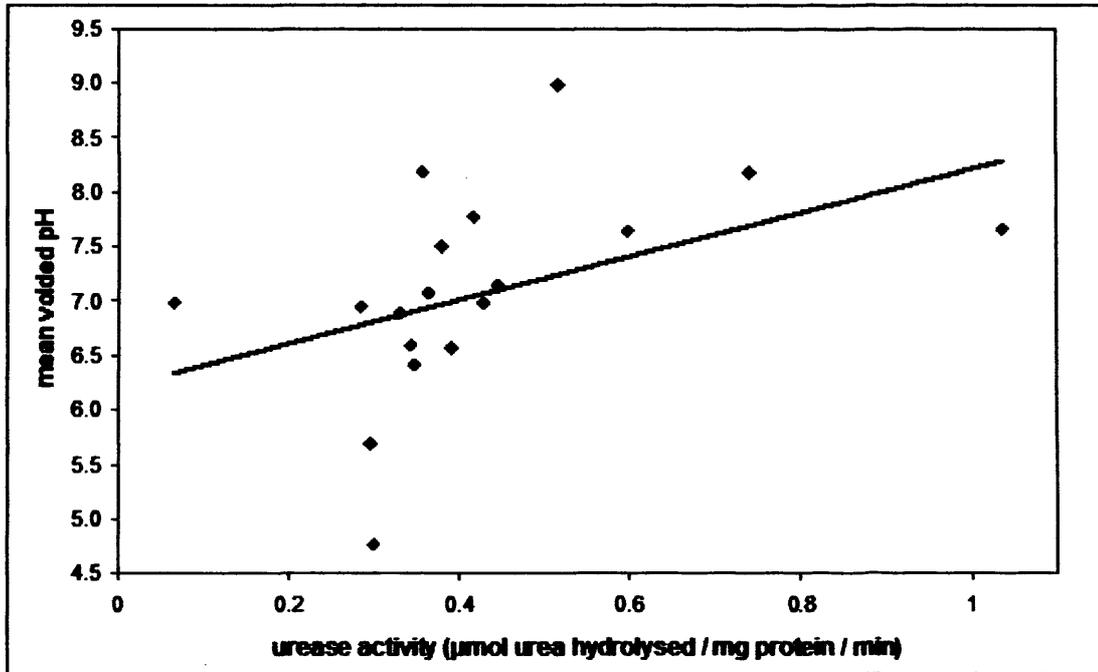
Table 3.20: Mann-Whitney U test - comparison of mean *Proteus* urease activity (μmol urea hydrolysed / mg protein / minute) between those catheter users with a mean catheter lifespan greater than and less than 28 days.

n 18				
urease by catheter lifespan	n	Rank sum	Mean rank	U
<i>catheter < 28 days</i>	7	72.0	10.29	33.0
<i>catheter > 28 days</i>	12	99.0	9.00	44.0
Difference between medians	0.042			
95.5% CI	-0.082 to 0.375			
Mann-Whitney U statistic	33			
2-tailed p	0.659			

There is also no significant correlation between urease activity and mean pH_v ($P = 0.093$), as shown in figure 3.33.

In conclusion, the differences in urease activity between different strains of *Proteus* are small, and the differences in catheter lifespan between catheter users with *Proteus* infections cannot be explained by the differences in urease activity of the strains they are infected with.

Figure 3.33: A scatter plot to illustrate the correlation between the mean urinary voided pH of an individual and the urease activity of their colonising *Proteus* strain



Pearson correlation coefficient $r = 0.408$

Coefficient of determination $r^2 = 0.166$

$P = 0.093$

Pearson's correlation demonstrates a significant positive correlation between urease activity and mean pH,

3.5 Behaviour of Bacterial Populations

3.5.1 The Persistence of *Proteus* Urinary Tract Colonisation

Urinary tract colonisation with *Proteus* seems to be persistent. In the 20 participants with a *Proteus* infection (19 cases of *Pr. mirabilis* and one of *Pr. vulgaris*) it was present constantly throughout the study, in urine and on catheters, in 17 cases. It was unaffected by seven out of the eight courses of antibiotics given to the participants in its presence. In participant 03 *Proteus* was not present at all, despite being isolated from the screening urine.

In one of these cases, participant 08, the *Pr. mirabilis* colonisation occurred in week 7 of the trial, but was then present in every urine sample and on all catheters until the end of the study 9 weeks later.

There were two other cases where *Pr. mirabilis* was not persistent. In participant 06 it was not present in the final urine sample of the trial, taken immediately following a course of amoxicillin, an antibiotic to which it was resistant. It was still isolated from the catheter removed during this course of antibiotics. This participant died from the respiratory infection the antibiotics were being used to treat, so it is not known if *Pr. mirabilis* would have been seen in any subsequent samples. In participant 11, the organism was only isolated from urine in weeks 5 and 6, and not from the catheter retrieved in week 5. The subject was only followed up for a further three weeks, but the organism did not reappear.

3.5.2 Frequency of Co-infections Associated with *Proteus*

Polymicrobial cultures were universally isolated from the urine samples collected during the study period. Table 3.21 shows the frequency of co-infection with other organisms in those 19 individuals with persistent *Proteus* colonisation. Participants 03 and 11 have been excluded.

The most common organism other than *Pr. mirabilis* was *Ent. faecalis*. Also commonly present were *E. coli* and *Ps. aeruginosa*. *Pseudomonas* colonisation appeared to be persistent, but *E. coli* was frequently present only fleetingly.

While it may appear from this table that slow encrusters are more likely to have persistent co-infection with organisms such as *E. coli*, *Ps. aeruginosa* and *Kl. pneumoniae*, these differences in frequency are not statistically significant (Fisher's Exact test).

Table 3.21: Number of participants with persistent *Proteus* colonisation from which other species were isolated from urine samples, either at any point during the trial or for three consecutive weeks (participants 03 and 11 excluded).

Rapid encrusters - mean catheter lifespan < 28 days

<i>Bacteria</i>	<i>Isolated at any Point</i>		<i>3 Consecutive Weeks</i>	
	<i>percent</i>	<i>number</i>	<i>percent</i>	<i>number</i>
<i>Ent. faecalis</i>	100%	9	89%	8
<i>E. coli</i>	67%	6	33%	3
<i>Ps. aeruginosa</i>	44%	4	44%	4
<i>Kl. pneumoniae</i>	22%	2	22%	2
<i>Staph. aureus</i>	22%	2	22%	2
<i>Ent. durans</i>	11%	1	11%	1
<i>Citrobacter spp</i>	-	-	-	-
<i>Prov. rettgeri</i>	-	-	-	-
<i>Staph. intermedius</i>	11%	1	11%	1
<i>Serr. marcescens</i>	11%	1	11%	1
<i>M. morgani</i>	11%	1	0%	0

Slow encrusters - mean catheter lifespan > 28 days

<i>Bacteria</i>	<i>Isolated at any Point</i>		<i>3 Consecutive Weeks</i>	
	<i>percent</i>	<i>number</i>	<i>percent</i>	<i>number</i>
<i>Ent. faecalis</i>	100%	10	100%	10
<i>E. coli</i>	80%	8	70%	7
<i>Ps. aeruginosa</i>	70%	7	70%	7
<i>Kl. pneumoniae</i>	40%	4	40%	4
<i>Staph. aureus</i>	20%	2	10%	1
<i>Ent. durans</i>	10%	1	10%	1
<i>Citrobacter spp</i>	20%	2	0%	0
<i>Prov. rettgeri</i>	10%	1	10%	1
<i>Staph. intermedius</i>	-	-	-	-
<i>Serr. marcescens</i>	-	-	-	-
<i>M. morgani</i>	-	-	-	-

3.5.3 Comparison of Cultures from Urine and Catheters

The tips of the catheters removed from participants in the study were cultured in order to determine the bacterial constituents of the catheter biofilms. The catheter flora was compared to the flora isolated from the urine sample taken in the same week. In this way it can be seen how accurate a reflection of the biofilm the urine cultures are. In total, 75 catheters could be analysed in this way, as, inevitably, some catheters were lost or were not stored correctly.

The most obvious result was the consistency of *Pr. mirabilis*. This organism was isolated from the urine in every case when it was also isolated from a catheter, and was isolated from the catheter when it was present in the urine in all but two cases, showing that urinary culture is an accurate reflection of the presence of a *Pr. mirabilis* biofilm. The two cases where *Proteus* was not isolated from the catheter occurred in different participants. In participant 15, a very frequent blocker, the *Proteus* was present in catheters removed a few days before and after. In participant 11 the *Proteus* was present in the urine for only two weeks, possibly being cleared from the urine as it failed to establish a biofilm, although this individual was recruited to the study on the basis of a urine culture positive for *Pr. mirabilis* a few weeks earlier.

Pr. vulgaris, the species seen consistently in participant 07, was not isolated from the retrieved catheters at all.

3.6 Antibiotic Sensitivities

The antibiotic sensitivities of isolates of urease producing organisms from study participants was compared with that of isolates obtained from urine specimens sent to a hospital pathology laboratory (Southmead Hospital, Bristol, UK) in the same area and during the same time period as the study specimens were collected. Breakpoint values for antibiotic resistance were taken from British Society for Antimicrobial Chemotherapy Guidelines, which were in use in Southmead Hospital at the time [208].

3.6.1 Sensitivity of *Pr. mirabilis* Isolates from Study Participants

Table 3.22 summarises the antibiotic resistance of *Proteus* samples from 19 long term catheterised individuals from the study group. Table 3.23 shows the antibiotic sensitivities, including minimum inhibitory concentration (MIC) results, for the study participant samples. Of the 19 *Proteus* samples, 2 (11%) were susceptible to all the antibiotics on test, 7 (37%) were resistant to one antibiotic, and 10 (53%) were multi-drug resistant. *M. morgani* SWB006, from participant 03, was also multi-drug resistant.

Table 3.22: Antibiotic resistance summary for *Proteus* isolates from the study group

Antibiotic	Resistance	
	percentage	number
Amoxicillin	47%	9
Nalidixic acid	11%	2
Trimethoprim	84%	16
Cefalothin	21%	4
Ciprofloxacin	0%	0
Gentamicin	16%	3
Co-amoxiclav	0%	0

Table 3.23: Antibiotic sensitivities of clinical isolates (MIC - minimum inhibitor concentration (µg/ml), R - resistant, I - intermediate, S - sensitive).

Gentamicin and cephalothin breakpoints are from systemic *Enterobacteriaceae* breakpoint data, others from UTI (gram negative rod) data.

Organism	Strain ID (participant)	Nalidixic acid		Cephalothin		Ciprofloxacin		Trimethoprim		Gentamicin		Co amoxiclav		Amoxicillin	
		MIC	S/R	MIC	S/R	MIC	S/R	MIC	S/R	MIC	S/R	MIC	S/R	MIC	S/R
<i>Pr. mirabilis</i>	SWB112 (21)	3	S	2	S	0.016	S	>32	R	0.125	S	3	S	>256	R
<i>Pr. mirabilis</i>	SWB108 (20)	3	S	3	S	0.023	S	0.19	S	0.38	S	0.75	S	0.75	S
<i>Pr. mirabilis</i>	SWB102 (19)	12	S	1.5	S	0.023	S	>32	R	4	R	32	S	32	S
<i>Pr. mirabilis</i>	SWB001 (02)	3	S	1.5	S	0.023	S	1.5	S	0.038	S	0.75	S	0.75	S
<i>Pr. mirabilis</i>	SWB085 (15)	6	S	6	S	0.032	S	>32	R	0.75	S	6	S	>256	R
<i>Pr. mirabilis</i>	SWB089 (16)	4	S	6	S	0.032	S	>32	R	0.25	S	3	S	>256	R
<i>Pr. mirabilis</i>	SWB083 (14)	>256	R	16	R	6	I	>32	R	12	R	6	S	>256	R
<i>Pr. mirabilis</i>	SWB098 (18)	3	S	1.5	S	0.016	S	>32	R	0.25	S	0.75	S	0.75	S
<i>Pr. mirabilis</i>	SWB093 (17)	6	S	1.5	S	0.023	S	>32	R	0.05	S	0.75	S	0.75	S
<i>Pr. mirabilis</i>	SWB053 (11)	3	S	3	S	0.012	S	>32	R	0.125	S	3	S	>256	R
<i>Pr. mirabilis</i>	SWB079 (13)	3	S	1	S	0.023	S	>32	R	0.5	S	0.5	S	0.5	S
<i>Pr. vulgaris</i>	SWB023 (07)	1.5	S	192	R	0.012	S	0.19	S	0.094	S	1.5	S	>256	R
<i>Pr. mirabilis</i>	SWB041 (09)	3	S	1	S	0.023	S	>32	R	0.38	S	0.5	S	0.5	S
<i>Pr. mirabilis</i>	SWB017 (05)	2	S	1	S	0.023	S	>32	R	0.25	S	0.38	S	0.38	S
<i>Pr. mirabilis</i>	SWB049 (13)	2	S	1	S	0.023	S	>32	R	0.25	S	0.5	S	0.5	S
<i>Pr. mirabilis</i>	SWB056 (12)	12	S	16	R	0.064	S	>32	R	1	S	32	S	>256	R
<i>Pr. mirabilis</i>	SWB046 (10)	>256	R	4	S	0.38	S	>32	R	16	R	2	S	>256	R
<i>Pr. mirabilis</i>	SWB037 (06)	8	S	>256	R	0.047	S	>32	R	0.75	S	16	S	>256	R
<i>Pr. mirabilis</i>	SWB016 (04)	3	S	1	S	0.016	S	>32	R	0.75	S	0.5	S	0.5	S
<i>M. morganii</i>	SWB006 (03)	>256	R	>256	R	0.08	S	>32	R	0.32	S	0.96	S	>256	R

3.6.2 Sensitivity of *Pr. mirabilis* Isolates from a Local Population

The levels of antibiotic resistance found in the population from which the study participants were drawn were determined by examining data routinely collected by the pathology laboratory of the local hospital covering that area over the time period of the study. This is summarised in tables 3.24 and 3.25. The first table shows the antibiotic resistance of *Proteus* isolates from the urine of 145 non-catheterised individuals, and the second, the antibiotic resistance of *Proteus* urinary isolates from 36 catheterised individuals. There is no significant difference between the two groups (P=0.166 for the most probable difference, trimethoprim sensitivity, by Fisher's exact test).

The hospital laboratory had determined amoxicillin, nitrofurantoin, trimethoprim, co-amoxiclav and ciprofloxacin to be the antibiotics which should be used as the initial treatment of the various infections of the urinary tract. Sensitivity to other antibiotics was only tested for if the isolate proved resistant to the majority of these first line agents. The sensitivity of these organisms, already shown to be multi drug resistant, to cefuroxime (70 isolates) and gentamicin (65 isolates) is shown in table 3.26, in both the catheterised and non-catheterised individuals.

Table 3.24: Antibiotic resistance of *Proteus* samples from 145 non-catheterised individuals submitted to a local hospital pathology laboratory

Antibiotic	Resistance	
	percentage	number
Amoxicillin	18%	26
Nitrofurantoin	99%	143
Trimethoprim	42%	61
Co-amoxiclav	5%	7
Ciprofloxacin	4%	6

Table 3.25: Antibiotic resistance of *Proteus* samples from 36 catheterised individuals submitted to a local hospital pathology laboratory

Antibiotic	Resistance	
	percentage	number
Amoxicillin	17%	6
Nitrofurantoin	100%	36
Trimethoprim	53%	19
Co-amoxiclav	6%	2
Ciprofloxacin	0%	0

Table 3.26: Resistance of organisms isolated from the local population to second line antibiotics. These isolates would have already proved resistant to some first line antibiotics.

Antibiotic	Resistance	
	percentage	number
cefuroxime		
<i>catheterised</i>	14%	6 of 42
<i>non-catheterised</i>	7%	2 of 28
<i>total</i>	11%	8 of 70
gentamicin		
<i>catheterised</i>	0%	0 of 28
<i>non-catheterised</i>	0%	0 of 37
<i>total</i>	0%	0 of 65

3.6.3 Differences in Antibiotic Sensitivity between Study

Participants and the Local Population

The antibiotic sensitivities of the study population and the local non catheterised population are summarised in table 3.27. The frequency of observed antibiotic resistance in the isolates from study participants was analysed using Fisher's exact test to compare the non-catheterised samples from the hospital laboratory and the samples from the study participants (table 3.28).

