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A case–control study of community-associated *Clostridium difficile* infection

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***Objectives*: The aim of this study was to determine the incidence of and risk factors for community-associated *Clostridium difficile* infection (CDI).**

***Methods*: Prospective surveillance of community-derived faecal samples for *C. difficile* cytotoxin, followed by a questionnaire-based case–control study in two distinct patient cohorts (one semi-rural and the other urban).**

***Results*: The proportion of randomly selected faecal samples positive for *C. difficile* cytotoxin was 2.1% in both patient cohorts (median ages 73 and 45 years for the urban and semi-rural cohorts, respectively). Exposure to antibiotics in the previous 4 weeks, particularly multiple agents ($P < 0.001$), aminopenicillins ($P < 0.05$) and oral cephalosporins ($P < 0.05$), was significantly more frequent among cases than controls. Hospitalization in the preceding 6 months was significantly associated with CDI (45% versus 23%; $P = 0.022$). However, almost half the cases had not received antibiotic therapy in the month before *C. difficile* detection, and approximately one-third neither had exposure to antibiotics nor recent hospitalization. Contact with infants aged ≤2 years was significantly associated with CDI (14% versus 2%; $P = 0.02$). Prior exposure to gastrointestinal-acting drugs (proton pump inhibitor, H2 antagonist or non-steroidal anti-inflammatory) was not significantly more common in CDI cases. *C. difficile* PCR ribotype 001 caused 60% and 13% of urban and semi-rural community-associated CDI cases, respectively.**

***Conclusions*: Reliance on antibiotic history and age (≥65 years) will contribute to missed diagnoses of community-associated CDI. Potential risk factors for community-associated CDI should be explored further to explain the large proportion of cases not linked to recent antibiotic therapy or hospitalization.**

Keywords: antibiotics, diarrhoea, community-acquired

Introduction

Clostridium difficile infection (CDI), characterized by symptoms varying from diarrhoea to life-threatening colitis, is a major complication of antibiotic therapy, particularly in the elderly. In this group, it causes marked prolongation of hospital stay and is associated with excess mortality.[1–3] CDI is usually considered to be a hospital-associated infection. The contribution of community-onset cases to the burden of disease and the associated risk factors are unclear.[4,5] Indeed, clinical laboratories do not routinely examine faecal samples submitted from patients seen in general practices for evidence of CDI, although recent guidance in England called for routine testing for *C. difficile* of community-based patients aged ≥65 years with diarrhoea.[6] Furthermore, the rates of reported infectious intestinal diseases (IIDs) from general practitioners (GPs) are underestimated, impacting significantly upon reports of community-onset CDI.[7]

A few studies have estimated the incidence of community-associated CDI, with rates ranging from 7.7 to 25 cases per 100 000 individuals.[5,8–10] This variation in observed incidence may be due to differing selection criteria for patient groups studied, as well as true geographical differences in infection rates.[5] Most of these studies reported a link between previous antibiotic exposure and community-associated CDI, although

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robust risk data are limited by methodological issues.[4,5,8–14] We carried out a prospective case–control study in two distinct UK populations (one semi-rural and one urban), initiated by routine submission of faecal samples from community patients to clinical microbiology laboratories, to determine the incidence of community-associated CDI, the role of antibiotics, hospitalization and other potential risk factors, and relatedness of *C. difficile* strains.

Methods

Case and control selection

Two cohorts, each of 1000 individuals aged over 2 years, were randomly selected (~20 per week by random number generation) throughout 1999 from patients who had a faecal sample submitted by their GP for microbiological testing. The cohorts represented two distinct geographical locations, 380 miles apart: one semi-rural (Truro, Cornwall, UK) and one urban (Leeds, UK), each served by one diagnostic microbiology laboratory. All the faecal specimens tested were routinely submitted to the laboratories for clinical reasons; none were solicited for the study. Approval for the study was obtained from the two respective local Ethics Committees.

In addition to the two randomly selected cohorts of patients as defined above, in Truro at the time of the study, the laboratory protocol included *C. difficile* cytotoxin testing of all diarrhoeal faecal samples from GP patients with a history of antibiotic use stated on the request form. This protocol was continued to obtain a subgroup of further *C. difficile* toxin-positive cases.

Cases of community-associated CDI were defined as patients who attended their GP with symptoms of diarrhoea and whose faeces were found to be *C. difficile* cytotoxin-positive. Three age- and sex-matched *C. difficile* cytotoxin-negative controls (patients with diarrhoea who attended a GP and were tested within 3 months of the case) were assigned to each case. Ages were matched within the bands 0–14, 15–25, 26–60, 61–74 and 75+ years. A postal questionnaire was sent to the GP of each case/control, within 1 month of diagnosis to ensure good recall, to determine demographic data, recent (1 month prior to onset of diarrhoea) antimicrobial therapy, other CDI risk factors, including recent (6 months prior to diarrhoea) hospitalization, and outcome (Table 1). We sent up to two reminders to all non-responders within 3 months of the questionnaire being sent.

