Whole-genome sequencing to determine the extent of *Clostridium difficile* transmission in a high incidence setting in North Wales in 2015

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24 **Running title**: *C*. *difficile* infection in North Wales

25

Abstract

Objectives

Rates of *C. difficile* infection (CDI) are higher in North Wales than elsewhere in the UK. We used whole-genome sequencing to investigate if this is due to increased healthcare-associated transmission from other cases.

Methods

Healthcare and community *C. difficile* isolates from patients across North Wales (February-July-2015) from glutamate dehydrogenase (GDH)-positive faecal samples underwent WGS. Data from patient records, hospital management systems, and national antimicrobial use surveillance were used.

Results

338/499(68%) GDH-positive samples were sequenced, and 299 distinct infections/colonisations identified, 229/299(77%) with toxin genes. Only 39/229(17%) toxigenic isolates were related within ≤2 SNPs to ≥1 infection/colonisation from a previously sampled patient, i.e. demonstrated evidence of possible transmission. Independent predictors of possible transmission included healthcare exposure in the last 12 weeks ($p=0.002$, with varying rates by hospital), infection with multilocus sequence types ST-1 (ribotype-027) and ST-11 (predominantly ribotype-078) compared to all other toxigenic STs ($p<0.001$), and cephalosporin exposure in the potential transmission recipient ($p=0.02$). Adjusting for all these factors, there was no additional effect of ward workload ($p=0.54$), or

failure to meet cleaning targets (p=0.25). Use of antimicrobials is higher in North Wales compared to England and the rest of Wales.

Conclusions

Levels of transmission detected by WGS were comparable to previously described rates in endemic settings; other explanations, such as variations in antimicrobial use, are required to explain the high levels of CDI. Cephalosporins are a risk factor for infection with *C. difficile* by another infected or colonised case.

Introduction

Using whole-genome sequencing in endemic settings only the minority of hospital and community *Clostridium difficile* infections, CDIs, are acquired from other symptomatic cases.[1,2] However, how acquisition from cases varies with increased *C. difficile* incidence is not known. Despite declines in CDI incidence over the last 15 years,[3] North Wales has some of the highest CDI incidence in the United Kingdom; in 2015/16 CDI incidence was 51.1 per 100000 population, compared to a Wales-wide rate[3] of 40.1, 25.8 in England[4] and 31.2 in Scotland[5] (calculated using total reported cases[3-5] and mid-2015 population estimates[6]). Surveillance methodologies in England[4] and Wales[3] are broadly similar. Reporting in Scotland[5] differs by including cases ≥15 years old, compared to ≥2 years in England and Wales.

To investigate the relatively high incidence of CDI in North Wales, a prospective WGS study was initiated to test the hypothesis that this was due to increased within-hospital *C. difficile* transmission. We also investigated whether risk factors for transmission could be identified, to suggest potential infection control and other preventative interventions.

Methods

Setting

Wrexham Maelor Hospital, Glan Clwyd Hospital, and Ysbyty Gwynedd are three district general hospitals providing secondary-level care to the entire region of North Wales. These hospitals serve a population of 694,473 (mid-year 2015 estimate), living in a mix of urban

and remote rural settings. All hospital and community samples submitted for *C. difficile* testing from these hospitals, smaller community hospitals in the same region, and general practitioners are processed by a single laboratory at Glan Clwyd Hospital. These hospitals are randomly identified as hospital A, B and C to anonymise study results. Hospital policy was to test inpatients >2 years with diarrhoea (≥3 unformed stools in 24 hours), without another identified cause, for *C. difficile* infection. Community testing was advised when *C. difficile* was suspected, in particular with a documented history of antibiotic exposure within 6 weeks, in patients from residential or nursing homes or hospital exposure in the last 2 months.

Microbiology

Faecal samples submitted for *C. difficile* testing underwent glutamate dehydrogenase, GDH, testing using C. DIFF Chek 60 (TechLab, Blacksburg, VA, USA). Positive samples underwent C. DIFF QUIK CHEK COMPLETE (TechLab) to confirm the GDH result and detect the presence of *C. difficile* toxins A and B by enzyme immunoassay. Samples were saved, selectively cultured for *C. difficile* as described previously,[7] and isolates obtained underwent WGS. GDH-positive patients were considered infected or colonised, and those who were faecal toxin-positive patients to be infected (i.e. have CDI).[8] Cases were denoted healthcare facility-associated, community-associated or indeterminant using standard surveillance definitions.[9] Cases were assigned to a given hospital based on inpatient exposure in the last 12 weeks, excluding the 48 hours immediately prior to diagnosis.

Sequencing

DNA was extracted after subculture of a single colony and sequenced using Illumina HiSeq2500. Sequence data were processed as previously,[1,10,11] mapping sequenced reads to the *C. difficile* 630 reference genome. Sequences were compared using SNPs, obtaining differences between sequences from maximum-likelihood phylogenies,[12] corrected for recombination using ClonalFrameML.[13] Sequence reads were also assembled *de novo* with Velvet,[14] using VelvetOptimiser. Toxin genes, *tcdA* and *tcdB*, were identified from *de novo* assemblies using BLAST searches, on the basis of >1kb of sequence identity to each gene. Multi-locus sequence types were predicted from *de novo* assemblies.[1] Sequence data have been deposited under NCBI BioProject PRJNA412541.