The results show that that the observed difference in trimethoprim resistance of *Proteus* isolates between the study population of long term catheter users (84%) and the general population of non catheterised individuals (42%) is significant ($P=0.001$).

The difference in amoxicillin resistance between study participants (47%) and the local population (18%) is also significant ($P=0.013$). The differences in sensitivities to the other antibiotics were not tested for significance due to the low numbers of samples obtained.

It seems reasonable to conclude that isolates of *Pr. mirabilis* from individuals with long term catheters are more resistant to commonly used antibiotics than isolates from non catheterised individuals with urinary tract infections.

Table 3.27: Comparison of the resistance of organisms isolated from the local population and the study population to common antibiotics

Antibiotic	Resistance	
	study participants	local population
Amoxicillin	47%	18%
Nalidixic acid	11%	-
Trimethoprim	84%	42%
Cefalothin	21%	-
Cefuroxime	-	7%*
Ciprofloxacin	0%	4%
Gentamicin	16%	0%*
Nitrofurantoin	-	99%
Co-amoxiclav	0%	5%

*The results for cefuroxime and gentamicin in the local population samples are from a subset of multi drug resistant isolates.

Table 3.28: Fisher's exact test – differences in antibiotic sensitivities for trimethoprim and amoxicillin between trial isolates and those seen in the local hospital pathology laboratory (R - resistant, S - sensitive)

Antibiotic	number		totals	difference between proportions	2-tailed p
	R	S			
trimethoprim					
study participants	16	3	19	0.421	0.001
local population	61	84	145		
<i>totals</i>	77	87	164		
amoxicillin					
study participants	9	10	19	0.294	0.013
local population	26	119	145		
<i>totals</i>	35	129	164		

3.6.4 Effects of Antibiotic Use on Urinary Bacterial Populations in the Study Group

Several of the study participants received courses of antibiotic during the trial, although not necessarily for urinary tract infections. Table 3.29 summarises the effect on the cultured urinary bacterial species.

After four of the seven antibiotic courses for which good data was available there was no change in flora at all. Of the 23 colonising organisms present before the antibiotic courses, 18 were still isolated from the urine afterwards. *Pr. mirabilis* was eliminated in one out of six cases, and in that case it was still isolated from the catheter removed during the antibiotic course. This participant died from the respiratory infection the antibiotics were being used to treat, so it is not known if re-colonisation of the urine with *Pr. mirabilis* would have occurred. Re-colonisation with the other successfully eradicated bacteria did not occur during the time period of the trial, except for the case of *E. coli* in participant 18. This occurred six weeks after the antibiotics and so was probably a new infection from a source external to the urinary tract.

Table 3.29: Effects of antibiotic use on urinary flora, showing the species isolated immediately before and after the course of antibiotic shown

participant	bacteria isolated before	antibiotic course	bacteria isolated after
06	<i>Pr. mirabilis, Ent. faecalis, E. coli</i>	amoxicillin	<i>Ent. faecalis</i>
08	<i>Ent. faecalis, E. coli, Kl. pneumoniae, Staph. aureus</i>	minocycline	<i>Pr. mirabilis, Ent. faecalis, Staph. aureus</i>
10	<i>Pr. mirabilis, Ps. aeruginosa</i>	co-amoxiclav	<i>Pr. mirabilis, Ps. aeruginosa</i>
16	<i>Pr. mirabilis, Ps. aeruginosa</i>	ciprofloxacin	<i>Pr. mirabilis, Ps. aeruginosa</i>
16	<i>Pr. mirabilis, Ent. faecalis, Ps. aeruginosa, E. coli</i>	ciprofloxacin	<i>Pr. mirabilis, Ent. faecalis, Ps. aeruginosa, E. coli</i>
18	<i>Pr. mirabilis, Ent. faecalis, Ps. aeruginosa, E. coli</i>	clarithromycin and ciprofloxacin	<i>Pr. mirabilis, Ent. faecalis, Ps. aeruginosa</i>
21	<i>Pr. mirabilis, Ent. faecalis, Kl. pneumoniae, Ps. aeruginosa</i>	trimethoprim	<i>Pr. mirabilis, Ent. faecalis, Kl. pneumoniae, Ps. aeruginosa</i>

Note: Participant 18 was one week in to a two week course of cefalexin when the study started. The bacteria cultured in that week and after the end of the course were the same: *Pr. mirabilis, Ps. aeruginosa* and *E. coli*.

Most worrying is the emergence of a *Pr. mirabilis* infection in participant 08 following a course of minocycline (directed by antibiotic sensitivities against a wound infection at her suprapubic catheter site) which removed *E. coli* and *Kl. pneumoniae* from the urine. While this could be a coincidence, it is also a possibility that the antibiotic allowed colonisation of the catheter once the existing flora had been removed. Following colonisation by the *Proteus*, this catheter user developed severe encrustation.

Ent. faecalis and *Ps. aeruginosa* survived in five out of five cases. *E. coli*, however, was cleared from the urine in three out of four cases.

Participant 16 was on a constant prophylactic course of nitrofurantoin, during which *Pr. mirabilis*, *Ent. faecalis*, and *Ps. aeruginosa* grew persistently, new colonisation with *E. coli* and *M. morganii* occurred, and he suffered two symptomatic urinary infections which required additional antibiotics as shown in the table.

3.7 Comparison of Urine and Stool Cultures

Pairs of faecal and urinary *Pr. mirabilis* isolates were obtained from catheter users in order to compare the strain present in the subject's urine with that present in their gastrointestinal flora. Both the faecal and urine sample from each subject were collected on the same day. From faecal cultures obtained from 18 subjects with *Pr. mirabilis* urinary tract colonisation, *Pr. mirabilis* was isolated from faeces in 10 cases. To assess whether they were the same strain of organism, and therefore whether the gastrointestinal tract was likely to be the source of the urinary tract colonisation, pairs were Dienes typed and then genotyped by Pulse Field Gel Electrophoresis (PFGE).

3.7.1 Dienes Typing

When two *Proteus* colonies are incubated on the same agar plate until their swarming fronts meet, they will either merge into one another or repel each other to form a clear line of demarcation. Those which repel are regarded as different strain types and the Dienes test is designated as positive. If the colonies merge the test result is negative and the isolates are regarded as the same strain type. Figures 3.34 and 3.35 show examples of negative and positive Dienes typing. Table 3.30 shows the results of the Dienes typing.

Phenotyping using the Dienes test showed no lines of mutual inhibition between any of the paired isolates from the urine and faeces of an individual participant. All sample pairs from the same subject merged, indicating that they were of the same Dienes type. Inhibition lines were seen between all isolates from different subjects.

Therefore, by Dienes typing, each subject was colonized by a distinct *Pr. mirabilis* strain, and this strain was present in both the urine and faeces of that individual.

Figure 3.34: A negative Dienes test result. Two strains of the same Dienes type merge when they meet.



Figure 3.35: A positive Dienes test result. Two different strains repel each other forming a Dienes demarcation line between them.



Table 3.30: Dienes typing results, showing isolate combinations which formed demarcation lines (+) and combinations which merged (-). Isolates are labelled as participant number followed by U for urinary origin and F for faecal origin.

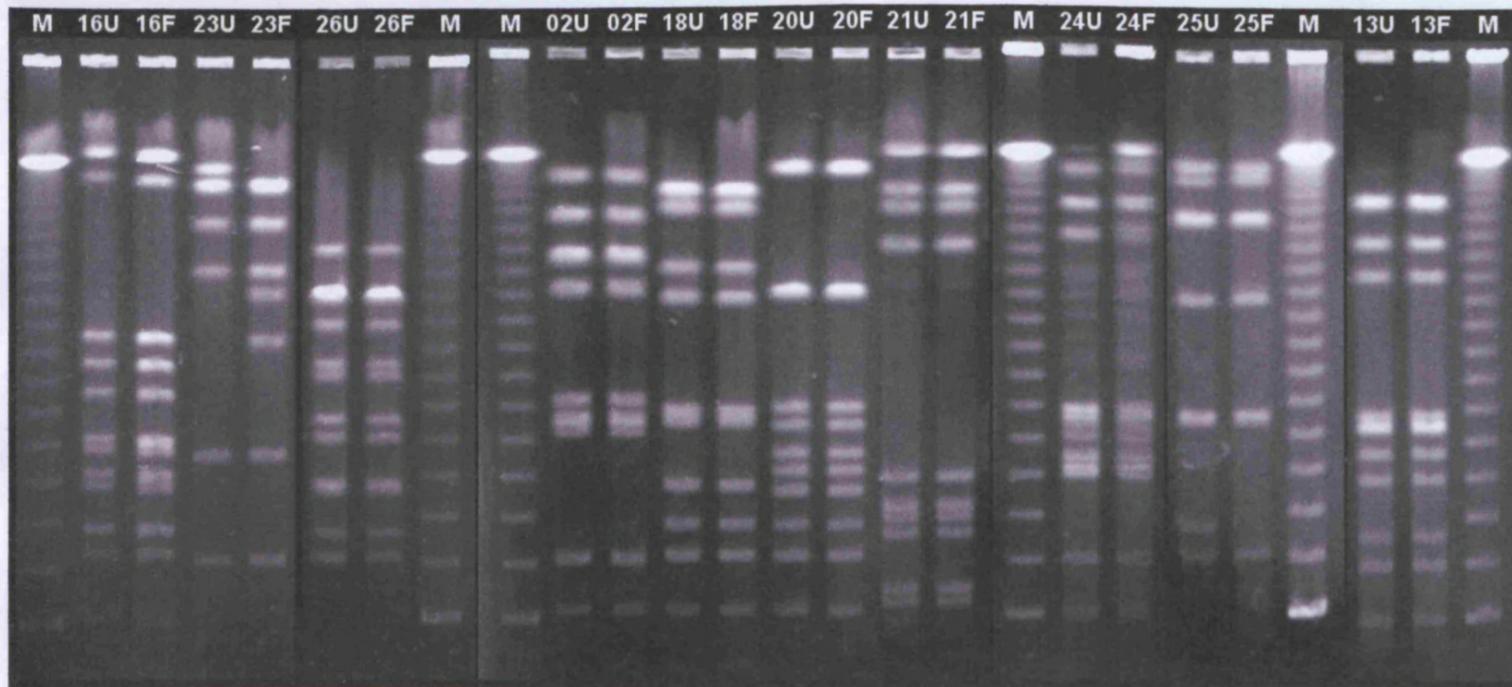
16U	16F	23U	23F	24U	24F	25U	25F	26U	26F	02U	02F	13U	13F	18U	18F	20U	20F	21U	21F		
-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16U
	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16F
		-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	23U
			-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	23F
				-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	24U
					-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	24F
						-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	25U
							-	+	+	+	+	+	+	+	+	+	+	+	+	+	25F
								-	-	+	+	+	+	+	+	+	+	+	+	+	26U
									-	+	+	+	+	+	+	+	+	+	+	+	26F
										-	-	+	+	+	+	+	+	+	+	+	02U
											-	+	+	+	+	+	+	+	+	+	02F
												-	-	+	+	+	+	+	+	+	13U
													-	+	+	+	+	+	+	+	13F
														-	-	+	+	+	+	+	18U
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																-	-	+	+	+	20U
																	-	+	+	+	20F
																		-	-	+	21U
																				-	21F

3.7.2 Pulsed Field Gel Electrophoresis

Genotyping by PFGE of bacterial DNA also showed that each subject was colonized by a distinct strain of *Pr. mirabilis*. Within the applied typing criteria, seven of the 10 subjects had genetically indistinguishable strains in both their urine and faeces. Three subjects had closely related strains, differing by a single genetic event (participants 23, 24, and 25). This degree of genetic difference can be found by repeatedly culturing a strain in a laboratory, or by examining multiple isolates from the same source taken over an extended period of time. It is therefore probable that these isolates originated from the same source. Figure 3.36 shows the results of the PFGE.

There was no relationship between the strains isolated from three subjects who were resident in the same nursing home at the same time (participants 02, 20 and 13).

Fig. 3.36: PFGE patterns of *Pr. mirabilis* DNA restriction fragments from pairs of isolates of urinary (U) and faecal (F) origin. Numerals identify the different subjects. Lanes marked M contain the molecular size markers.



Section 4 - Discussion

4.1 The Prospective Study

Noteworthy participants and variation in catheter lifespans

4.2 Urinary pH and Encrustation

Variability of measured values and assessment of novel measurement techniques

4.3 Factors Influencing Nucleation pH

The chemistry of crystal precipitation in the context of nucleation pH

4.4 Urease Activity

The contribution of the urease enzyme

4.5 Behaviour of Bacterial Populations

Changes in urinary and catheter flora

4.6 Antibiotic Sensitivities

The antibiotic resistance problem in the context of other studies

4.7 Comparison of Urine and Stool Cultures

The origin of *Pr. mirabilis* colonisation

Refining our Understanding of Catheter Encrustation

The simple view of catheter encrustation has long been that crystal precipitation occurs when the pH of the catheter user's urine became alkaline as a result of urinary tract colonisation with a urease positive organism. Strategies to control catheter encrustation have therefore been directed towards preventing this rise in pH, by aiming to prevent colonisation or eradicate the bacteria, to acidify the urine, or to inhibit the actions of bacterial urease. None of these methods has proved successful.

What was obvious to the clinician, however, was that despite being colonised by the same bacteria and having alkaline urine, catheter users blocked their catheters at markedly different rates. Unfortunately, by comparing 'blockers' with 'non-blockers' in an attempt to discover the factors leading to catheter encrustation, most previous studies had compared those with urease positive bacteriuria to those without, but in the absence of organisms such as *Proteus*, encrustation is unlikely irrespective of other urinary factors. This study aimed to define the limits of the variation in the severity of encrustation in long term catheter users with *Proteus* bacteriuria and investigate whether different individuals had different susceptibilities to encrustation which may be exploited in future treatments.

4.1 The Prospective Study

4.1.1 Noteworthy Participants

Among the individual participants in the study, several show interesting patterns of results which may provide insight into the problem of catheter encrustation.

Participant 03

Although participant 03 tested positive for *Pr. mirabilis* in the pre-trial screening, this organism was not isolated again during the study follow up, either from catheters or urine. Both the *M. morganii* and the *Ps. aeruginosa* isolates were urease positive according to the standard urease test used during the identification process, but both organisms have very low urease activity compared to *Proteus*. This is reflected in the low pH, and minimal catheter encrustation seen. Although this study focuses on *Proteus* as the leading cause of encrustation related catheter blockage, it should be noted that urease positive organisms other than *Proteus* can certainly cause encrustation.

Participant 06

Participant 06 was a 101 year old individual resident in a nursing home (see figure 3.6). Despite her age, she was reasonably healthy until her final illness. She had previously suffered a stroke, limiting her mobility and suppressing her gag reflex. As well as requiring a urinary catheter for functional incontinence due to her limited mobility, she was unable to swallow thin liquids without risk of choking. She was able to eat normally, but had quite a suppressed appetite. Because of these two issues,

she was fitted with a percutaneous endoscopic gastrostomy (PEG) tube. This enables liquids to be fed directly into the stomach, bypassing the throat and swallowing mechanism. In this lady's case, it was used to increase the amount of water she could drink as well as providing some enteral feeding to top up her slightly inadequate daily nutrition. As a result of this, she had a greater daily fluid intake than many other participants in the study.

Her results show a consistently high voided pH (mean 7.65), ranked 6th overall. She maintained a nucleation pH which was always higher than this (mean 8.96), and was the highest recorded in the study. This gave a mean safety margin of 1.33, the 6th highest in the study, and led to her first catheter only blocking after 13 weeks, and then showing only a small area of encrustation. Her mean urinary magnesium concentration was among the lowest in the trial group, and her calcium concentration was the lowest. This might suggest dilute urine, with the obvious factor contributing to this being her PEG feed.

Morris and Stickler studied the effects of increased fluid intake and urinary dilution [211]. Pooled urine was collected from test subjects who drank normally, or who drank an extra 1000 ml of water per day, and was tested in an artificial catheterised bladder model. The urine was inoculated with *Pr. mirabilis*, and encrustation, as the amount of calcium and magnesium recovered from the catheters in the model after a set time period, was measured. Significantly less encrustation was formed using the urine from the subjects who supplemented their fluid intake. In a similar test by the same authors, artificial urine was diluted to various strengths to model an increased fluid intake. It was run through the catheterised model bladders at increased rates according to the degree of dilution such that the same quantity of solute flowed through the catheter per unit time in each case. Those catheters tested with the dilute, high flow urine took longer to block. This suggests a highly beneficial role for

fluid intake. In a clinical survey, Getliffe found no significant difference in fluid intake between blockers and non-blockers as defined by the degree of observed encrustation [46]. However, in those with no trace of encrustation their fluid intake is irrelevant. Without urease producing organisms in their urinary tract they will not encrust, at least by the crystalline biofilm mechanism. Comparing possible risk factors for encrustation between blockers and non-blockers is unhelpful unless a group of non-blockers colonised with urease positive organisms is found.

Participant 12

In contrast is participant 12 (see figure 3.12). This gentleman was a resident at the same nursing home as participant 06. He had a urethral catheter for bladder outlet obstruction caused by locally advanced prostate cancer. He was fed normally, although he also had a poor appetite and low fluid intake. Like participant 06, he had a long catheter life.

Although he had the third highest mean nucleation pH (8.58) in the group, probably partly due to having the lowest and second lowest mean urinary magnesium and calcium concentrations respectively, he also had a consistently high voided pH (mean 8.98), the highest in the trial. This led to a negative safety margin of -0.5, which would have been thought to encourage more severe encrustation than actually occurred. It is unclear as to why there was not more encrustation. This pH_v result is an obvious outlier among the other mild encrusters.

This participant dropped out of the trial after only five weeks, so there is less confidence in the data than with the other trial participants, and it could be that his usual urinary state was as shown in his first and last urine samples, with his nucleation pH clearly above his voided pH. Due to the short period of time that he

provided samples, the catheter whose lifespan was used as part of this participant's data set had been in for three weeks when urine was not being analysed, and this could explain the discrepancy. It should be noted that the period of time he was in the trial was not obviously unrepresentative, looking at his past health records. His catheter lifespan was typical for him, and there was no change in diet, fluid intake, medication, level of function, or health. What is clear is that the low calcium and magnesium concentrations he exhibited could not be due to dilute urine. Although urine output was not formally measured in this trial, he was being cared for by the same nursing staff as participant 06. They reported his urine output to always be extremely poor, as compared to participant 06's which was reported to be very good. This agrees with the experience of this researcher. Part of the reason for lack of data on this gentleman is that even after clamping his catheter for an hour, sufficient urine could not be obtained to perform a nucleation pH analysis.

Participant 08

It is a common finding that catheter users do not suffer from encrustation when initially catheterised. They describe a few months, or sometimes even years, of using a catheter without any problems with encrustation, before suddenly the catheter starts to block. This is thought to be due to the colonisation of the urinary tract with a urease positive organism, and is demonstrated by participant 08 (see figure 3.8).

This lady grew *Ent. faecalis*, *E. coli*, *Kl. pneumoniae* and *Staph. aureus* for the first six weeks of the study before she was given a course of minocycline for a wound infection at her suprapubic catheter site in week 6. This cleared her urine of the *E. coli* and *Kl. pneumoniae*, but immediately following the antibiotics, a urease positive *Pr. mirabilis* was isolated. After two weeks the *Staph. aureus* disappeared from the urine leaving only *Ent. faecalis* and the *Proteus* for the last seven weeks of the study.

While the *Kl. pneumoniae* isolate was urease positive according to the standard urease test used to identify bacteria, this organism has a considerably lower urease activity than *Proteus*. The urinary pH_v increased noticeably over the two weeks following the arrival of *Pr. mirabilis* from a mean of 5.23 in the first six weeks to a mean of 8.27 in the last seven. The pH_n, however, remained approximately the same at means of 6.94 compared to 7.11 over these same periods. This resulted in the voided urine changing from being comfortably below the nucleation pH to being above it. The mean safety margin changed from 1.71 in the first six weeks to -0.85 in the last seven.

This resulted in a change from a catheter non-blocker to a blocker. The catheter that had been in place for eight weeks since the start of the study blocked two weeks after the colonisation with *Proteus*. Catheters then started to block on a weekly basis, being heavily encrusted each time. Each of these catheters was colonised with *Pr. mirabilis* and *Ent. faecalis*. The catheter recovered in week 1 was colonised only with *Ent. faecalis*. While calcium deposits were found on the first catheter there was very little magnesium. In contrast, the catheters colonised with *Proteus* showed high levels of magnesium deposits, perhaps indicating a change to struvite from a previously different mineral type.

Participant 15

Participant 15 was the most rapid encruster in the study (see figure 3.15). She was an 88 year old female with a urethral catheter for functional incontinence. She had extremely poor mobility following several strokes, and was cared for at home by her husband. She received treatment with Suby G (3% citric acid) catheter maintenance

solutions throughout the course of the study. Despite this, she had a mean catheter lifespan of 3.2 days, with a consistent range of 2 to 4 days.

Her pH_v was average for a catheter user with *Pr. mirabilis*, at a mean of 6.98. She did, however, have the highest mean urinary calcium (16.2 mM) and magnesium (20.2 mM) levels in the study group, and, probably as a result of this, had the second lowest mean pH_n at 6.89. Her pH_v was normally higher or only slightly lower than her pH_n , resulting in extensive crystal precipitation.

Once again, the reason for this particularly high concentration of calcium and magnesium is likely to be concentrated urine. Urine output was not formally measured, but judging by the frequency with which the drainage bag was emptied it was very low, and fluid intake was reported to be poor.

She received twice weekly instillations of an acidic catheter maintenance solution throughout the trial. The lack of clear evidence for the effectiveness of acidification has been discussed, but in this case there is the practical point that these catheters blocked so quickly that many were subject to no installations, and even those that were received only one treatment.

Participant 16

Participant 16 was a reasonably fit 80 year old gentleman with a suprapubic catheter for a combination of bladder outlet obstruction and detrusor underactivity (see figure 3.16). He had a history of suffering from recurrent episodes of symptomatic urinary tract infection. As a result, he was prescribed a regular treatment of prophylactic low dose nitrofurantoin to try and prevent these episodes. He had been taking this for several months before the study and continued taking it throughout the period of

investigation. Urinary isolates of *Pr. mirabilis* collected from the local area, either from catheterised individuals or those with uncomplicated urinary tract infections, had resistance rates to this antibiotic of almost 100%. It was used as more common uropathogens such as *E. coli* were still overwhelmingly sensitive. It should be noted that this individual did develop colonisation with *E. coli* during the study, suggesting the selective pressures which may be placed on bacterial populations by persistent antibiotic use. The possible advantages of not eliminating reasonably easy to treat, urease negative organisms such as *E. coli* are discussed further in this section with regards to participant 19.

Even with this prophylaxis, participant 16 developed two symptomatic urinary infections towards the end of the study period, both of which were treated with ciprofloxacin. It is unclear which of the several urinary tract organisms carried by this gentleman was responsible for his symptoms, as is often the case with catheter associated infection. What is clear, however, is that despite his symptoms resolving with treatment, his urinary flora remained completely unchanged on both occasions. The strain of *Pr. mirabilis* he was colonised with was sensitive to ciprofloxacin when tested, but despite this it persisted along with *Ps. aeruginosa*, *E. coli* and *Ent. faecalis* (of unknown sensitivities). This is also despite the suggestion that ciprofloxacin and the other fluoroquinolones may be effective anti-biofilm agents, as demonstrated in experimental models of *Ps. aeruginosa* catheter biofilms [212,213] and in a clinical trial previously discussed in section 1.4.4 [112].

Participant 19

This gentleman had a urethral catheter because of bladder outlet obstruction caused by benign prostatic enlargement. He declined an operation on his prostate as he had a degree of cardiac failure which would have increased the risks of anaesthesia. He

lived in his own home and was fairly independent. His records show no problems with catheter encrustation and blockage despite being catheterised for several years.

He had a mean nucleation pH of 7.87 which was fairly average for the study participants, with average urinary calcium and slightly above average magnesium concentration (see figure 3.19). What was surprising, given his persistent urinary tract and catheter colonisation with *Pr. mirabilis*, was his extremely low mean voided pH of 4.77, the lowest in the group and over two standard deviations from the mean. Both the pH_v and pH_n also showed little week to week variability. It is clear to see why crystals did not precipitate from this gentleman's urine to form encrustation on his catheters, given the mean difference between pH_n and pH_v was 3.11.

What is interesting is why his pH_v was not raised higher by his *Proteus* infection. The *Proteus* strain he carried had a urease activity which was slightly below average, but still higher than the two most rapid encrusters in the study, so this does not offer an explanation. A greater than average fluid intake may reduce encrustation, but this would likely be by lowering the calcium and magnesium concentrations and raising the nucleation pH. The voided pH may not change substantially as the urease enzyme should be able to increase urea hydrolysis to compensate for the ammonia dilution, although this may be limited by the flow through the system. Even so, this participant did not seem to have a significantly higher than normal fluid intake.

The low pH may be due to the presence of other bacterial species. An *in vitro* study by Edin-Liljegren *et al* demonstrated that urease added to urine inoculated with *E. coli* produced less ammonium, less of a pH rise, and fewer precipitating crystals than urease added to sterile urine [214]. This gentleman did have *E. coli* isolated from his urine almost every week, and it was isolated from both his catheters, even when not concurrently present in the urine. In most trial participants isolation of *E. coli* was not

this consistent. The presence of *E. coli* on this participant's catheters may have been reducing the urease activity of the *Proteus*. However, reasonably consistent *E. coli* colonisation was also found from the urine samples of participants 02, 04, 09, and 14, and they had average or above average voided pH. Except in the case of participant 02, and on one occasion from participant 04, *E. coli* was not found on their catheters, and this may be more important.

The *Pr. mirabilis* in this gentleman's urine showed colony counts of around 5.0×10^6 . While colony counts were not performed routinely on the urinary isolates, on those occasions when it was tested, the *Proteus* species in the urine of other participants had similar counts. For example, in participant 20, *Pr. mirabilis* counts were found to be 6.8×10^6 . What is perhaps more interesting is the counts of the other organisms present in the urine. In participant 20, *Proteus* was the dominant organism, with *Prov. rettgeri* and *Ent. faecalis* having counts of 9.0×10^5 and 1.0×10^5 respectively. In participant 19, the other organisms had higher counts than the *Proteus*, with *Kl. pneumoniae* and *Citrobacter* spp having counts of 1.3×10^7 and 1.37×10^8 . It is possible that the *Proteus* was prevented from increasing in numbers by the presence of other organisms on the catheter, or in the urine.

The phenomenon of bacterial interference may offer an explanation. This involves clinically using deliberate colonisation with non-pathogenic organisms to prevent symptomatic infection caused by pathogenic organisms [215]. Trautner *et al* have investigated the role of bacterial interference in catheter colonisation in an *in vitro* model. Lengths of hydrogel coated latex catheter were incubated in broth with *E. coli* 83972, an avirulent strain, for 24 hours. These sections, along with controls which had been incubated in sterile broth, were then exposed to pathogenic urinary tract organisms, isolated from individuals with symptomatic infections, for 30 minutes. The colony counts of pathogenic organisms were significantly lower on the pre-inoculated

E. coli catheters than the sterile catheters. The *E. coli* 83972 was shown to resist colonisation by *Ent. faecalis*, pathogenic *E. coli*, *Prov stuartii*, and the fungal uropathogen *Candida albicans* [216,217]. It could be argued that the ability of an existing biofilm to reduce the colony counts of recently invading organisms over a period of 30 minutes, even in the growth enhancing medium of culture broth, may not translate into an ability to resist colonisation in a clinical situation over a period of many months. Investigations into bacterial interference in non-catheterised individuals suggest that the effects could be long lasting. By purposefully colonising the bladders of spinal cord injured individuals with *E. coli* 83972, the safety and efficacy of this approach as a therapeutic tool was demonstrated in prospective clinical trials [218,219]. A significant reduction in the incidence of symptomatic urinary tract infections was seen when those whose bladders were successfully colonised were compared with their own pre-colonisation infection rates and the rates in those individuals where colonisation failed. A moderate reduction in infection rates of women suffering from recurrent urinary tract infections was also observed after deliberate vaginal colonisation with non-pathogenic *Lactobacillus* spp [220,221]. Would an organism such as *E. coli*, whether pathogenic or not, be capable of suppressing the growth of *Pr. mirabilis*?

Work by Sabbuba *et al* has shown that *Pr. mirabilis* is capable of swarming over catheters in the presence of several other bacterial species [146]. Two separate blocks of agar were linked by a bridge of catheter material. At one side of the bridge the agar had been colonised by a bacterial species isolated from a catheterised individual. *Pr. mirabilis* was inoculated to this side of the bridge, and its ability to migrate through the other species, across the catheter bridge, and to colonise the other agar block was assessed. Isolates of *E. coli*, *Kl. pneumoniae*, *Staph. aureus* and *Ent. faecalis* did not significantly inhibit the *Proteus*, although the isolates of *Serr. marcescens* and *Ps. aeruginosa* did. *Kl. pneumoniae* and *Staph. aureus*, unlike the

other organisms, were shown to migrate along with the *Proteus*, perhaps indicating a degree of cooperation between the species. Again, there may be important differences in the mechanisms interacting in such an *in vitro* experiment, and those which govern species dominance in a catheter biofilm over a period of months.

As well as *E. coli*, this participant also had persistent urine and catheter isolates of *Kl. pneumoniae* and *Ps. aeruginosa*, so if bacterial interference was responsible for the low *Proteus* counts, it may not have been due to, or solely due to, *E. coli*.

Even though the urease activity of this strain of *Pr. mirabilis* was not unusual, and it possessed normal swarming activity, it is possible that it lacked some virulence factor which may have allowed other *Proteus* strains to more fully dominate their environments despite the presence of other organisms. It is also possible that one of the other species present may have possessed an uncommon virulence factor allowing it to suppress the growth of other bacteria.

4.1.2 Variation in Catheter Lifespans in Individuals

The classic study on catheter lifespans was produced by Norberg *et al* [178]. They conducted a survey of 20 inpatients with indwelling catheters from long stay geriatric wards in a Swedish hospital. The patients all suffered from forms of dementia. They used silicone coated latex catheters of various sizes which were aimed to be changed routinely every 30 days, although this was not always done. They were selected as problem patients from a larger group of 50 because they had shortened catheter lives, due either to 'catheter blockage' or 'the patients wrenching their catheters out'. The authors demonstrated considerable variation in catheter lifespans both between individuals and within the experience of a single patient. They describe the scatter of catheter lifespans as being wide and skew in most patients, with those

who tended to have long lifespans occasionally having very short ones and vice versa. They did, however, conclude that a useful measure of catheter lifespan in a patient could be obtained from measuring the time to blockage of 3 to 5 catheters.

In their study, the median value of median catheter lifespans was 9 days (range 3-35, interquartile range 6-16). In the present study, the distribution of catheter lifespan means (and medians) was normally distributed, possibly due to not imposing an upper limit to catheter lifespan of 30 days, but for comparison the median of median catheter lifespans was 29 days (range 3-70, interquartile range 15-47.5). The mean of means was 32.7 days.

The different selection criteria in the two studies are probably responsible for a large part of this disparity. In the Norberg study, patients were recruited whose catheters tended to be changed more often than the planned period of 30 days, which was the desired interval in their hospital. They selected the worst affected patients. In the present study, recruitment was on the basis of urine microbiology, with no reference to catheter lifespan. This was deliberate, as it was intended to take account of those slow encrusters who were normally excluded from studies which define catheter encrustation by a short catheter lifespan. The usual definitions of catheter blockage used in studies are catheters lasting less than either one month or six weeks. As can be seen from figure 3.23 and table 3.1, up to eight of the participants in the present study could have been excluded using a 30 day criteria, even though they did suffer from unpredictable encrustation related blockage. The present study demonstrates that encrustation does occur at median or mean intervals greater than 30 days, and indeed greater than six weeks. Getliffe also found that a few individuals whose catheters usually lasted longer than six weeks had encrustation visible on those catheters [46]. As it is arguably the unpredictability of blockage, more than the frequency, which is actually inconvenient to the catheter user, it is important to

recognise these individuals who encrust slowly. Also, by not studying those with less severe problems, the mechanisms by which these individuals are resistant to encrustation will not be appreciated. As these mechanisms may be applicable to all catheter users, opportunities to improve the management of this problem may be missed.