Genomic analysis

Based on *C. difficile* evolutionary rates and within-host diversity[1,15] >95% of transmission pairs sampled ≤123 days apart are expected to have ≤2 SNPs between them and cases up to 124-364 days apart ≤3 SNPs, but with 3 SNPs uncommon.[1] Therefore, during the 5.5 months of the study ≤2 SNPs were expected between the large majority of transmitted isolates. Where patients had multiple samples, subsequent isolates >10 SNPs different to previous isolates from the same patient were considered to represent a new acquisition of a distinct *C. difficile* strain. This higher SNP threshold almost completely excludes isolates being from the same infection within the study. We used a previously described correction factor[11] to adjust for sequencing only a subset of GDH-positive samples, assuming sequenced and non-sequenced cases transmit onwards at the same rate and cases are missing at random.

Risk factor analysis

Data from paper and electronic patient records was extracted into a Public Health Wales data warehouse. Data were available on admissions and ward movements for infected/colonised patients from the three district general hospitals and for smaller community hospitals and nursing homes. Additional data were available on prescribing, ward workload (ward admissions per day), and from mandatory audits of cleaning compliance within the hospital setting.

Multivariate logistic regression was used to identify independent predictors of a case being genetically-related to ≥1 previous case within ≤2 SNPs, selecting a final model from factors shown in Table 1 using backwards elimination with an exit p-value of >0.1. Multiple fractional polynomials were used to allow for non-linear effects of continuous factors. Following initial model selection, each excluded variable was added back to model one at a time and retained if its Wald p-value was <0.1. Interactions between main effects in the final model were retained where interaction p <0.01. 1All analyses were performed using Stata 14.1 (Stata Corp, College Station, TX, USA).

Publicly available demographic[16,17] and antimicrobial surveillance data[18,19] were used to investigate alternative explanations for variation in CDI incidence. Sex and age adjusted rates of primary care antibiotic use were compared using items prescribed per 1000 Specific Therapeutic group Age-sex Related Prescribing Units (STAR-PUs).[20]

Ethics

Ethical approval was not required as the work formed part of the Betsi Cadwaladr University Health Board's response to *C. difficile* infection. Sequencing was carried out on *C. difficile* isolates following routine isolation.

Results

Between 01-February-2015 and 16-July-2015, 499 *C. difficile* GDH-positive samples were obtained from 417 patients. 182 (36%) samples from 159 patients were faecal toxin-positive and considered to represent infections. One patient had evidence of a genetically distinct second infection. Of these 160 CDIs, 33 (21%) were community-associated, i.e. had no healthcare facility exposure for >12 weeks, representing a rate of 4.8 per 100000 population per year. 118 (74%) were healthcare-facility associated (healthcare exposure within 4 weeks) and 9 (6%) indeterminate (healthcare exposure 4-12 weeks ago), together representing a rate of 5.7 per 10000 bed-days. Monthly CDI incidence, with historic rates,[3] is shown in Figure 1.

Of 499 GDH-positive samples, 338 (68%) underwent WGS (144/182 [79%] faecal toxin-positive samples and 194/317 [61%] faecal toxin-negative samples). Rates of GDH-positive sample retrieval were similar by hospital, 95/136 (70%), 55/81 (68%), 92/134 (69%) at hospitals A, B and C respectively, 5/6 (83%) for patients exposed to both hospital A and C. 6/7 (86%) of samples from patients with only community hospital exposure were retrieved and 85/135 (63%) of samples from patients without recent hospital exposure.

Considering all GDH-positive samples, irrespective of faecal toxin status, the 338 sequenced samples contained 299 distinct infections/colonisations in 290 patients. Of these, 229/299 (77%) had detectable toxin genes on WGS, and within these potentially toxigenic isolates, 114/229 (50%) were from consistently faecal toxin-positive patients, 103/229 (45%) from consistently faecal toxin-negative patients, and 12/229 (5%) from patients with both faecal toxin-positive and negative results on different samples. Of the 70 distinct colonisations without detectable toxin genes on WGS, 65 (93%) were consistently faecal toxin-negative, 4 (6%) were faecal toxin-positive and 1 (1%) had both faecal toxin-positive and toxin-negative results on different samples.

Genetic and epidemiological links between samples

Of the 299 sequenced distinct infections/colonisations, 43 (14%) were within ≤2 SNPs of ≥1 infection/colonisation from a previously sampled patient, i.e. had evidence of possible transmission (Figure 2). 39/43 (91%) genetically-linked cases were toxigenic (i.e. had toxin genes), and 39/229 (17%) distinct toxigenic infections/colonisations were within ≤2 SNPs of ≥1 infection/colonisation. Figure 3 shows the relationship between donor and recipient faecal toxin status. Faecal toxin-positive cases were not more likely to have a faecal toxin-positive donor; instead faecal toxin-negative recipients had predominantly positive donors, and some faecal toxin-positive recipients had faecal toxin-negative donors (p=0.006 versus no relationship between donor and recipient toxin status). Of the 43 potentially transmitted infections/colonisations, 26 (60%) had a single possible source, 9 (21%) had 2 possible sources, and 4 (9%), 3 (7%), and 1 (2%) had 3, 4 and 5 possible sources respectively. The