The goal of the Norberg study was to investigate shortened catheter life from all causes, not specifically to study catheter encrustation. As well as those who had their catheters changed prematurely by the nursing staff, the researchers also recruited patients who had removed their own catheters. This would include those who suffered from spontaneous catheter expulsion due to urethral sphincter damage, as well as those who pulled out their catheters because of their dementia. It is not difficult to remove a catheter by simply pulling on it, even if the balloon is inflated. The proportion of the study group made up by these patients is not specified. In the clinical experience of the current author, catheter expulsion would probably not be particularly common. However, in a study consisting entirely of patients hospitalised with dementia, self-removal of catheters may occur quite frequently. This could affect the data in two ways: a patient with a normally long catheter lifespan may experience occasional short lifespans due to catheter removal, or a confused patient particularly irritated by their catheter may remove it very regularly. Both of these patterns are seen in the Norberg data. However, both of these patterns are also seen in the present study, represented by participants 04 and 15, where it is known that the catheters were blocked by encrustation.

Another area of possible inaccuracy is the common phenomenon of detrusor overactivity causing catheter bypassing and so mimicking a catheter blockage. A carer will often assume that a blockage has occurred and change the catheter, leading to a shortened catheter lifespan unrelated to encrustation. It is unclear how

many catheters are changed for this reason. In a retrospective study, Cools and Van der Meer found that only 18% of long term catheters were changed at the planned one month interval. 54% were changed early because of blockage, 25% because of bypassing, and 3% removed by the patient [44]. The most common replacement interval for blocked catheters was 2 weeks and for bypassing catheters, 3 weeks. Data about catheters rather than average lifespan values per patient will, of course, over-represent those individuals with particularly short catheter lives. In the present study, looking only at encrusted catheters, only 25% lasted for 30 days or more, but mean catheter lifespans greater than 30 days were found in 12 of the 21 participants. Another indication of the frequency of bypassing mimicking blockage is a study by Kunin *et al* [45]. Of catheters which were removed prematurely, only 50% were blocked or showed poor flow on subsequent testing. Getliffe found that 21% of those whose catheters were changed more often than every six weeks had no visible catheter encrustation, although the reasons for these changes were not explored [46]. It is vital that in studies of encrustation, catheters are examined to exclude those who suffer from other problems.

Even with these caveats, it appears that the present data follows the pattern previously described by Norberg *et al*, except that it is possible for encrustation to be a slower process than sometimes appreciated.

4.2 Urinary pH and Encrustation

4.2.1 Variability in Measured Values

Individual values of nucleation pH, voided pH, calcium and magnesium concentration showed considerable week to week variability. Individual measured values cannot be used as if they were representative of a catheter user. Several measurements must be taken in order to use a mean value, which can then be used to characterise an individual.

Considerable differences exist in the mean pH_v values of the participants, even between those colonised with *Pr. mirabilis*. In the literature, average values of pH_v for non-blockers (however defined) range from 5.97 to 6.42, and for blockers, from 6.88 to 7.85 [46,148,149,222]. Most of these non-blockers did not have urease positive bacteriuria. The mean pH_v values from the *Proteus* colonised individuals in the present study overlap this entire range. It is clear from the individual patient graphs (figures 3.02 to 3.22) that individual values of pH_v are unreliable for determining the severity of the encrustation suffered by a *Proteus* colonised catheter user. It appears that even mean values of pH_v are not reliable, given the lack of significant difference between the rapidly and slowly encrusting groups. Participant 05, with a mean pH_v of 6.42, has a very short mean catheter lifespan, while participant 06, with a mean pH_v of 7.65, has a considerably longer one.

Hedelin *et al* suggest that struvite precipitation occurs predominantly above a critical pH_v value of 6.7 to 6.8, based on their findings that very little encrustation is deposited on the catheters of those with a mean pH below this [223]. Choong *et al* show mean pH_n values for non-blockers of 7.46 (SD 0.73) and for blockers with

urease positive bacteriuria, 8.15 (SD 0.80) [148]. These last results are based on a single urine specimen per subject. These investigators also identified a small subset of blockers without detectable urease positive organisms. They found that they had a significantly lowered nucleation pH of 6.45, and hypothesised that crystal formation might occur in some catheter users due to this abnormality of urinary chemistry rather than a rise in urinary pH, much as calculi occur in the upper urinary tract largely in the absence of infection. This group did have a raised pH_n , however, at 7.36, which would have put them at risk of encrusting even had they had the mean pH_n of the non-blocking group. It is therefore possible that urease positive bacteria were present on the catheters, which were not analysed, but not found in the urine on the single sample taken. The results of the present study show that pH_n is variable between individuals with urease positive bacteriuria, even when means of values sampled over many weeks are compared. This study has uncovered individuals with consistently high values (participants 06 and 17), as well as those with consistently low values (participants 05 and 15). This demonstrates that real differences exist in this value between the urines of different individuals, contributing to their differing susceptibilities to encrustation. This is reflected in the highly significant difference in pH_n between rapid and slow encrusters. However, in some subjects, this value varied dramatically from week to week (participants 20 and 22), suggesting that it is not fixed in an individual.

Overall, the variability of mean pH_n follows a normal distribution, not suggesting a clear distinction between a normal pH_n and an abnormally low pH_n due to some distinct metabolic defect in this group. It would seem logical, however, that those metabolic defects responsible for aseptic calculus formation in the upper urinary tracts of some individuals would predispose them to encrustation, even in the absence of urease positive bacteriuria, should they ever be catheterised. None of the participants in this study had a history of non-infective renal calculi.

This data seems to confirm the theory that the difference between those with rapid catheter encrustation and those without is due to a narrowing of the gap between the pH of the urine and the pH at which the salts in it will precipitate out of solution. In those who have urease producing organisms in their urine, at least in some cases this narrowing can be explained by a lower value of the nucleation pH rather than an increased urinary pH. For example, from figure 3.25 it can be seen that the mean pH_v for participants 15 and 18 is similar (6.98 and 6.89 respectively), but that because of a much lower mean pH_n in participant 15 (6.89) compared with participant 18 (8.73), the mean safety margin seen in figure 3.24 is negative for participant 15 (-0.10) and positive for participant 18 (1.83). This explains why participant 15 was a very rapid blocker and participant 18 rather slow (shown in figures 3.15 and 3.18).

It therefore seems that there may be a way of treating the problem of catheter encrustation other than simply lowering pH_v , which is the effective goal of current strategies. It is possible that if the factors determining these differences in pH_n could be determined, it may be possible to raise pH_n to a point at which little crystal deposition would occur.

4.2.2 Catheter Encrustation Rate

Dividing the participants into rapid and slow encrusters on the basis of their calculated encrustation rate demonstrated the same significant differences in urinary constituents as when they were divided by using their mean catheter lifespan, except that the difference in safety margin was no longer significant. There was also considerable variability in the results for each individual participant (table 3.10).

Encrustation rate was supposed to provide a more useful and consistent measure of the problem of catheter blockage than the catheter lifespan, for the purposes of experimental data collection. Theoretically, all catheters could be used to calculate the value. Even if they had been removed before blockage had occurred, either routinely or accidentally, some encrustation would have formed that would allow a value to be calculated. It was also hoped that it would prove more consistent between catheters in any individual than the value of catheter lifespan. Norberg *et al* showed that catheter lifespan was a useful parameter for measuring catheter function, but that the scatter of individual catheter lifespan results for any given catheter user meant that isolated readings could not be used [178]. This was found to be quite variable in this study, as is widely recognised clinically, and there are several reasons which might account for this.

In the clinical situation, catheters may be changed because of a number of conditions which mimic blockage. The catheter may be blocked by twisting of the tubing, or a severe episode of spasm may imitate a blockage. These conditions can occur in those known to have catheter encrustation. In these cases where the exact problem is not known, the care staff will change the catheter. If the cause was encrustation, the immediate problem is solved. If it is not, no harm is done. It is the safe option to change the catheter, and as most catheters are not inspected for encrustation, catheter blockage will be recorded in the notes as the 'reason for change'.

The final event leading to catheter blockage may not actually be the formation of new crystals. While a steady rate of crystal deposition will gradually narrow the catheter lumen until it is completely occluded, it is possible that this rarely occurs in the *in vivo* situation. Once the catheter lumen is sufficiently narrowed, it may become suddenly occluded by a piece of debris. The random size of these debris particles would lead to obstruction with differing amounts of encrustation at each blockage event. The

debris could be a dislodged piece of encrustation from more proximally in the catheter, a piece of bladder stone, a blood clot, or a mucus plug. Mucus is formed in the catheterised, bacterially colonised bladder in large quantities, and this is commonly seen being passed through the catheter tubing. This random and uncontrollable final blockage event would add considerable variability to the catheter lifespan, masking the underlying and more important process of encrustation which is what actually needs to be measured.

The differences in luminal diameter between catheters of the same size, even when made of the same material, may not be appreciated [224], and this may lead to differences in catheter lifespan.

Several authors have used a measure similar to encrustation rate. Desgrandchamps *et al* used mass of deposited calcium and magnesium to assess levels of encrustation on different types of ureteric stent and urethral catheter *in vitro* using human urine and purified urease [225]. Choong *et al* used mass of deposited calcium to develop an *in vitro* encrustation model to test ureteric stents and urinary catheters using either artificial or human urine, but again, this wasn't an infected system [171]. Choong *et al* later advised that both calcium and magnesium should be measured as both are important in encrustation [148]. Calcium and magnesium mass has been used in a *Proteus* infected model of urinary catheterisation using artificial or human urine and given equivalent results to catheter lifespan [226]. In their clinical study demonstrating the high voided pH of those with encrustation, Hedelin *et al* measured encrustation as magnesium and phosphate per catheter, irrespective of the size of catheter or duration of placement, and achieved meaningful results [223].

In the present study, encrustation rate was much more variable between the catheters of a given participant than expected. The variability could be explained by

several mechanisms. Most importantly, the encrustation rate concept assumes that encrustation is being laid down in a linear fashion. That is, the same amount of mineralization occurs in the first week as in every other. If the pattern is exponential, for example, a greater mass of encrustation will form in latter weeks. This would lead to calculated rates being lower for those catheters changed before they block, removing one of the supposed advantages of this method. Even when used over a fixed time period, as in some of the *in vitro* studies using this method, it makes comparison of different materials less effective. With exponential encrustation, a material with half the encrustation of another after a set time period may not be that much further away from blocking.

As with catheter lifespan, variability will also be caused by using different catheters. The differences in lumen diameter between catheters of different size, constructed from different materials, between different manufacturers, and even between different batches from the same manufacturer may lead to variation in both catheter lifespan and encrustation rate. Cox has described the surfaces of various catheters as viewed by electron microscopy and demonstrated considerable variation in surface roughness between different manufacturers, especially in the internal surfaces of latex based devices [16]. However, an increased lumen diameter may lead to a far greater increase in encrustation rate than catheter lifespan. This is because, assuming that encrustation is laid down uniformly and at a constant rate on the luminal surface, catheter lifespan will increase in proportion to luminal diameter, whereas encrustation rate will increase in proportion to luminal surface area.

The distribution of encrustation down the length of the catheter will also be very important in determining encrustation rate. Someone who forms encrustation only at the catheter eyeholes will have a much lower encrustation rate than someone who deposits crystals along the full length of a catheter. In their artificial bladder models,

Morris *et al* described a fairly consistent pattern of encrustation on different types of catheter [168]. Deposits are mainly laid down in the 10 cm below the catheter eye holes. The maximum deposition, and therefore the area which blocks, is in the 2 cm immediately below the eye holes. From the present study, and the author's clinical experience, this fits with the pattern seen in many catheter blockers, but certainly not all. Encrustation can develop along varying lengths of the catheter and the blockage point can be some distance from the eyeholes. In model bladders, the catheters are straight, and are not moved, unlike in the clinical situation. The catheter in the patient will be curved depending on how they wear it, and this may set up areas of turbulent flow or repeated flexing which could produce differing patterns of encrustation.

If encrustation rate is no less variable than catheter lifespan, why should it be used? It is time consuming to measure, and it relies on catheters being collected rather than discarded. Encrustation rate also has some conceptual disadvantages. Firstly, the catheter user is not worried about their encrustation rate. They are worried about their catheter lifespan. This is the clinical endpoint to be altered by any proposed intervention. Secondly, crystalline biofilms have an organic as well as an inorganic component. The secreted bacterial extracellular polysaccharide matrix makes up a considerable bulk of the biofilm, and this may possibly vary in volume between catheter users, and between catheters from the same user. This inorganic component will not be measured by calculating the encrustation rate.

Where the two techniques may be useful is in the comparison of individuals using catheters with different drainage lumen diameters. As explained above, as compared to a hypothetical situation in which every catheter lumen was identical, both encrustation rate and catheter lifespan should be too high in those with larger catheters. This would give the impression that the encrustation problem was more severe than it was when using encrustation rate, but less severe than it was when

using catheter lifespan. Combining both techniques may help correct for differences in catheter lumen. The exact method for doing this would have to be worked out in a controlled environment, probably *in vitro*, before being applied to the clinical situation.

In conclusion, calculated encrustation rates can be used to distinguish between mild and severe encrusters, however, this method does not appear to offer any real advantages over the far simpler technique of measuring catheter lifespan.

4.2.3 Catheter Specific Safety Margin

To compensate for any week to week variability in safety margin which may be responsible for that part of the variability in mean catheter lifespan not accounted for by comparison with mean safety margin, a catheter specific safety margin (CSSM) term was calculated. This would give the mean safety margin experienced by any one particular catheter, and this could be compared with the lifespan of that catheter. This method, however, only produced a minimal improvement over the comparison of mean catheter lifespan with mean safety margin (figure 3.29).

The obvious flaw in the measurement of CSSM is that readings of pH_n and pH_v were only taken at weekly intervals. Variation could have occurred between these measurements, and the magnitude of the variation this would produce in the safety margin on a daily or hourly basis is not known. It could be possible that CSSM does not give a better indication of the average safety margin environment to which a catheter is exposed than does mean safety margin for the full study period. It is known that pH_v undergoes a small diurnal variation in both normal subjects and those with long term catheters, being higher in the early morning [223]. Measurements were taken at the same time of day for each participant to account for this in this study. The possibility of diurnal variation in pH_n has not been studied, however. It is also

clear that for some catheters in very rapid encrusters, no readings were taken during its lifespan. These would have to be excluded from analysis. For others, the reading was taken at the start of a catheter's lifespan, but it was changed immediately before the next reading, which happened to give a markedly different safety margin. It could not be confidently assumed that the reading taken at the start of that catheter's lifespan represented the environment to which it was exposed.

It was also considered possible that the variation in catheter types used by participants may obscure a correlation. The analysis was repeated using only catheters of the most common types, but no improvement in the correlation was evident.

4.2.4 The Blocking pH

The concept of blocking pH, as used by many District Nurses in the Bristol area, relies on the hypothesis that pH_v will gradually increase over the lifetime of a catheter, that this will be measurable, and that above a certain value catheter blockage will be imminent.

The results shown in tables 3.12 to 3.16 demonstrate that even when there appears to be such a pH_v rise, it is small in magnitude. Even for those severe encrusters with the greatest mean pH_v changes, this severely limits the clinical usefulness of this method of predicting catheter blockage. For all catheters the mean difference in pH_v is only 0.39 pH units. For those with the heaviest encrustation the mean difference in pH_v is greater, but is still just 0.81 pH units. A standard urine dipstick as used by most District Nurses is calibrated in intervals of 0.5 pH units, so using this as a method of registering these small changes would be unreliable. Even using a more accurate measure of pH_v may not prove useful given the variability of individual pH_v changes

around these means (sample standard deviations of change in pH_v were 1.12 for all catheters and 1.06 for 28 day blockers) set against the background week to week variability in pH_v already described (see figure 3.25). It seems likely that such an inconsistent increase in pH_v seen before catheter blockage, at least measured by weekly urine sampling, would be disguised by the noise of the normal variability in pH_v . It may be that this could be overcome by more frequent pH sampling and by calculating a moving average to smooth out the variability, perhaps combined with a more accurate method of pH measurement than a urine dipstick. This would require increased effort and expenditure on the part of catheter users and care staff.

Another problem is that while the pH_v did, on average, increase slightly during the lifespan of a catheter, there did not seem to be an obvious 'blocking pH' in any given participant which could be used as a cut off value to signal that the catheter should be changed. It may be that this would become apparent with more catheter samples from each participant, or that a rise in pH above the baseline for each catheter taken at the time of catheter insertion could be used.

From the data collected in the present study, it is clear that when there is an increase in pH_v with catheter life, this is too small to be picked up on the measuring devices used by District Nurses, and, more importantly, smaller in magnitude than the normal variability in urinary pH_v readings. This could explain the apparent success of this technique in those areas where it has been used. Once a blocking pH is set, the normal random variability in pH_v will tend to produce a reading higher than this value before the catheter encrusts to the point of blockage. This will trigger a catheter change, and the result will be that catheters are changed before they block, apparently validating the technique. It is possible that the pH_v would have fallen the next week in any case as a result of random variation. Although catheters will probably be changed before they block, they will likely be changed long before this

would have occurred. The technique really results in catheter changes which are essentially random, but for many catheter users, at a higher mean frequency than catheter blockage would normally occur. The standard recommended technique of establishing a mean catheter lifespan and changing the catheter slightly more often than this [46] would be expected to give as good a result, but with fewer catheter changes, reducing health care expenditure and inconvenience to the catheter user.

A more direct trial of the scientific basis behind this technique would be to establish a blocking pH, but still allow a catheter user to keep their catheter in place until blockage. The difference in the time at which the blocking pH is reached and the time at which the catheter actually blocks could be evaluated to determine if there is any useful relationship between blocking pH and catheter blockage, or whether it really is as random as suggested by this study. At the very least, this technique must be trialled against the standard 'mean catheter lifespan' technique before it could be recommended.

4.3 Factors Influencing Nucleation pH

4.3.1 Crystal Formation

Hedelin *et al* measured the amount of phosphate precipitated onto catheters which had been removed from 11 long term catheterised patients after three weeks [223]. They found that there was a critical urinary pH of between 6.7 and 6.8. Those with a mean urinary pH above this level precipitated 10 times more phosphate onto their catheters than those with a mean pH below this. This concept of a critical pH of 6.8 above which a catheter user will become a catheter blocker is widely quoted in articles on management of catheter blockers as almost a definition of the problem. Experiments on model systems by Cox *et al* using artificial urine supplemented with urease showed precipitation of calcium phosphates and then struvite as the pH rises above 7.2 [147]. Choong *et al* measured the nucleation pH of catheter blockers and non-blockers, as defined by whether their catheters lasted longer than six weeks or not. The mean pH_n of blockers was 7.58, similar to the 7.66 measured in the non-blockers [222].

These figures have all been taken as static values, both within an individual patient and between different patients. While it is certainly true that catheter blockers have a higher mean urinary pH than non-blockers [150,151], and that precipitation of the phosphate containing crystals of encrustation occurs at high pH [227], it is not evident from the experimental literature that the pH above which precipitation occurs is fixed.

It is useful to describe the theory of crystal formation. This is detailed in many chemical and urological texts [227-229], and is summarised here.

As described earlier, crystallisation depends on the availability of the ions which make up that crystal, not on the pH, although the pH may influence the availability of some of these ions. Whether crystal formation (*nucleation*) or crystal growth (*aggregation*) occurs in a solution is determined by the product of the concentrations of the constituent ions which form the crystal. This is known as the *ionic product*. A more concentrated solution has a higher ionic product. Changing the concentration of only one of the ions which make up the crystal will change the ionic product, and if any of the constituent ions is missing from the solution the product will obviously be zero.

Consider a system in which solid crystal is added to an aqueous solution of the constituent ions of that crystal. At a low ionic product, the solid will be completely dissolved. Any solid crystal added to the solution will lose ions from its surface at a faster rate than they are deposited, so the crystal will dissolve. If the concentration of ions in solution is increased to a certain critical point, ions from solution will be deposited onto any solid crystal at exactly the same rate as they are lost. The mass of any solid will remain constant as the system is in a stable equilibrium. This is known as a *saturated* solution. The value of ionic product at which this occurs is termed the *solubility product*.

At values of ionic product above the solubility product, any added crystals will increase in mass as deposition onto their surface will be faster than loss of ions to solution. This will occur until enough material has precipitated out of solution so that the ionic product of the ions remaining in solution drops to equal the solubility product. In this situation, the ionic product cannot exceed the solubility product for any length of time.

Interestingly, if a solution can be produced that has an ionic product greater than the solubility product, but that contains none of the solid crystal, spontaneous precipitation of solid will not necessarily occur. A solution in which the ionic product is maintained above the solubility product without crystal precipitation is known as a *supersaturated* solution. Such a solution could obviously not be produced by adding solid crystal to a saturated solution. It is possible, though, if ions are added in a form different from the crystal under study. For example, adding extra chloride ions in the form of sodium chloride to a saturated solution of silver chloride will not cause the precipitation of silver chloride crystals, even though the ionic product of silver chloride will now be greater than the solubility product, because no solid silver chloride was ever present. If the ionic product is increased by the addition of individual ions, for example by the ion pumps present in the cell membranes of the renal tubules, the same effect occurs. Precipitation does not occur because there is no solid crystal on which to precipitate, and a supersaturated solution may be formed.

There is, however, a limit to the degree of supersaturation that is obtainable. Once the concentration of the solution increases further it encounters another critical level above which spontaneous precipitation of crystals occurs. This spontaneous precipitation is known as *homogeneous nucleation*, and the ionic product above which this occurs is known as the *formation product*. Solutions with ionic products above the formation product are unstable.

The solubility product of a solution is thought to be a constant (at a given temperature), although experimentally measured values of the more complex crystals found in biological systems show some variability. The value of the formation product is quite variable, for reasons that are not fully understood.

Between the solubility product and formation product, in a supersaturated solution, crystallisation may occur under certain circumstances. Crystal growth will occur if crystals are already present. Crystals may precipitate onto the surface of other solid materials which may be added to the solution, such as cellular debris, foreign objects or other species of crystal (this is known as *heterogeneous nucleation*). Spontaneous nucleation may also occur, but is very slow. In these solutions, certain substances known as nucleation inhibitors can prevent crystal formation by binding to tiny crystals before they can aggregate (there is also another group of substances that can reduce crystal formation, but these work by chelating ions such as calcium, so are actually reducing the ionic product). Above the formation product, these inhibitors tend to be overwhelmed.

In the case of crystal precipitation in the human urinary tract, many healthy individuals have urine which is supersaturated with respect to various minerals which make up renal calculi, yet they do not form stones. Some combination of the presence of nucleation inhibitors, the absence of heterogeneous nucleation sites, and the rapid transit of urine out of the body before spontaneous nucleation can occur keeps most individuals free of stones.

In catheterised individuals, precipitation could occur even if the urine is only supersaturated. It is possible that the catheter, the biofilm organisms, or the extracellular polysaccharide matrix may act as heterogeneous nucleation sites. Small pockets of urine may remain pooled for long enough for spontaneous nucleation to occur. The biofilm may act as a microenvironment in which the increased concentration of certain species could raise the ionic product above the formation product locally, even if the ionic product is lower in the bulk of the urine. Once crystallisation starts it will continue until the ionic product drops to the solubility product, which is unlikely as the urine will be being continually refreshed and urease

will continue to act on it. Once started, catheter encrustation will probably continue to form.

Dumanski *et al* conducted a study on the ability of the extracellular polysaccharide produced by *Pr. mirabilis* to influence struvite formation [230]. They used a model system with purified capsule polymers from several organisms including *Pr. mirabilis*, *Kl. pneumoniae* and *E. coli* in artificial urine, with the pH raised using ammonium hydroxide. They found that the *Pr. mirabilis* capsule polymers enhanced struvite precipitation at moderately raised pH, up to 8.5, compared to controls or the capsule polymers of other species. They actually found that *E. coli* polymer inhibited crystal formation. They theorised that the anionic bacterial polymers attract the magnesium cations. Strongly anionic polymers, such as those produced by *E. coli*, will bind the magnesium, chelating it and so preventing it from complexing with other ions to form crystals. The weakly anionic *Pr. mirabilis* polymers, however, will attract the magnesium ions and concentrate them in a localised area, raising the ionic product, but without binding them so tightly that they are unavailable to form crystals. At very high pH, struvite precipitation will occur rapidly even without this concentrating effect, due to the increased concentrations of the other ionic species which form struvite.

The ionic product of struvite is given by multiplying the concentrations of the constituent free ions:



In the case of the precipitation of struvite in urine containing urease, it is thought that the huge amount of ammonium generated raises the ionic product above the formation product, making spontaneous nucleation inevitable provided the pH is high enough to provide some dissociation of hydrogenphosphate species to free

phosphate ions. Studies and calculations by Elliot *et al* suggest that struvite will not precipitate below a pH of 7.1 [231].

The ionic product of apatite involves raising the concentration of the free ions to the power which represents their relative abundance in the crystal matrix:



As can be seen, changes in concentrations of calcium and phosphate produce correspondingly greater changes in ionic product. In the case of carbonate apatite, as either carbonate or phosphate ions can be incorporated into the matrix, both their concentrations contribute to the ionic product.

It can be seen from this outline of crystal formation that there are factors which affect the likelihood of crystal precipitation which are not pH dependent.

The concentration of free magnesium and calcium ions are largely dependant on the concentrations of total dissolved calcium and magnesium (the calcium and magnesium which is not ionised exists as stable ion pairs with positively charged species, from which, in most cases, they can easily dissociate). The total quantity of phosphate species available to dissociate may be fixed, but the proportion of these species which exist as free phosphate ions is pH dependent as described earlier.

Ammonium, and much of the carbonate present, is derived from the urease catalysed hydrolysis of urea. All of these may vary in concentration at any particular pH. At a given pH, for example, ionic products and the chance of crystal precipitation will be lowered in more dilute urine. By varying the concentrations of the non pH dependent species in these ionic product equations, the concentrations of the pH dependent

species needed, in other words the pH needed, to achieve a total ionic product of a particular level will change.

Urine may also vary in its concentration of crystallisation inhibitors and chelating agents. Glycosaminoglycans, protein macromolecules and a variety of low molecular weight substances are thought to prevent precipitation in supersaturated urine, although the exact mechanisms of action have not been determined. They may prevent initial crystal nucleation, or bind to formed crystals to prevent further growth. A lack of these factors has been suggested to lead to stone formation in susceptible individuals, although the contribution which this has to the overall burden of stone disease is not known. Most of this work has also been done with regards to non-infective urinary tract calculi, although pyrophosphate, citrate and albumin have been shown to inhibit struvite growth in some *in vitro* studies [230,232,233].

Alterations in these factors would provide a reason for why urine from different individuals may precipitate crystals at differing pH levels, thus explaining the differences in nucleation pH measured.

4.3.2 Determinants of Nucleation pH

It would seem intuitive that the concentrations of calcium and magnesium in the voided urine would correlate with a urease positive catheter user's propensity to form encrustation, as their supply is not virtually unlimited as is the case of ammonium, or dramatically increased by small rises in pH as is the case with phosphate, carbonate and hydroxide.

As expected, in an *in vitro* study using human urine incubated with urease, Hugosson *et al* found a positive relationship between the precipitation of magnesium ammonium

phosphate and calcium phosphate, and the urinary concentration of magnesium and calcium, respectively [234]. However, in studies comparing the urine of catheter blockers and non blockers, no differences in calcium or magnesium concentrations were found by Choong *et al* [148], or Getliffe [46], but significant differences in urinary calcium were found by Kunin *et al* [45], and Burr and Nuseibeh [150]. This variation in results may be because the non-blockers in these studies were not colonised by urease positive organisms and so were unlikely to encrust irrespective of their urinary composition.

In the present study of catheter encrusters with persistent *Proteus* colonisation, there was found to be a significantly higher urinary calcium concentration in rapid encrusters as compared with slow encrusters (table 3.7), and a significant correlation between mean calcium concentration and mean catheter lifespan (figure 3.26).

What the concentration of calcium is affecting is the propensity of the urine to precipitate crystals of carbonate apatite, which is reflected in the nucleation pH. The causative relationship is shown by the addition of calcium, in the form of calcium chloride, to samples of uninfected human urine, which decreases the nucleation pH [206]. The same effect was seen with the addition of magnesium, also as a chloride. As changes in both magnesium and calcium concentrations, along with variations in other factors, should influence the propensity of crystals to precipitate, nucleation pH as measured by optical density changes should provide a more valuable measure of crystallisation than calcium concentration alone.

The study showed that it is possible to have a *Proteus* infection leading to a rise in pH above the levels at which encrustation should occur, but to have few problems as a result, due to having urine possessing a high nucleation pH. One avenue for future treatment is to accept that voided pH cannot be lowered in the presence of urease

positive organisms, that urease positive organisms may not be eradicated, and that urease itself may not be inhibited, but that the nucleation pH may be raised sufficiently to alleviate the problem of encrustation.

The multiple linear regression analysis showed that both calcium and magnesium concentrations were important determinants of nucleation pH in this group of patients (table 3.18). Calcium was found to have a greater influence than magnesium on pH_n . Certainly, calcium containing carbonate apatite crystals precipitate at lower pH than the magnesium containing struvite [148], and so could be expected to have more influence on the pH above which encrustation occurs. There may be considerable therapeutic value in altering calcium concentrations, or those urinary constituents which chelate calcium. The regression model also seems to show that the effects of calcium reduction will only be pronounced if urinary magnesium is also low, due to the interaction term. Lowering both of these constituents may be necessary to achieve any noticeable improvement. In catheter users where both these concentrations are high, such as those with a low urine output, improvement may be impossible.

4.3.3 Calculated Theoretical Variability of Nucleation pH

The equation derived in section 3.3.2 can be used to predict the possible effects of varying urinary constituents on the nucleation pH:

$$pH_n = 10.55 - 0.606 [Ca] - 0.130 [Mg] + 0.027 [Ca]*[Mg]$$

This relationship shows that over the commonly encountered range, as the concentration of calcium or magnesium in the urine increases, the pH_n of the urine

decreases. This effect is markedly more pronounced for calcium concentration than for magnesium concentration.

This effect of a change in the concentration of one element is less pronounced, however, in the presence of a pre-existing high concentration of the other element. For example, if the magnesium concentration is high, increasing calcium concentration results in a less pronounced lowering of pH_n than if the magnesium concentration is low. At even higher concentrations, this retardation of the effects of concentration change would actually become a reversal of the effect. According to the equation, while in the range of magnesium concentrations above 22.1 mM, increasing calcium concentration would be predicted to increase pH_n rather than decrease it. With calcium concentrations remaining above 4.7 mM, increasing magnesium concentrations would be predicted to increase pH_n . Mean magnesium concentrations this high are not found in the data, but mean calcium concentrations above 4.7 mM do occur.

Therefore, when using the equation within the range of data found in the study participants, it can be seen that the effect of a change in calcium concentration is an inverse change in pH_n , whereas a change in magnesium concentration will affect pH_n to a lesser extent but in either direction. This is shown in figure 4.1, where the reversal of the effect of magnesium is seen at high calcium concentrations.