192 median (IQR) [range] time from the most recently sampled case within ≤2 SNPs of the
193 potential recipient was 21 (7-47) [0–117] days.
194
195 Healthcare exposure in the 12 weeks prior to diagnosis was an important predictor of
196 genetic linkage to a previous case; 40/217 (18%) patients with healthcare exposure were
197 genetically linked to a previous case, compared to 3/82 (4%) without (p=0.001, Figure 4A).
198 Rates of genetic linkage to previous cases varied at the 3 hospitals: 9/80 (11%), 11/51 (22%),
199 20/75 (27%) at hospitals A, B and C respectively (p=0.04, Figure 4A). Transfers between
200 hospitals were uncommon; five patients were exposed to both hospital A and C, and six
201 patients only to smaller community hospitals; none of these 11 patients were genetically
202 linked to a previous case. Genetic linkage did not correspond to the overall rates of
203 healthcare-associated/indeterminant GDH-positive *C. difficile* colonisation/infection at
204 hospitals A, B and C, which were 16.8, 11.3, 12.2 per 10000 bed-days respectively or to
205 faecal toxin-positive CDI, occurring at 7.0, 5.2, 5.2 per 10000 bed-days, respectively.
206
207 Of the 43 genetically linked cases, 11 (26%) shared time and space on the same hospital
208 ward with their potential donor between the dates of their diagnoses, 9 in a district general
209 hospital and 2 in a community hospital (Figure 2). A further 2/43 (5%) patients shared time
210 and space on the same district general hospital ward before either were diagnosed. Another
211 8/43 (19%) patients shared the same ward location at different times within the 28 days
212 prior to diagnosis, 5 in a district general hospital, 2 in a community hospital and 1 in a
213 nursing home. Finally, 2/43 (5%) patients without any other link shared time in the same
214 district general hospital between the dates of their diagnoses, but not specific wards. Thus
215 20/43 (47%) potential recipients had no recent or concurrent shared healthcare exposure

with any previous case within ≤2 SNPs even at the broadest level of the hospital, and accounting for smaller community hospitals and nursing homes.

The most commonly occurring toxigenic STs were: ST-6 (30/229 toxigenic infections/colonisations, 13%); ST-2 (27, 12%); ST-8 (21, 9%); and ST-44 (18, 8%) all from *C. difficile* clade 1[21]; and ST-11 (19,8%, equivalent most commonly to ribotype-078) and ST-1 (18,8%, ribotype-027). Rates of genetic linkage were higher in ST-1 and ST-11 than the combined group of all other toxigenic STs (Figure 4B, p<0.001). Rates of genetic linkage were lower for non-toxigenic *C. difficile* despite all tested patients having diarrhoea.

Similar percentages of sequenced infections/colonisations after the first 3 months of the study were within ≤2 SNPs of an earlier sequenced case (20/123 [16%] versus 23/176 [13%] before, p=0.27) even though cases earlier in the study may have been less likely to have had their source sampled. We applied a previously published correction[11] to adjust for having only sequenced 68% of *C. difficile*-positive samples. This provided a corrected estimate for the percentage of cases after the first 3 months of the study that were genetically-linked to a prior case of 24% (i.e. 20/123*1/0.68). Restricting only to potentially toxigenic cases, this figure was 30% (16/87*1/0.68).

Risk factors for transmission

Independent risk factors for genetic linkage within ≤2 SNPs to a previous case (Table 1) included healthcare exposure in the last 12 weeks, in hospital A (OR 3.15 [95%CI 0.77-12.9]), in hospital B (5.63 [1.40-22.7]), and in hospital C (10.1 [2.75-37.4]), compared to no

healthcare exposure (p=0.002). *C. difficile* genotype was also associated with genetic linkage (p<0.001); compared to all other toxigenic STs, ST-1 cases were independently more likely to be linked to a previous ST1 case (OR 7.61 [95%CI 2.50-23.2]), and there was some evidence for similar associations for ST-11 (2.27 [0.67-7.68]). Older patients were somewhat more likely to be genetically linked to a previous case (p=0.06). Second/third-generation cephalosporin exposure in the last 90 days in the potential transmission recipient increased the risk of genetic linkage (OR 6.03 [95%CI 1.42-25.5, p=0.02]), however only 5/43 (12%) of cases and 6/256 (2%) of controls were exposed. Adjusting for all these factors, within the limits of the power of the study, we found no evidence for any additional effects on transmission of ward workload (p=0.54), or failure to meet cleaning audit targets (p=0.25).

Population risk factors for CDI

We considered explanations other than increased transmission for rates of CDI in North Wales. The majority of antibiotics in the UK are prescribed in primary care by general practitioners. Rates of community antibiotic use (in the second quarter of 2015) were higher in North Wales (296.7 per 1000 STAR-PUs) and Wales overall (296.9 per 1000 STAR-PUs), compared to England (243 items per 1000 STAR-PUs) (Figure 5).[18] Comparing acute hospital total antibiotic use in defined daily doses per 1000 bed-days in 2015, for the 17 acute hospitals in Wales, Ysbyty Gwynedd had the 2nd highest rate, Ysbyty Glan Clwyd the 6th highest and Wrexham Maelor the 12th.[19]

Similarly, age is another CDI risk factor. The population in North Wales is older than Wales as a whole, 22.6% of the population are >65 years old,[16] compared to Wales 20.4% and England 17.9% (mid-2016 data).[17]

Discussion

Despite high CDI incidence in North Wales, based on WGS only 39/229 (17%) of toxigenic infections/colonisations could have been plausibly acquired from another case. Adjusting for only sequencing 68% of isolates, this proportion was still only 30%. This is higher than in a study of six English hospitals, where rates of genetic linkage to previous cases were between 7% and 24% by hospital and 20% overall.[11] However these differences are insufficient to explain CDI incidence being nearly double in North Wales compared to England.[3,4] Therefore, higher incidence is likely to be driven predominantly by factors other than lapses in infection control.

Antimicrobial exposure is an important CDI risk factor.[22] Rates of antibiotic use in primary care are higher in Wales than in England, but similar in North Wales to Wales overall, potentially explaining some of the differences between North Wales and England, but not between North Wales and elsewhere in Wales. Additionally, two of the three hospitals in North Wales are among the highest users of antibiotics of all the acute hospitals in Wales. Similarly, increasing age is another important risk factor for CDI,[22] and the population in North Wales is older than Wales as a whole and England. Other factors may also be important; the area of the country served by the three hospitals contains extensive areas of livestock farming. Disease causing *C. difficile* strains have been isolated from livestock,[23]

with overlap seen between isolates from CDI cases, healthy humans and livestock on WGS.[24] However a large scale environmental survey 20 years ago in South Wales identified relatively little *C. difficile* in livestock.[25] Asymptomatic patients are another potential source of *C. difficile* infection, however it is not known if rates of *C. difficile* colonization differ across the UK.

Recent healthcare exposure was an important risk factor for potential acquisition from a previous case; 40/43 (93%) of genetically-related cases were in hospital in the 12 weeks prior to their diagnosis. The median (IQR) time between genetically-related cases was 21 (7-47) days. However shared space and time on the same hospital ward could only explain the minority of genetically-related cases, and nearly half such cases had no healthcare contact including allowing for shared time in hospital resulting in overlap outside of wards, e.g. diagnostic areas. Additionally, although our study is only moderately powered, we found no signal that failure to meet cleaning audit targets or high levels of patient turnover were associated with more transmission. However, the proportion of cases linked to a previous case varied between 11% and 27% at the three main hospitals suggesting potential for reductions in overall incidence. Supporting the previously described role in transmission of GDH-positive patients without detectable faecal toxin,[26] 7/39 (18%) of toxigenic *C. difficile* acquisitions could only be linked to consistently toxin-negative sources. Therefore, all patients with toxigenic *C. difficile* should be a focus of infection control efforts, not just those with detectable faecal toxin. ST-1 (ribotype-027) and ST-11 (ribotype-078) were associated with higher rates of genetic linkage replicating previous findings from England[27] and for ST-1 from Canada.[28] The underlying reasons for this may be multifactorial including more severe disease[29] leading to greater environmental contamination, enhanced

environmental persistence, and also a greater likelihood of clinically detectable disease in transmission recipients.

Antimicrobials are risk factors for CDI.[22] We investigated more specifically the effect of recent antimicrobial exposure on acquisition of *C. difficile* from another case. Second/third generation cephalosporin exposure, but not antibiotics in general or any other specific antibiotic class, increased the risk of being a transmission recipient. The effect of cephalosporins may reflect intrinsic resistance in *C. difficile*,[30] and more variable susceptibility to other antibiotics in the population studied.

The main limitation of this study is that only 68% of samples tested were available for sequencing; this was due to a failure by the research team to ensure all samples taken for diagnostic purposes were successfully processed prior to sequencing within the study. This will have reduced the observed rates of linkage to previous cases, as demonstrated in previous simulations.[11] However, by applying a correction factor for missing data we were able to estimate the true proportion of cases linked. As rates of sample retrieval for sequencing were similar between the three hospitals, the differences observed in linkage rates are unlikely to have been differentially affected by sample retrieval rates at each site. The small number of samples, 5/299 (2%) infections/colonisations, that were faecal toxin positive, but yielded isolates that lacked toxin genes on sequencing may have arisen as a result of mixed infections, laboratory error or a false-positive faecal toxin assay. Mixed infections are a potential additional source of underestimates of the extent of transmission from other cases, but previous work suggests this is uncommon in *C. difficile*.[31]

This study was based on prospective storage of samples, culture of isolates and sequencing in response to a period of high CDI incidence. An alternative approach which may allow similar methods to be applied more widely is the storage of *C. difficile* GDH-positive faecal samples, e.g. on a rolling annual basis. These can then be cultured and sequenced retrospectively if increased incidence is noted, as demonstrated recently in six English hospitals.[11] The development of surveillance systems that interpret CDI incidence and sequencing data and present it back to clinicians in a timely manner is essential to guide local and national infection prevention and control responses.

In summary, despite relatively high CDI incidence in North Wales, levels of transmission detected by WGS were comparable to previously described rates in endemic settings; other explanations, including variations in antimicrobial use, are required to understand the reasons for the high levels of CDI.

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Transparency declaration

No author has any conflict of interest to declare.