Figure 4.1: Predicted effect of changing calcium concentration in the presence of a magnesium concentration of 10 mM (dotted line), 13 mM (dashed line) and 16 mM (solid line)

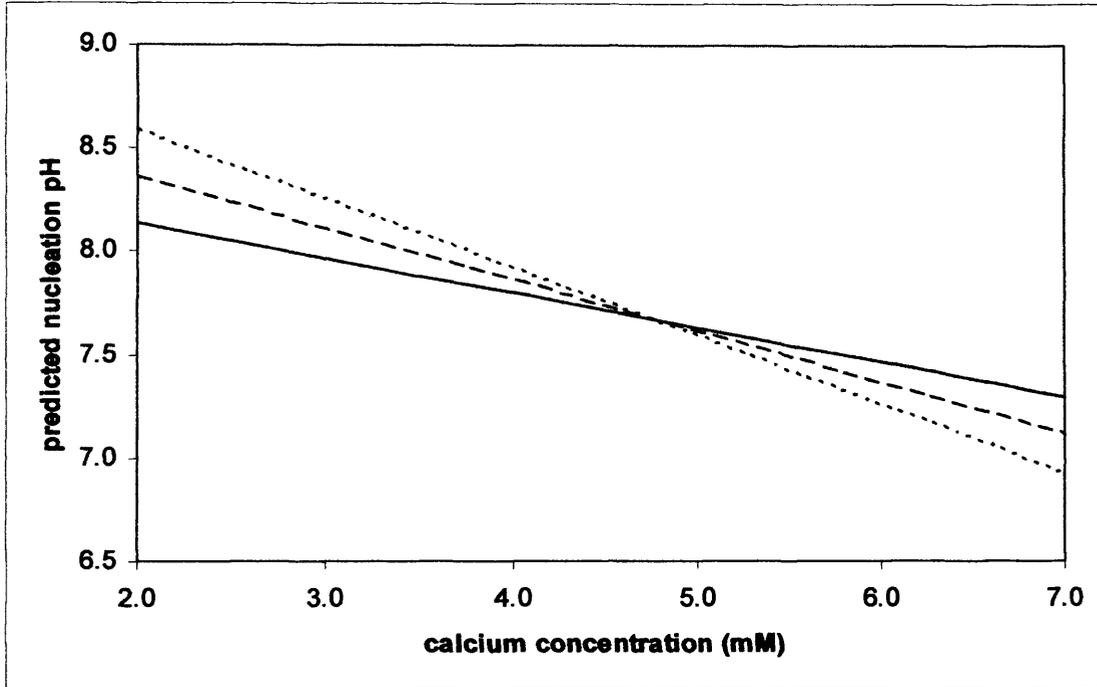
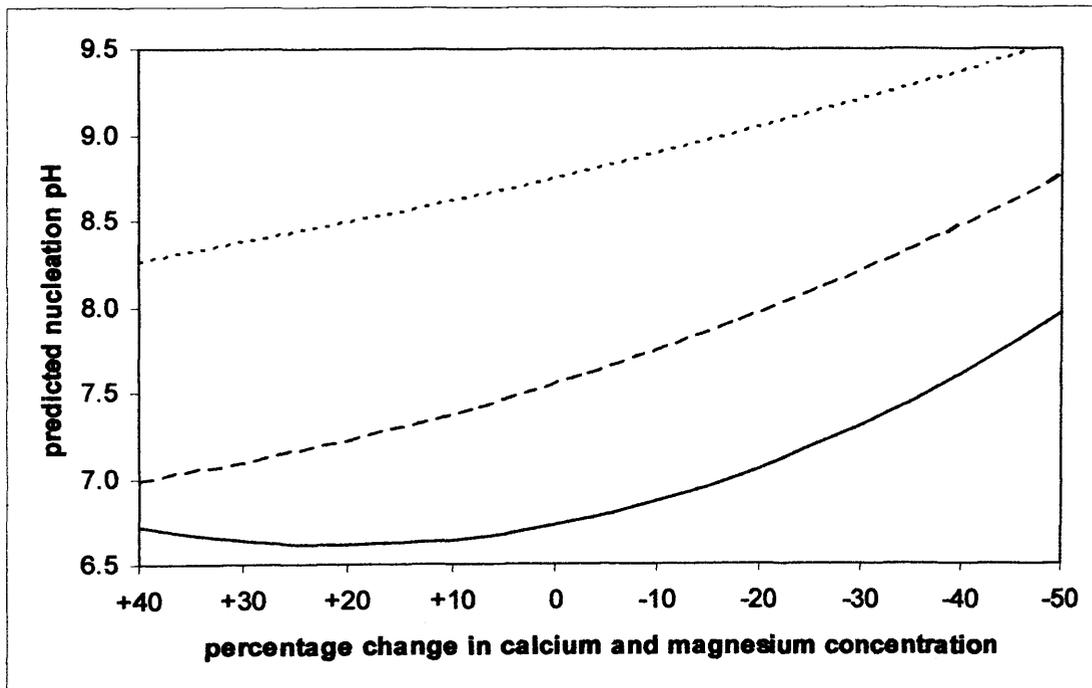


Figure 4.2: Predicted change in nucleation pH as concentrations of calcium and magnesium are varied in proportion from the mean figures for participants 02 (solid line), 16 (dashed line) and 06 (dotted line)



The effects of varying both calcium and magnesium concentration simultaneously, and in proportion, are demonstrated in figure 4.2. This may simulate the effects of diluting or concentrating the urine by varying fluid intake. The initial concentrations of solute were chosen from participants 02, 06 and 16 and give the pH_n results shown at the value 0 on the x axis. As the solutes are diluted, pH_n is predicted to increase. The majority of graphs generated from the participants' results are similar to those for participants 06 and 16, with slight variations in gradient, intercept and degree of curvature. They predict that a rise in pH_n of around 0.3 to 0.4 pH units could be achieved by a 25% reduction in calcium and magnesium concentration in most cases, and a rise of 0.8 to 1.0 pH units with a 50% reduction. For comparison, the difference in mean safety margin between the rapid and slow encruster groups was 1.04, and the difference in mean pH_n was 0.71.

Interestingly, the graphs created using a few participants' results, such as that for participant 02, demonstrate the possibility of pH_n increasing with more concentrated urine within a range which may occur clinically. This could be a real phenomenon, or it is possible that it is an artefact of an incomplete mathematical model. The model may not be accurate for values outside, or at the limits of, the range of variables used to derive it. It may be imperfectly simulating the behaviour of urinary variables other than calcium and magnesium which may not be diluted proportionally, or may have an effect on pH_n which is not directly proportional to their concentration. Further experimental work is needed to refine the model, although the potential for altering pH_n by varying calcium concentration over the range found in the majority of subjects in this study is clear.

It should be noted, however, that the values for calcium concentrations found in the present trial are higher than those found in some other studies. Mean values of 2.2 to 4.4 mM are reported from catheterised populations with standard deviations of 1.0 to

2.2 mM [46,149,150]. This compares with a mean of 5.48 mM (median is 4.59 mM as the data is skew) and standard deviation of 3.42 mM in this study. A greater difference is noted in the magnesium concentrations, although these values have been less frequently reported in the literature on catheter users. Concentrations recorded in this study (mean 13.3 mM, standard deviation 3.8 mM) were considerably higher than those found in other literature on catheter users (mean 1.91 mM, standard deviation 1.54 mM [46]), although higher values are found in studies on normal populations (mean 3.91 mM, standard deviation 2.5 mM [234]) and there is considerable overlap in the ranges. For comparison, examples of the values used in artificial urine recipes during *in vitro* studies on encrustation are calcium 4.3 mM and magnesium 3.2 mM [157]. The obvious cause for differences between the results would be differences in the characteristics of the populations being sampled. A sizeable proportion of the catheter users in the present study were elderly nursing home residents with very limited mobility and low fluid intakes compared to the general population. The other studies on catheter encrustation quoted have looked at either spinal cord injured patients, who are generally young and fit and often quite mobile despite their injuries, or catheter users living independently or in warden controlled accommodation who would have had considerably less co-morbidity than the group in the present study. Differences in diet and medication which could alter urinary composition would also be expected between these populations. The result of these differences, however, may be that there is less potential for reducing encrustation by urinary dilution in these other populations as compared to those studied in this present trial. As all the studies, including the present one, used small numbers of participants, it is not clear that the typical range of urinary calcium or magnesium for catheter users has been determined.

4.3.4 Theoretical Inaccuracies of the Nucleation pH Method

The experimental method used in this, and other, studies for determining the propensity of urine samples to precipitate catheter blocking crystals has limitations.

The pH of the urine test samples in the experimental protocol was obviously not altered by varying their hydrogen ion concentration in isolation, but by the addition of chemicals which would have this effect. These chemicals, hydrochloric acid and sodium hydroxide, are not the substances which would alter the pH *in vivo*. A decrease in hydrogen ion concentration in the urine of a catheter blocker *in vivo* would be accompanied by a rise in the ammonium concentration, but in the test protocol *in vitro* it would be accompanied by a rise in sodium ion concentration. Sodium ions do not contribute to the ionic product of the crystals of catheter encrustation, but ammonium ions do. This would result in the pH_n derived experimentally being higher than the pH at which crystals would tend to precipitate *in vivo*. This discrepancy may be more pronounced in those samples where the measured pH_v was furthest below the pH_n , as to cause crystal precipitation more sodium ions would need to be added experimentally, as opposed to ammonium ions being produced naturally, than in those samples where the pH_v was close to the pH_n . The degree to which this actually affects the results is unclear, but could be checked by repeating the experiment using ammonium hydroxide in place of sodium hydroxide.

It should also be noted that the reactions involved in crystal precipitation are not instantaneous. The time taken for the products to reach equilibrium following addition of alkali to raise the pH may be long enough that different results would be obtained if the time taken to complete each step of the protocol varied significantly between batches. If the protocol were run through very quickly the crystals may not have time

to precipitate before the optical density reading was taken, and the pH_n reading would be erroneously high compared to if the sample were left to equilibrate for a long period of time between each alkali addition. Although timings of alkali additions were not standardised in this study, the time taken between additions was approximately equal. However, for future work it would seem sensible to include more rigid timings in the protocol design.

4.3.5 Alternative Methods of Estimating the Potential for Crystal Precipitation from Urine

The present study has deliberately adopted an empirical approach to the measurement of crystal precipitation: the pH of a urine sample was altered experimentally to determine the point at which crystallisation occurred and this nucleation pH value was correlated with the catheter lifespan and the concentrations of two of the chemical species present in urine. An alternative strategy is to calculate the likelihood of crystal precipitation for a urine sample mathematically based on the concentrations of chemical species present and known thermodynamic principles.

Urine contains many chemical species participating in various equilibrium reactions in addition to those discussed earlier. Indeed, most species are involved in more than one equilibrium reaction. For any given species, a proportion will be complexed to various other species in a variety of chemical compounds, and the remainder will be free in ionic form and therefore contributing to the ionic product of one or more crystal varieties. For any single equilibrium reaction in a dilute solution it is a relatively simple matter to calculate the ratio of complexed to free ions given the total amount of the species present and the *equilibrium constant* of the reaction. This constant can be thought of as the tendency of the reaction to occur in a particular direction, and will indicate the position of the equilibrium in terms of the proportion of products and the

proportion of reactants in a mixture which has been left to settle to a steady state. With multiple species participating in multiple reactions in more concentrated solutions the calculations to determine the free ion concentrations and hence the ionic products become much more complicated.

In order to perform the complex calculations required in biological systems, computer based speciation programs are available. The programs EQUIL2 and JESS have both been used to model the risk of formation of the common non-infective calcium oxalate urinary tract stones [235,236]. As an example, EQUIL2 requires the input of the total concentrations of Na, K, Ca, Mg, NH₄, PO₄, SO₄, C₂O₄, citrate, urate, pyrophosphate, CO₂, Cl in a sample, along with the solution's pH [237]. The program then calculates the concentrations of free and complexed species using a database of thermodynamic parameters and an iterative mathematical technique. From this the ionic products for the common crystal types are calculated, and compared to a database of solubility products to determine whether the solution is supersaturated for any crystals. The degree of supersaturation can be quantified to give the tendency of the solution to precipitate crystals, either as a simple supersaturation ratio, or by calculating a thermodynamic potential known as the *Gibbs free energy* of the transfer of the supersaturated solution to one which is saturated and contains crystals. More recent updates of the EQUIL software model crystal surface chemistry effects as well as the chemical equilibria in solution [238].

Like nucleation pH, the degree of supersaturation for struvite or apatite should indicate which urines might be at greatest risk of precipitating crystals, and this should be expected to show a correlation with the catheter lifespan. The theorised effects of dilution of urine could also be studied in a similar way to that using the regression model in the previous section, by varying the concentrations of urinary constituents used as input data.

The disadvantage of using a speciation program, as opposed to measuring precipitation empirically, is that it obviously cannot predict the effects of any substances not included in the calculation. Many urinary constituents are not accounted for in the mathematical model, including some proposed nucleation inhibitors such as proteins and glycosaminoglycans. While it is clear from the r^2 value of the regression model that some factors affecting the nucleation pH are not being accounted for, it is not necessarily the case that these would be accounted for in a speciation program model. In addition, many more urinary constituents must be measured to use these programs which complicate the experiments, certainly beyond the scale of the current study. The speciation programs also model the state of urine at equilibrium, and given the continuous flow through the urinary tract it is likely that not all reactions have time to establish equilibrium (calcium oxalate equilibrium, for example, requiring 6 to 10 hours [239]) which would lead to the estimated free ion concentrations being inaccurate to some degree. However, it is probable that in the *in vitro* nucleation pH test the equilibria are not at the state found in the urinary tract.

The advantage of speciation programs is that they allow changes in crystal precipitation to be explained using well established chemical principles rather than simply observed, especially in terms of changes in chemical species only indirectly involved in crystal formation which may nonetheless affect free ion availability for the crystals of encrustation in ways which would be unexpected without this fuller consideration of the complexity of urinary chemistry. Even so, this sophisticated chemical model would still be expected to fall short of a comprehensive description of a crystalline bacterial biofilm. Both empirical and theoretical methods have the common failing that they measure what occurs in the bulk urine, which may differ from conditions found in the biofilm matrix.

4.4 Urease Activity

The urease enzyme is probably the most important factor in the production of catheter encrustation, providing both ammonium ions for struvite formation and hydroxide ions to raise the pH. Strains of *Proteus* isolated from the study participants were tested for urease activity to test the hypothesis that catheter users colonised with high urease activity strains would encrust more rapidly.

In a study of the levels of urease activity of different strains of *Pr. mirabilis*, Mobley and Chippendale found no difference in those isolated from the urine of patients with symptomatic acute pyelonephritis, the urine of those with catheter associated bacteriuria, and 20 faecal samples [240]. Sabbuba *et al* also found no difference in urease activities between isolates from retrieved catheters, catheter associated bacteriuria, other clinical sources, and environmental sources [226]. This group did find, however, a significant positive correlation between urease activity and the ability of a strain of *Pr. mirabilis* to encrust test catheters in an *in vitro* bladder model using artificial urine, with encrustation measured as the summed mass of calcium and magnesium deposited within defined sections of the catheter lumen over the test period or per hour.

It could be argued that the lack of difference between the urease activities in rapid and slow catheter encrusters in the present study (table 3.20), and between isolates from different environments in the other studies mentioned above, is an artefact of the method used. The cells tested were grown in suspension in Luria broth in a shaking incubator, and were therefore in their planktonic form. *In vivo*, cells are present in both the planktonic and biofilm mode. As described earlier, the change from planktonic to biofilm form results in changes in protein expression [95], and this

may include a change in the rate of urease production. Urease production is known to increase in the swarmer state of *Pr. mirabilis* as compared to the vegetative or swimmer states [194]. If the biofilm form of *Proteus* expresses different levels of urease activity, and these are not proportional to those levels expressed in the planktonic state measured in this protocol, then this would explain the lack of correlation.

It could be that the experimental set up does not represent the clinical situation in other important respects. The enzyme assay involves a reaction in a static, fixed volume of solution over a fixed period of time. In the clinical situation the reaction is taking place in a flow through system in which the substrate is being continuously supplied. The environment in the biofilm may differ from that in the bulk of the urine, and may even expose the urease enzyme to a high local pH which could radically reduce its activity.

If these factors were significant criticisms, however, it does not seem likely that a correlation would have been achieved in the case of Sabbuba's experiment with catheter encrustation in the artificial bladder model. This result would seem to validate this urease assay technique, as there were no obvious variables other than urease activity. It seems more likely that in the clinical situation other factors are a more important determinant of encrustation than urease activity. In the artificial urine model, factors such as urinary ion concentrations would be standardised, and host immune defences and possible nucleation inhibitors would be entirely absent, yet these may play a vital role in the clinical setting.

It could be that in a clinical setting, once a low threshold activity has been crossed, any amount of urease will be sufficient to cause severe encrustation if other factors are favourable. It is also possible that in a polymicrobial setting, other organisms in

the catheter biofilm may produce substances which could inhibit urease. This is discussed in more detail in section 4.5.

4.5 Behaviour of Bacterial Populations

The individuals in the study were not selected randomly, but because of the presence of urease positive organisms in their urine, and in particular, the presence of *Proteus* spp. Even so, the other organisms present in these individual's urinary cultures were typical of those previously found in long term catheter users: *Ent. faecalis*, *E. coli*, *Ps. aeruginosa*, *Kl. pneumoniae* and *Staph. aureus* being isolated as persistent urinary tract colonists in many cases [38,70,116]. The less common *Providencia*, *Morganella* and *Serratia* were also seen, again as persistent urinary tract colonists.

A casual study of table 3.21 perhaps suggests that slow encrusters are more likely to have persistent co-infection with other organisms, such as *E. coli*, *Ps. aeruginosa* and *Kl. Pneumoniae*. These differences in frequency are not statistically significant, but this seems worthy of further investigation. The phenomenon of bacterial interference, discussed in section 4.1.1 with regards to participant 19, may provide a reason to expect a difference in frequency of stable co-infections between these groups. As establishing that an individual is a rapid encruster and that their various co-infecting organisms are stable involves considerable time in a clinical setting, it may be worthwhile conducting such a study in a laboratory encrustation model initially.