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Figure legends and table

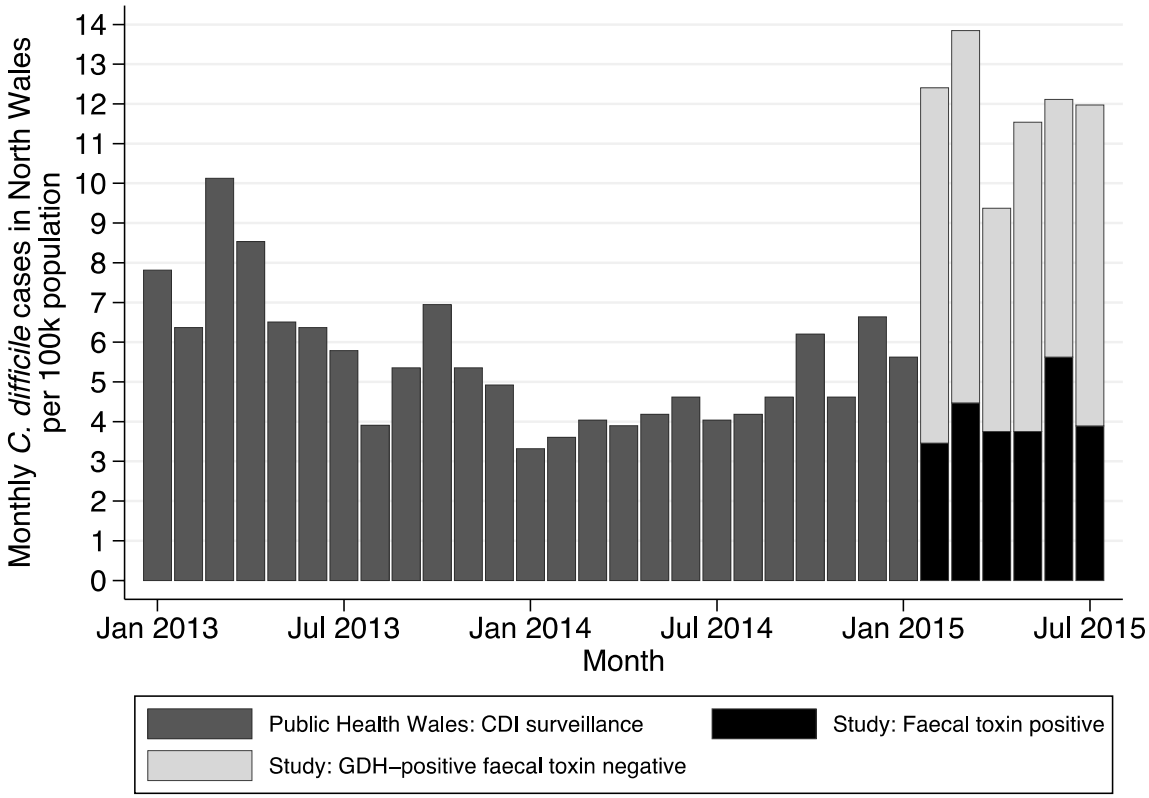
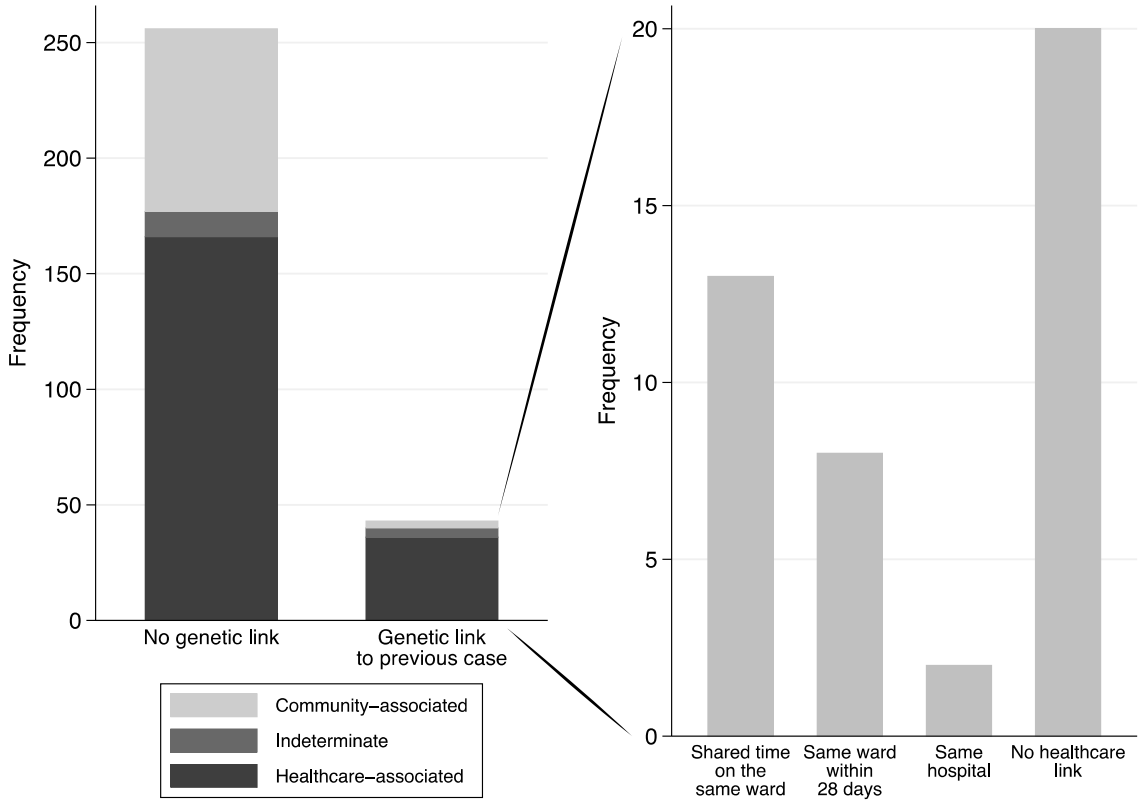