Mobley and Warren conducted a survey of long term catheter users using weekly urine samples [241]. They found that the most commonly isolated organisms were *Pr. mirabilis* and *M. morgani*, each found in more than half of all specimens. They found that *Pr. mirabilis* was conclusively associated with the problem of catheter blockage, but that *M. morgani* had a more complex role, perhaps acting in some way to inhibit catheter encrustation even in the presence of high counts of *Proteus*. Other urease positive organisms were not associated with catheter blockage.

It may be that as well as restricting the growth of other species of bacteria, and so, in the case of *Proteus* numbers being diminished, reducing the amount of urease available, organisms might act to reduce encrustation in other ways. Some bacteria may alter urinary composition or pH. For example, some strains of *E. coli* can break down citrate. Citrate has been shown in a study by Mclean *et al* to retard the *Pr. mirabilis* induced growth of struvite crystals in artificial urine [232]. The presence of *E. coli* might therefore increase the catheter encrustation caused by *Proteus*. However, an *in vitro* study by Edin-Liljegren *et al* demonstrated that urease added to human urine inoculated with a strain of *E. coli* produced less ammonium, less of a pH rise, and fewer precipitating crystals than urease added to sterile urine [214]. It was found that this *E. coli* did not alter urinary pH, phosphate, magnesium, calcium, ammonium or citrate in the absence of urease, and it was concluded that the organism was not exerting its effect by altering urinary composition. It was suggested that a direct action on urease may be occurring. It should be noted that Jack bean urease was used in this experiment, rather than *Proteus* urease or *Proteus* bacteria.

4.6 Antibiotic Sensitivities

4.6.1 Resistance of Urinary Isolates

There was no significant difference in antibiotic sensitivities between the *Proteus* isolates derived from catheterised and non-catheterised individuals sent to the local hospital pathology laboratory (tables 3.24 and 3.25). This could possibly be explained if the majority of these individuals had only short term catheters. They would not have had time to develop the multi-organism colonisation characteristic of long term catheter users. They may not have been previously exposed to antibiotics, so the resistance profile of their infecting organisms would echo that of the local population they originated from. Hospital laboratories also tend to report multi-organism cultures as 'mixed growth' and do not report sensitivities, assuming them to be contaminated samples. Isolates from long term catheters may have been missing from the local population data for this reason.

The antibiotic resistance of urinary organisms isolated from elderly people and residents of long term care facilities has been studied by several authors. The general elderly population share many similarities with the long term catheterised population, and indeed, a significant minority will use long term catheters. In order to acquire antibiotic resistance there must be a pool of organisms present to develop resistance, and exposure to suboptimal concentrations of antibiotic. In the population aged over 65, asymptomatic bacteriuria is observed commonly with a prevalence ranging from 5% in older men living independently in the community to 50% in some studies of long term care facilities [242]. These individuals are often treated on the basis of these urinary cultures, even if not suffering from a symptomatic urinary tract infection. They may also be treated for a presumed infection without cultures being

taken, due to the high incidence of chronic non-infective genitourinary symptoms also suffered by this group [243]. This population is also susceptible to other infectious diseases, such as respiratory tract infections, which may require antibiotic treatment. This may be given in such a way as to eradicate the infection being treated, but deliver suboptimal therapy to those organisms coincidentally colonising the urinary tract, thus leading to the development of a pool of resistant urinary organisms. Penicillins and fluoroquinolones are commonly used antibiotics for both respiratory and urinary infection. Respiratory infections are also commonly treated with antibiotics when, in fact, they have a viral origin. This elderly group is also more likely to need care by community medical staff, or to be admitted to a care facility, which allows transference of bacteria from other individuals who may have previously acquired resistant strains.

Chronic bacteriuria and chronic medical conditions leading to repeated antibiotic and medical care exposure are even more common findings in the long term catheterised than the general elderly population, as previously described, and so their risks of developing antibiotic resistance may be correspondingly higher.

Wingard *et al* conducted a study of levels of trimethoprim resistance of asymptomatic colonising gram negative bacilli from 67 residents in a nursing home setting [244]. The majority of organisms were isolated from urinary or perineal sources. They found that colonisation with resistant organisms was significantly associated with decreased mobility, poor functional status, length of stay and the presence of a urinary catheter. In multivariate analysis, however, the only independent predictor was functional status.

Other studies have looked at the rates of antibiotic resistance among those presenting with a symptomatic urinary tract infection, rather than merely having

resistant colonising organisms. Terpenning *et al*, looking at urinary tract infection with gentamicin and ceftriaxone resistant Gram-negative bacilli in a long term care facility, found resistant organisms significantly associated with intermittent and indwelling urethral catheterisation [245].

Wright *et al* conducted a study of patients presenting to a hospital emergency department with symptomatic urinary infection [246]. After multivariate analysis, the risk factors for infection with multi-drug resistant organisms were age > 65, recent or current antibiotic use, and the presence of a urinary catheter. In this study, the isolated urinary tract pathogen was multi-drug resistant (resistant to more than one of nitrofurantoin, co-amoxiclav, gentamicin, ampicillin, TMP/SMX or a quinolone) in 12 to 14% of otherwise healthy individuals under the age of 50. The multi-drug resistance rate for otherwise healthy individuals aged 65 or older was 23%, but for all individuals aged 65 or older it was 45%, and for those from long term care facilities it was 61%.

While these studies suggest that long term catheter users are at risk from multi-drug resistant organisms, the study of Wazait *et al* has examined the changing patterns of antibiotic resistance in urinary isolates from catheterised individuals over the period 1996 to 2001 in the United Kingdom [247]. The isolates examined were obtained in the same way as those local hospital pathology laboratory samples used in the present study, and suffer from the same problems. A large, and unknown, proportion of them were probably from hospitalised patients with short term catheters, rather than community based long term catheter users. Overall, organisms have shown increased resistance to cefalexin and ciprofloxacin over this period, while resistance to co-amoxiclav and nitrofurantoin has remained steady. Specifically, the organisms associated with short term catheterisation such as *E. coli* and *Enterococci* showed a marked increase in ciprofloxacin resistance, probably due to its increasingly frequent

use, but remain susceptible to nitrofurantoin. Bacteria associated with long term catheterisation, such as *Proteus*, which has consistently had almost universal resistance to nitrofurantoin, are still largely susceptible to ciprofloxacin. The most recent *Proteus* data from this paper, for the year 2001, is shown in table 4.1.

Two studies have looked at resistance rates for *Proteus* spp in residents of long term care facilities. Smith *et al* looked at routine isolates from those with urinary catheters, tracheostomies, gastrostomies or wounds across several different facilities in Nebraska and Iowa, USA [248]. 39 of 104 *Proteus* isolates were from the urinary tract. Muder *et al* conducted a five year survey of isolates from blood cultures of symptomatic individuals in a single large institution in Pittsburgh, USA, 15 of the 26 *Proteus* isolates were of a confirmed urinary tract origin [249]. Table 4.1 compares the results from these studies and the study of Wazait *et al* with those obtained from long term catheter users in the present study.

Table 4.1: Percentage of *Proteus* isolates resistant to various antibiotics from the studies of Muder *et al* (1992), Smith *et al* (2000), Wazait *et al* (2003), and the present study.

<i>Antibiotic</i>	<i>Muder et al</i>	<i>Smith et al</i>	<i>Wazait et al</i>	<i>Present study</i>
<i>ampicillin</i>	39%	2%	-	-
<i>amoxicillin</i>	-	-	39%	47%
<i>piperacillin</i>	22%	1%	-	-
<i>co-amoxiclav</i>	-	-	21%	0%
<i>trimethoprim</i>	-	-	46%	84%
<i>TMP/SMX</i>	46%	-	-	-
<i>nalidixic acid</i>	-	-	-	11%
<i>ciprofloxacin</i>	-	10%	4%	0%
<i>gentamicin</i>	8%	0%	-	16%
<i>cefalexin</i>	-	-	18%	-
<i>cefalothin</i>	21%	-	-	21%

Ampicillin and amoxicillin are closely related penicillin compounds having very similar spectrums of antibacterial activity. TMP/SMX is a mixture of trimethoprim and sulfamethoxazole, commonly used as a first line agent for uncomplicated urinary tract infection in the United States, but rarely used in the UK where trimethoprim alone is favoured. Piperacillin is a penicillin with increased activity against gram negative bacilli such as *Proteus* and *Pseudomonas*, and, again, would not be used routinely in the UK.

Although the antibacterials tested in the present study differ slightly from those in the American studies, they do reflect the antibiotic classes used commonly in the UK, just as those drugs tested in the American studies reflect those commonly used in the USA. They should therefore give an indication of resistance against those drugs likely to be used against the isolates in question. It seems as if antibiotic resistance among *Proteus* is a more serious problem in long term catheter users than even the high risk group in long term care facilities. It is comparable with the data from catheter users in the Wazait study in the UK, except for the higher trimethoprim resistance and lower co-amoxiclav resistance in the present study. There does seem to be a higher level of antibiotic resistance in the Wazait samples than in the hospital laboratory samples used in the present study, however. It is interesting to note that the Wazait results show a much higher resistance to co-amoxiclav, possibly reflecting different patterns of prescribing between the two areas, as well as perhaps a different selection of patients.

4.6.2 Effects of Treatment

As can be seen from table 3.29, the study participants received several courses of antibiotics, some for non-urinary infections, and some directed at the organisms in the urinary tract. All the agents which were used are usually active against a broad

range of urinary pathogens and, with the exception of minocycline, are commonly used to treat urinary tract infections.

Participants 06 and 10 were treated for respiratory tract infections with penicillins, with courses which would also have been adequate for treatment of an uncomplicated urinary tract infection. Although the previously consistent *Pr. mirabilis* cleared from participant 06's urine at this time, the strain was resistant to the amoxicillin used, suggesting that this may have been unrelated to the antibiotic. *E. coli* was also cleared, although it had only recently appeared in the urine. As this participant died before another urine sample could be collected, it is not known if the bacteria would have returned. Participant 10 showed no effect of co-amoxiclav on his urinary tract organisms, although his *Pr. mirabilis* was sensitive to it.

Participants 16, 18 and 21 were treated specifically for catheter associated urinary tract infection. Participant 21 was treated with trimethoprim which had no effect on urinary or catheter flora, and to which her *Pr. mirabilis* strain was resistant. Due to pre-existing antibiotic resistance, and the possibility of promoting more, it is recommended that catheter associated urinary tract infection should only be treated before the results of antibiotic sensitivities are known in the most severe cases [42], which this was not. Even then, trimethoprim is not recommended as empirical therapy due to the high levels of resistance among many uropathogens, and its use is even being questioned in uncomplicated infections [250]. Both participants 16 and 18 were treated with courses of ciprofloxacin, an effective treatment for even complicated urinary infection, and considered useful for empirical treatment due to low, although increasing, levels of resistance [247]. The *Pr. mirabilis* isolates in these individuals were both sensitive to ciprofloxacin, but in both cases, were not eradicated.

Participant 18's *Proteus* also survived a course of cefalexin (despite being sensitive

to the similar cefalothin) and clarithromycin. Other than participant 18's *E. coli*, the other bacteria present also survived these antibiotics.

It would seem that this study supports the view that biofilm resident bacteria have a decreased susceptibility to antibiotics which is not based on classical antibiotic resistance. While the planktonic bacteria sampled by urine culture would be eradicated by the antibiotic treatment, provided the correct antibiotic was chosen, organisms could survive in biofilm communities on the catheter. While these surviving organisms may not necessarily reinvade the patient's tissues to cause a recurrent symptomatic infection, this would be a risk. Raz *et al* conducted a randomised study of the technique of catheter replacement in combination with antibiotics or antibiotics alone in the treatment of long term catheterised patients with symptomatic urinary infection [251]. Replacing the catheter immediately before the start of antibiotic therapy was hypothesised to be beneficial for two reasons. Firstly, it would remove the protected biofilm organisms which could rapidly repopulate the urine. Secondly, it would allow a urine culture specimen to be taken from the new catheter which would sample only those bacteria which were planktonic. Those which were purely biofilm resident and therefore not responsible for symptoms would not be sampled, potentially giving fewer polymicrobial cultures and therefore allowing more accurately directed therapy. The most accurately directed therapy would of course be obtained if the infecting organism could be identified in the patient's tissues, but even this group, who advocate that treatment should be reserved for markedly symptomatic infections, only report successful blood culture in 25% of these cases. Patients were initially treated empirically with fluoroquinolones, and these were then changed if necessary on the basis of the urine culture results. The study found that catheter replacement did lead to less complex initial urinary cultures, a more rapid resolution of symptoms and a lower recurrence rate at one month.

Treatment of the participants in the present study did not follow the Raz protocol. Even so, In the Raz study, recurrence did sometimes occur even when the catheter was changed. Catheters are regularly changed in all patients and yet the new catheters are immediately colonised by, in most cases, the same organisms. Certainly, this occurs too quickly to be ascending bacteria from the perineum. Participant 16 had a catheter change the day after the start of a course of antibiotics to which his *Pr. mirabilis* strain was sensitive, yet, after the antibiotic course finished his new catheter was found to be colonised with *Pr. mirabilis* again. It would seem that there are other sources of bacterial re-colonisation than just the catheter. In cases where antibiotics are not used, this could just be planktonic organisms in small pools of residual urine, but in this case the numbers of species should decrease as biofilm resident only organisms would not reappear. In cases where antibiotics are used, the organisms must be in a protected environment. Suggestions for this could be bladder calculi, which have been shown to harbour the same *Proteus* strain as seen in the patient's catheter biofilm [49]. They could also survive in biofilm communities on the bladder mucosa, or possibly in small pieces of biofilm, crystalline or not, which break off from the old catheter during its removal. These may be more vulnerable to antibiotics than a calculus or well developed catheter biofilm, but this would still explain the difficulty in eradicating bacteriuria, even temporarily.

4.7 Comparison of Urine and Faecal Cultures

The present study was not primarily designed to assess the rate of faecal carriage of *Pr. mirabilis* in catheter users, and the numbers surveyed were small for this purpose, but it appears that isolation of faecal *Pr. mirabilis* may be more common among those with *Pr. mirabilis* urinary tract colonization than in the general population. The normal faecal carriage rates reported in the literature in studies by Muller [183], Peerbooms *et al* [180], Krickler [182], and Rustigian and Stuart [181] were, respectively, 2.7%, 8.5 %, 17%, and 24%, whereas in this study *Pr. mirabilis* was isolated in 56% (10 out of 18 samples). This may be because the organism is found more frequently in these catheterised individuals, or because it exists in larger numbers allowing it to be isolated more easily. It is not possible to say with certainty that those from whom we did not isolate faecal *Pr. mirabilis* did not have this organism in their gastrointestinal flora based on a single culture. Half of long term catheter users are not troubled with encrustation, and it is possible that this is because they are not faecal carriers of *Pr. mirabilis*, or that they carry it at low counts. In the current study there was an equal chance of a participant from whom faecal *Pr. mirabilis* was isolated being a rapid or a slow encruster. It could be hypothesised that faecal carriage of *Pr. mirabilis* is a risk factor for urinary colonisation and catheter encrustation, but that once colonisation is established other factors determine the severity of that encrustation.

Urinary tract colonisation usually occurs by the ascending route via the catheter, either through the internal drainage lumen as a result of contamination of the drainage apparatus, or along the external catheter surface by bacteria from the urethral meatus [109]. This external route is likely to be more important since closed drainage systems have become standard, as the lumen of the catheter is not exposed. However, it is usual in the United Kingdom to change catheter drainage

bags every week for long term catheter users, and this cannot be regarded as a closed system. Bacteria may be introduced by those changing the drainage bag. Long-term catheter users are frequently elderly or disabled and often reside in care facilities or have their catheter managed by visiting health care staff [24], who may facilitate transmission of uropathogens between patients [42]. *Pr. mirabilis* is, however, a common constituent of the normal human gastrointestinal flora, and a phenotyping study of catheter associated bacteriuria in short-term catheterisation has suggested a gastrointestinal origin for several common species [252]. The majority of catheter users develop *Proteus* colonisation and encrustation only after having been catheterised for many months or years, and so, conceivably, it arises from a different source than the initial colonising organisms. It is therefore unclear whether the origin of *Proteus* colonisation is mainly endogenous, from the patient's own gastrointestinal flora, or exogenous, possibly as a result of transmission by health care staff.