Figure 1. *C. difficile* incidence in North Wales 2013-2015. Public Health Wales surveillance data are for faecal toxin positive CDI cases.



454

455 **Figure 2. Proportion of cases genetically linked within ≤2 SNPs, classified by surveillance**

456 **definitions and epidemiological relationships between linked cases**. Cases sharing the

457 same ward or hospital did so with their potential donor between the dates of their

458 diagnoses, or prior to the diagnosis of either case. For cases sharing the same ward within

459 28 days, the potential recipient spent time on the same hospital ward after the discharge of

460 an already diagnosed donor.

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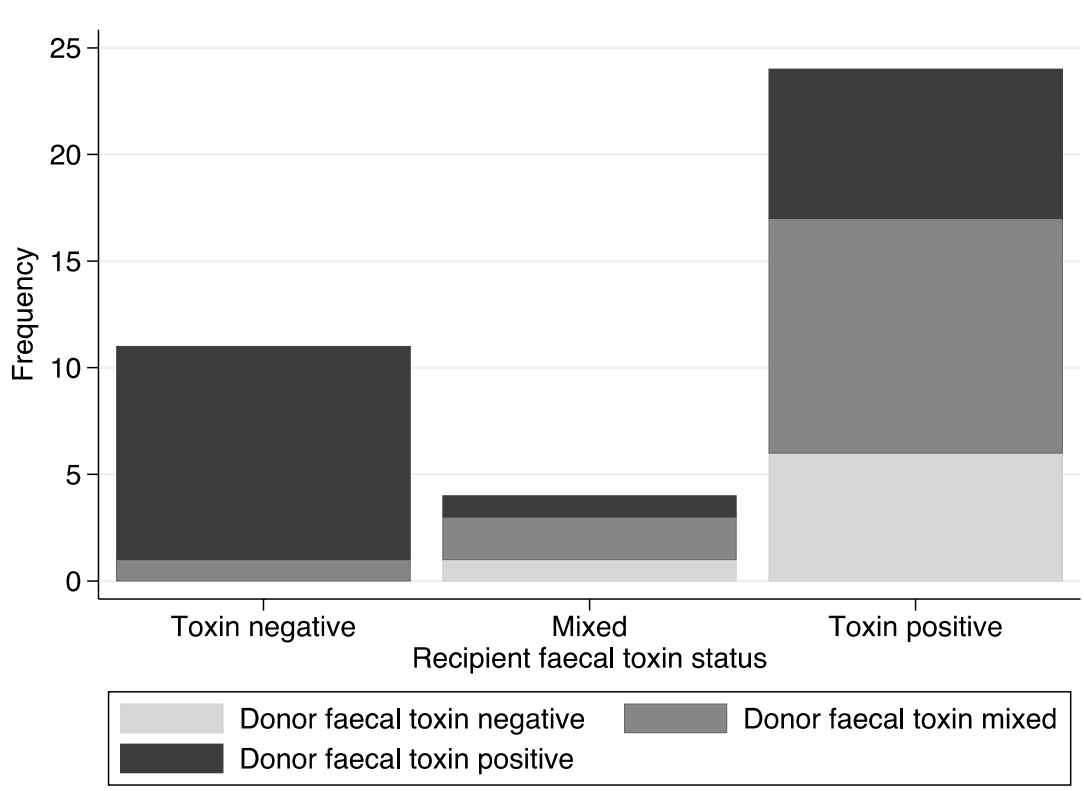
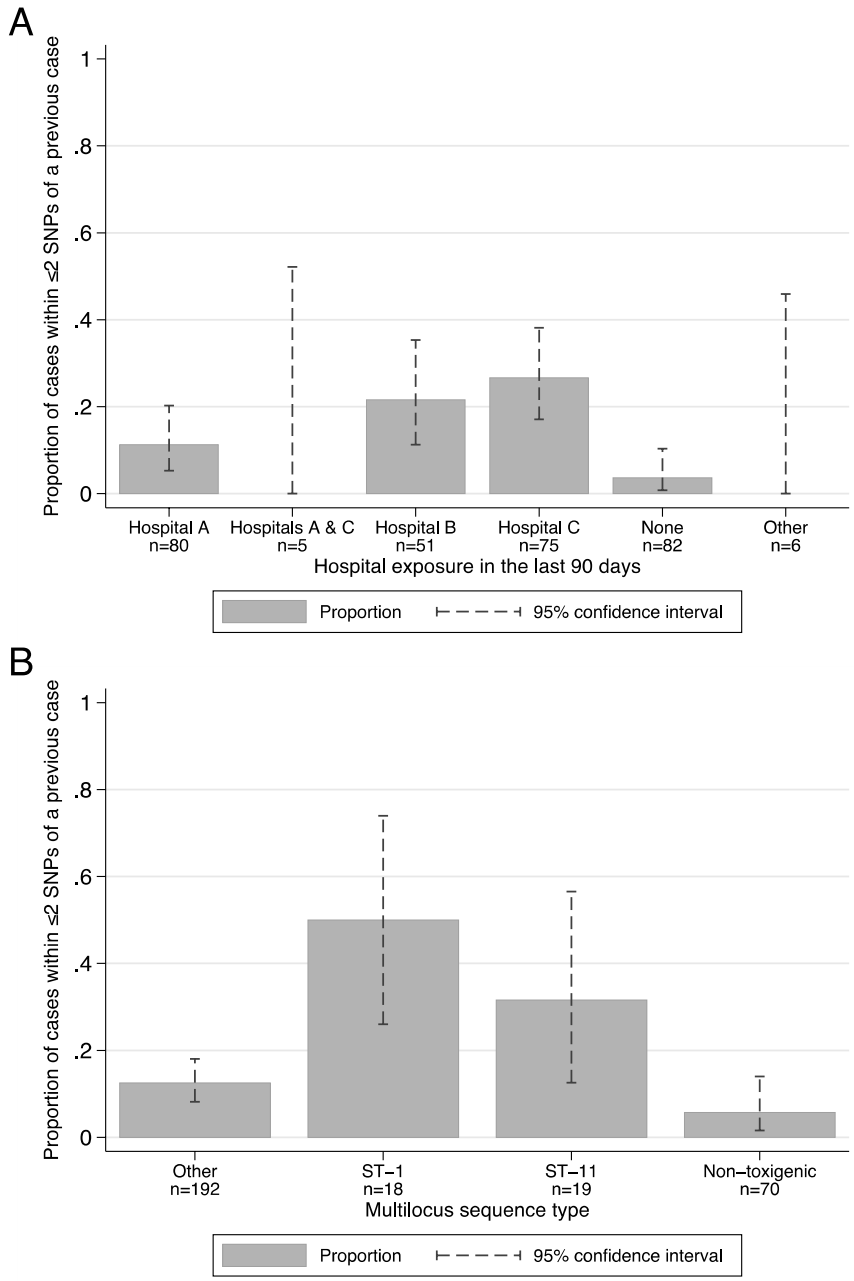


Figure 3. Relationship between potential transmission donor and recipient faecal toxin status. A mixed toxin status patient had ≥1 faecal toxin positive and ≥1 faecal toxin negative sample. Overall p value = 0.006.



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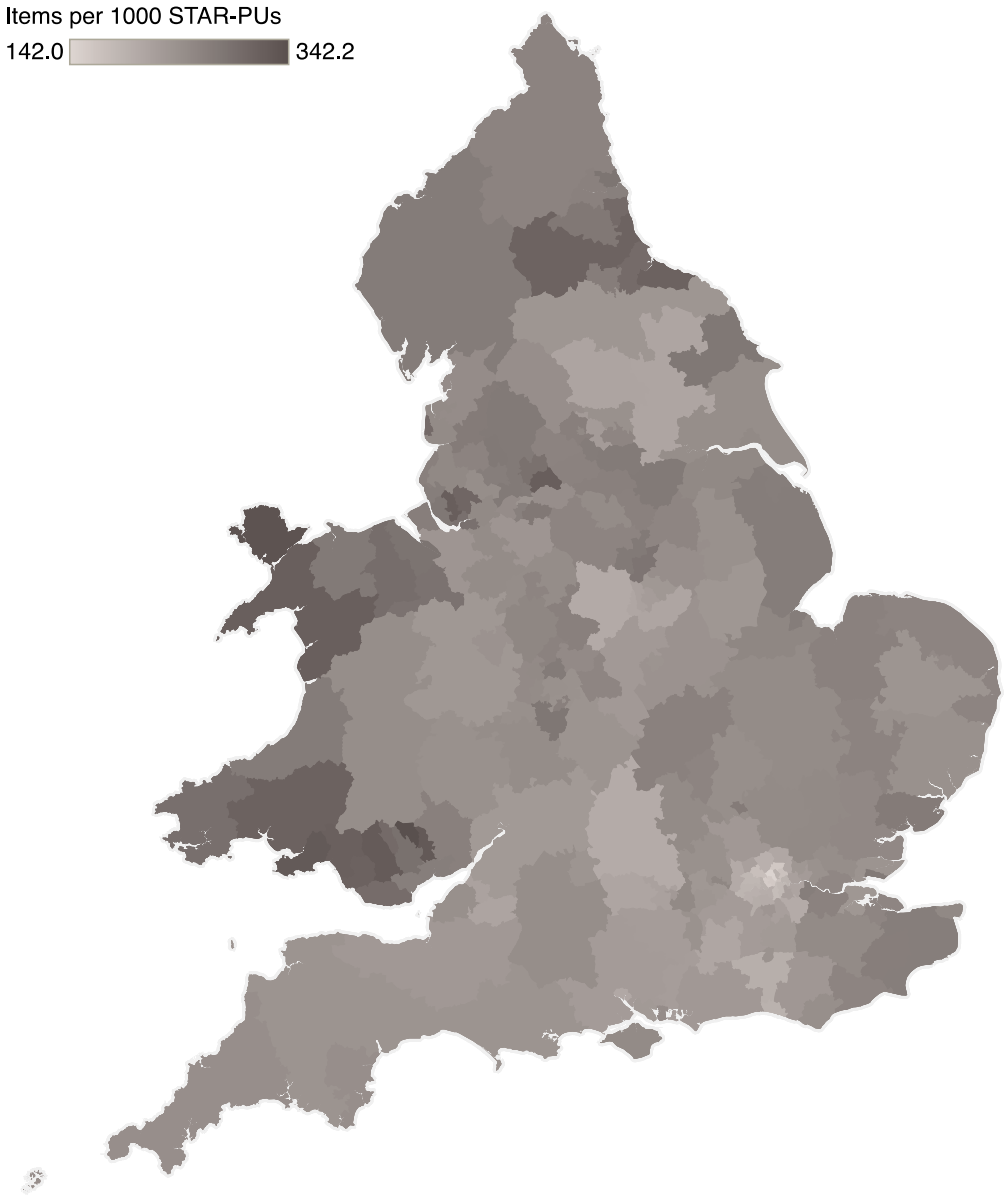
469