It has been shown previously that the *Pr. mirabilis* strains isolated from urine culture are identical to those found in the crystalline biofilms and bladder calculi which develop in those patients with catheter encrustation [49,191]. The current study suggests that the strains responsible for this urinary colonisation commonly originate in the catheter user's gastrointestinal tract. From there they most likely contaminate the perineum and urethral meatus, before ascending to the bladder along the external catheter surface and causing bacteriuria, biofilm formation and then encrustation. A technique to prevent colonisation with urease producing bacteria would therefore be of great benefit, even if bacteriuria with other species could not be prevented.

The origin of encrustation-causing urinary tract colonisation in individuals in the community or long term care facilities does not seem to be an exogenous source such as spread by health care workers in the majority of cases. Such routes of transmission are effectively managed by simple and well established techniques such

as hand hygiene. While this does not exclude the possibility of poor technique by care staff leading to contamination of the catheter drainage apparatus with faecal organisms, the demonstrated ease with which bacteria can ascend from the perineum to the bladder in the face of a rigidly applied closed drainage technique [99], and the ability of *Pr. mirabilis* to rapidly colonise any available catheter surface even in the presence of other organisms [146], leads to the conclusion that *Proteus* colonisation would arise irrespective of the level of care taken.

A solution to preventing colonisation from the perineum may be a regime of meatal cleansing or the application of topical antibacterial agents to this area. As described in section 1.4.4, these techniques have proven ineffective in short term catheterisation, or have actually promoted the emergence of urease positive or multi-drug resistant organisms. In these trials the goal has been to prevent the initial colonisation of the previously sterile urinary tract, but as the mechanism of colonisation is the same as in the acquisition of *Proteus* their results should be applicable. The acquisition of *Pr. mirabilis* reported in some of these studies suggests that this method of meatal care may actually increase the risk of catheter encrustation if this policy was adopted in long term catheter users.

The route of colonisation of the urinary tract in suprapubic catheters has not been studied in detail. Initial onset of bacteriuria seems to be delayed slightly with suprapubic as compared to urethral catheters in short-term use [133], but in the long-term, the same rates of bacteriuria are seen with the same type of organisms isolated. These results demonstrate a gastrointestinal origin for *Pr. mirabilis* in the five patients tested who used suprapubic catheters. It could be postulated that urethral ascension of perineal organisms occurs in these cases, as in the majority of non-catheterised individuals who develop urinary tract infection. However, in the current study two of the females with suprapubic catheters had undergone a urethral

closure procedure, eliminating this route of infection. It would seem that contamination of the cystostomy site may occur. It may be that cleansing of this site would be as ineffective as cleansing the urethral meatus, but further study in this area is warranted.

Section 5 - Conclusions

5.1 Conclusions

A summary of conclusions from the study

5.2 Future Work

Possible further investigation of the topics examined in this study

5.1 Conclusions

- There is considerable variation in mean catheter lifespan between individuals with *Pr. mirabilis* urinary tract colonisation, and within any particular individual. Lifespans ranging from less than 48 hours to over 3 months were recorded.
- Individuals exist who have persistent colonisation with *Pr. mirabilis* and yet have little or no problem with encrustation related catheter blockage.
- In catheter users with *Proteus* bacteriuria, rapid encrustation is associated with a lowered nucleation pH and a voided pH which is above or only just below their nucleation pH, rather than solely a raised voided pH. The difference in pH_v between rapid and slow encrusters is not significant, but the difference in safety margin ($pH_n - pH_v$) is. Rapid blockers had a mean safety margin of only 0.13, compared to 1.17 in those who encrusted more slowly.
- The pH_n is a variable factor both between and within individuals. Rapid encrusters had a mean pH_n of 7.3 compared to 8.0 in those who encrusted more slowly. This raises the possibility of altering pH_n as a treatment for catheter encrustation.
- While the pH_v of urine does seem to increase slightly between the start and end of a catheter's lifespan in those with *Proteus* colonisation and rapid encrustation, the 'blocking pH' method of predicting catheter blockage is not useable in its present form due to the small magnitude of this rise (0.8 pH units in the most rapid encrusters, 0.4 pH units overall) compared with the

normal variability of urinary pH, readings in an individual (standard deviations ranging from 0.12 to 1.56). Alkaline urine does not necessarily indicate that catheter blockage is imminent, or, indeed, that it will occur at all.

- In catheter users with *Proteus* urinary colonisation, the urinary nucleation pH is dependent on the calcium concentration and, to a lesser extent, the magnesium concentration of urine. Rapid encrusters have higher urinary calcium concentrations than slow encrusters. The calculated regression equation predicts that a rise in pH_n of around 0.3 to 0.4 pH units could be achieved by a 25% reduction in calcium and magnesium concentration in most cases, and a rise of 0.8 to 1.0 pH units with a 50% reduction.
- Strains of *Proteus* with higher urease activities do not necessarily produce voided urines with a higher mean pH, and there is no relationship between increased urease activity and a shorter catheter lifespan.
- Once acquired, *Proteus* appears to be a stable component of a polymicrobial urinary and catheter flora.
- *Proteus* isolates from long term catheter users have higher levels of antibiotic resistance, particularly to trimethoprim and amoxicillin, than those from urinary infections in the general population. Courses of antibiotic appear ineffective at altering the urinary flora.
- The source of *Pr. mirabilis* urinary colonisation in long term catheter users is commonly their own intestinal flora.

5.2 Future Work

The variability of pH_n and the safety margin

The variability of pH_n , and therefore the safety margin, is unknown beyond the weekly data presented in this study. In order to more fully understand the environment to which an encrusting catheter is exposed, this variable needs to be studied more closely. An examination of any diurnal variation and the degree of day to day variation is required. This could be used to study the concept of catheter specific safety margin in greater detail.

Manipulating pH_n as a means of controlling catheter encrustation

It has been shown that pH_n is variable, and is determined to some extent by factors which may be controllable. The extent to which this value can be deliberately manipulated, and the effect this would have on catheter encrustation rate, could be studied in a clinical trial. A study has recently shown what should be a beneficial effect of citrate on the pH_n of healthy subjects [206]. It would be interesting to see if this effect is also seen in those with *Proteus* colonisation. Attempts could also be made to lower urinary calcium with dietary modification, or perhaps by simply increasing fluid intake to dilute the urine.

Developing measurements of catheter encrustation rate

The concept of encrustation rate, measuring the amount of calcium and magnesium deposited on a catheter per unit time, failed to provide a more useful measure of the severity of catheter encrustation in the clinical setting than the existing method of

catheter lifespan. A technique to compensate for the frequent removal of catheters for reasons other than catheter blockage would prove invaluable in future clinical trials of strategies to reduce encrustation. Laboratory studies of the development of *Pr. mirabilis* biofilms on catheters over time, using artificial urine for consistency, would be the logical first step in this process. The mathematical pattern of growth of the measurable portion of the biofilm would need to be defined. It may be necessary to limit measurement to a short section of catheter at a standard point, or at the point of maximum occlusion. The effects of different catheter materials and sizes would need to be compensated for. This model would then need to be confirmed and assessed for consistency in human urine.

The effects of co-infecting organisms on encrustation

The possibility exists that some organisms present in stable catheter biofilm communities in addition to *Pr. mirabilis* may influence the degree of encrustation produced in an individual, either by reducing the numbers of *Proteus* organisms present, or by inhibiting or counteracting their effect on the urine. This could be studied in an artificial catheterised bladder model by comparing time to blockage for catheters exposed to pure *Pr. mirabilis* inoculated urine, with urine inoculated with *Pr. mirabilis* in addition to strains of other organisms such as *E. coli*, *Ps. aeruginosa* and *Kl. Pneumoniae*. Using standard strains of bacteria in known proportions under controlled conditions would be more efficient than further clinical investigation, now that it is known from this and other studies which organisms are commonly present.

Faecal Proteus carriage as a risk factor for encrustation

The source of *Pr. mirabilis* colonisation in long term catheter users appears to be their own gastrointestinal flora in the majority of cases, and certainly in those with

faecal colonisation. A larger survey is needed to determine the rates of faecal carriage of these organisms in long term catheter users, both those with and without catheter encrustation and urinary *Pr. mirabilis*. This would assess the degree to which faecal carriage of *Pr. mirabilis* is a risk factor for catheter encrustation.

The effects of bladder calculi

It was hoped to find catheter users with bladder calculi for recruitment into the study. Unlike in many regions, catheter users in the area where the study was conducted are regularly checked and these calculi removed, so it is perhaps unsurprising that no calculi were found in this group. It would be interesting to analyse the effects of the acquisition and removal of calculi on catheter lifespan, voided urinary pH and bacterial populations.

Section 6 - Appendices

Appendix 1: Example Calculation of Nucleation pH

The following example demonstrates a calculation of nucleation pH according to the method described in section 2.3.2. The data is from the urine sample collected from participant 21 in week 2, and is typical of the samples studied.

Figure 6.1 shows the plot of optical density of the acidified urine sample as it is re-alkalised. As the pH approaches 7.0, a sudden rise in optical density can be observed causing an abrupt increase in the gradient of the trace. This increase in optical density is the point during the protocol at which crystals start to precipitate from solution, and it is the pH of the sample at this point which is defined as the nucleation pH. As the urine is alkalised further, precipitation continues, resulting in a steady rise in optical density.

It can be observed that the two segments of the trace representing pH values immediately above and below this value are straight lines. The point at which the trace changes gradient will be the intersection of these two line segments, and determining this point will therefore give the nucleation pH. The data points contained in the ranges of these straight line segments can be used to calculate the regression equations which describe the lines of best fit through them. These lines have been plotted graphically in figure 6.2.

The graphical and statistical tools in Microsoft Excel were used.

Figure 6.1: Changes in optical density of a urine sample as it is alkalinised

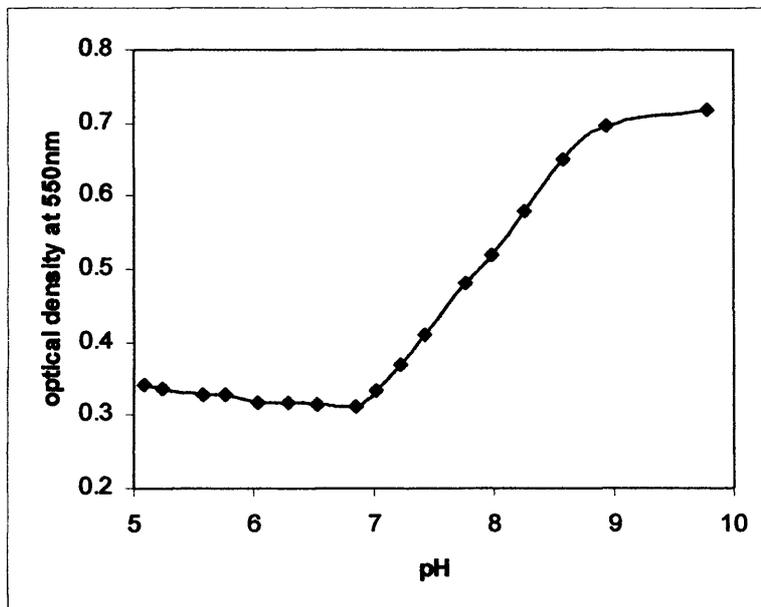
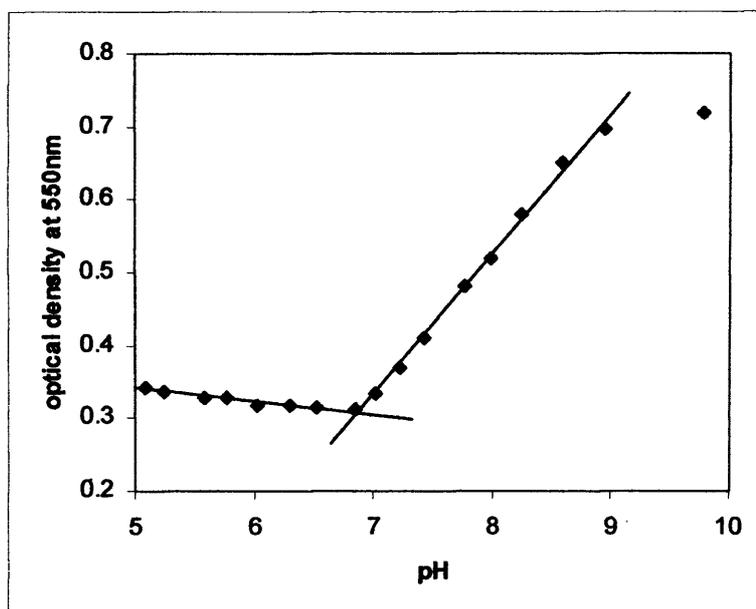


Figure 6.2: Regression lines for the straight segments of the trace shown in figure 6.1 for pH values immediately above and below the abrupt change in optical density. The intersection of these lines gives the nucleation pH.



The regression equations for these two line segments are, for the low pH segment:

$$\text{optical density} = -0.019\text{pH} + 0.438$$

...and for the high pH segment:

$$\text{optical density} = 0.192\text{pH} - 1.006$$

Solving these as a pair of simultaneous linear equations will give their point of intersection. The value of pH at this point is the nucleation pH. Substituting for optical density gives an expression which can be solved for pH. The optical density at this point does not need to be calculated.

$$\text{pH}_n = \frac{0.438 + 1.006}{0.192 + 0.019}$$

$$\text{pH}_n = 6.84$$

In this case the value of pH_n is 6.84. This can also be determined by plotting the graph of the two straight line segments, as in figure 6.2, and reading off the pH value at the intersection. If plotted using computer software and mathematically calculated best fit lines, the graph can be magnified to a point where accurate readings can be taken visually.

Appendix 2: Analysis Excluding Participants 03 & 11

Participants 03 and 11 did not have *Proteus* colonisation during the majority of the trial. The results shown in section 3.2 used the data from all participants. Excluding values for participants 03 and 11 made no difference to the results of comparisons between rapid and slow encrusters (table 6.1) or correlation tests (table 6.2).

Table 6.1: Comparison of the p-values for statistical tests comparing rapid and slow encrusters using data from all participants, and for tests excluding data from participants 03 and 11

Statistical test	all participants	excluding 03 & 11
t test - comparison of mean safety margin	0.003	0.014
t test - comparison of mean pH _v	0.385	0.680
t test - comparison of mean pH _n	0.004	0.002
U test - comparison of mean urinary calcium	0.007	0.010
U test - comparison of mean urinary magnesium	0.464	0.400
U test - comparison of mean total calcium and magnesium	0.129	0.133

Table 6.2: Comparison of the p-values and Pearson's correlation coefficient r between correlation tests using data from all participants, and for tests excluding data from participants 03 and 11

Correlation	all participants		excluding 03 & 11	
	r	p	r	p
mean catheter lifespan and mean urinary calcium	0.60	0.002	0.59	0.004
mean catheter lifespan and mean pH _n	0.56	0.004	0.61	0.003
mean catheter lifespan and mean safety margin	0.47	0.017	0.46	0.023
mean urinary calcium and mean pH _n	0.70	0.000	0.74	0.000
mean urinary magnesium and mean pH _n	0.52	0.008	0.53	0.009
mean urinary calcium and magnesium and mean pH _n	0.66	0.001	0.68	0.001

Appendix 3: Analysis Using Encrustation Rate

The independent samples t tests and Mann-Whitney U tests detailed in section 3.2.1 were repeated to determine whether differences in mean pH_v , pH_n , safety margin, urinary calcium, magnesium, and summed calcium and magnesium concentrations were significant between rapid and slow encrusters when the two groups were defined by encrustation rate rather than mean catheter lifespan. In most cases, the same results, with the same general level of significance, were obtained using an encrustation rate of greater than or less than 0.7 mg/day as when using a mean catheter lifespan of greater than or less than 28 days to divide the participants into two groups. The only exception is the comparison of mean safety margin, where the difference is significant using catheter lifespan but not encrustation rate. This is shown in table 6.3.

Table 6.3: Comparison of the p-values for statistical tests comparing the grouping of participants into rapid and slow encrusters as defined by either mean catheter lifespan or encrustation rate

Statistical test	catheter lifespan	encrustation rate
t test - comparison of mean safety margin	0.003	0.062
t test - comparison of mean pH_v	0.385	0.964
t test - comparison of mean pH_n	0.004	0.002
U test - comparison of mean urinary calcium	0.007	0.007
U test - comparison of mean urinary magnesium	0.464	0.370
U test - comparison of mean total calcium and magnesium	0.129	0.080

Section 7 - References

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