470 **Figure 4. Unadjusted proportion of cases with a previous case within ≤2 SNPs, by hospital**

471 **exposure in the last 12 weeks (panel A) and multilocus sequence type (panel B).**

472



473

474 **Figure 5. Antibiotic prescribing in primary care in England and Wales, items prescribed per**

475 **1000 Specific Therapeutic group Age-sex Related Prescribing Units (STAR-PUs)**. Data are

476 presented for July – September 2015. Areas shaded are Welsh Unitary Authorities and

477 English Clinical Commissioning Groups. Source: [18].

478

	Genetically-unlinked (N=256)		Genetically-linked (N=43)		Univariate			Multivariate		
	n / median	% / IQR	n / median	% / IQR	Odds ratio	95% Confidence interval	p value	Odds ratio	95% Confidence interval	p value
Any hospital exposure in last 12 weeks										
- None	79	31%	3	7%	1.00	Baseline	0.001	1.00	Baseline	0.002
- in hospital A	71	28%	9	21%	3.34	(0.87, 12.82)		3.15	(0.77, 12.87)	
- in hospital B	40	16%	11	26%	7.24	(1.91, 27.44)		5.63	(1.40, 22.68)	
- in hospital C	55	21%	20	47%	9.58	(2.71, 33.80)		10.13	(2.75, 37.39)	
- in both hospitals A and C	5	2%	0	0%	*					
- in community hospital only	6	2%	0	0%	*					
Multilocus sequence type										
- Other	168	66%	24	56%	1.00	Baseline	<0.001	1.00	Baseline	<0.001
- ST-1	9	4%	9	21%	7.00	(2.53, 19.38)		7.61	(2.50, 23.16)	
- ST-11	13	5%	6	14%	3.23	(1.12, 9.30)		2.27	(0.67, 7.68)	
- Non-toxigenic	66	26%	4	9%	0.42	(0.14, 1.27)		0.36	(0.11, 1.17)	
Sex, female	166	68%	28	65%	0.91	(0.46, 1.80)				
Age, years	79	69 - 86	82	71 - 88	1.02	(1.00, 1.05)	0.06	1.03	(1.00, 1.05)	0.06
Recipient faecal toxin positive	101	39%	28	65%	2.86	(1.46, 5.63)	0.002			
Inpatient days in last 90 days	12	3.5 - 25	17.5	8 - 41	1.02	(1.00, 1.04)	0.03			
Any antibiotic	142	58%	28	65%	1.36	(0.69, 2.67)	0.37			
Fluoroquinolone	21	9%	5	12%	1.47	(0.52, 4.14)	0.46			
Cephalosporin, 2nd/3rd generation	6	2%	5	12%	5.48	(1.59, 18.85)	0.007	6.03	(1.42, 25.50)	0.02
Beta-lactam/beta-lactamase inhibitor	61	25%	15	35%	1.54	(0.78, 3.06)	0.22			
Meropenem	10	4%	4	9%	2.52	(0.75, 8.44)	0.13			
Proton pump inhibitor	35	14%	6	14%	1.02	(0.40, 2.60)	0.96			
Laxative	18	7%	3	7%	0.99	(0.28, 3.52)	0.99			
Cleaning audit, per day below target	10	2 - 24.5	16.5	7 - 36	1.01	(1.00, 1.03)	0.06			
Admissions, per admission exposed to	56	21.5 - 110	85	37 - 141	1.00	(1.00, 1.01)	0.07			

Table 1. Risk factors for genetic linkage (≤2 SNPs) with a previous case. Antibiotic and proton pump exposures are ever receiving the relevant agent in the 90 days prior to diagnosis, and laxative exposure in the 30 days prior to diagnosis. Cleaning audit exposure is the total number of days in the 90 days prior to diagnosis spent on a ward that had failed to meet the audit standard at the last available audit. Ward workload was

judged by the total number of other patient admissions that occurred during all inpatient days in the 90 days prior to diagnosis. *These hospital exposure categories had no genetic links and so an odds ratio cannot be calculated.