Microbiological testing

Only diarrhoeal samples were examined. All faecal samples were examined for bacterial enteropathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Escherichia coli* O157) using standard methods. Faeces were examined for *C. difficile* cytotoxin using a Vero cell assay and were cultured anaerobically for 48 h on CCEYL agar: cycloserine (250 mg/L) and cefoxitin (8 mg/L) agar (Lab M, Bury, UK) plus 5 mg/L lysozyme (Sigma, Poole, UK) and 20 mL lysed horse blood. *C. difficile* isolates were stored frozen at −70°C and later DNA fingerprinted using ribosomal spacer PCR (RS-PCR) and arbitrary-primed PCR (AP-PCR), as described previously.[15]

Statistical analysis

All data were analysed with Mann–Whitney *U*-test, χ^2 test or Fishers' exact test, where appropriate. If individual questions on the GP questionnaire were not answered, then blank responses were excluded from the analyses.

Results

Forty-two cases of community-associated CDI (21 in Leeds and 21 in Truro) were detected in the two cohorts totalling 2000 randomly tested faecal samples. Thus, the prevalence of *C. difficile* cytotoxin-positive cases was 2.1% in both Leeds and Truro. Using local population demographic data (Leeds Teaching Hospitals Trust and The Royal Cornwall Hospitals Trust), we calculated annual incidences of 29.5 cases per 100 000 individuals in the urban setting of Leeds and 20.2 cases per 100 000 individuals in the semi-rural setting of Truro. In Truro, testing faeces from community patients with a history of antibiotic use ($n = 103$) yielded a further 19 CDI cases.

Follow-up of each patient via GP questionnaires was obtained for 57/61 CDI cases, including 40/42 randomly selected cases (44% males, median age 78 years, range 4–100 years) and 156/183 age- and sex-matched controls (including 112/126 controls for randomly selected cases). Twenty-six of the 42 case patients (62%) detected by random selection were aged <65 years. The median age of urban cases (73 years) was significantly higher than that of semi-rural cases (45 years; $P = 0.02$). Interestingly, those cases selected because of their history of antimicrobial therapy were found to be of a similar median age (78 years) to the randomly selected urban cohort.

Analysis of the randomly selected cases and controls showed that significantly more cases had received antibiotic therapy in the month prior to the onset of diarrhoea when compared with controls (52% versus 18%; $P = 0.001$) (Table 1). Aminopenicillins (16%) and oral cephalosporins (16%) were the most commonly prescribed antibiotics, and each were received by cases significantly more often than by controls ($P = 0.02$ and 0.045, respectively) (Figure 1). Overall, a significantly higher proportion of CDI cases (35%) were prescribed β-lactams when compared with controls (26%; $P = 0.001$). Only one CDI case was exposed to a β-lactam-inhibitor combination. Exposure to ciprofloxacin was low in both CDI cases and controls (6% versus 0.8%, respectively; $P = 0.06$). Those individuals in receipt of more than one antimicrobial agent were significantly more likely to be *C. difficile* cytotoxin-positive (26%) than cytotoxin-negative (1%; $P = 0.0006$). When the data for those cases identified in Truro by testing faecal samples from patients with a history of antibiotic use (on the request form were analysed), the same antimicrobial agents/classes were significantly associated with CDI when compared with controls (data not shown).

Hospitalization in the preceding 6 months was significantly associated with CDI within the randomly selected cohort (45% versus 23%; $P = 0.022$). Cases who had either been previously hospitalized in the preceding 6 months or in receipt of antimicrobial agents were significantly more likely to develop CDI when compared with controls (65% versus 28%; $P = 0.001$). Notably, approximately one-third (35%) of CDI cases neither had exposure to antibiotics nor hospitalization. There was no significant difference in the proportion of cases when compared with controls that had been recently hospitalized, but had received no antimicrobial therapy (3% versus 12%; $P = 0.2$).

Data pertaining to other potential risk factors for community-associated CDI cases and their controls are summarized in

Table 1. Questionnaire-based risk factor analysis for community-associated CDI cases (as identified by random sampling, total $n = 40$) and controls (total $n = 112$)

	Case % (n)[a]	Control % (n)[a]	P
Residence			
own home	87 (32)	91 (96)	0.51
nursing home	8 (3)	7 (7)	
other	5 (2)	2 (2)	
Foreign travel	11 (4)	14 (14)	0.73
Other person with diarrhoea at home	3 (1)	7 (7)	0.34
Contact with infant $\leq$2 years old	14 (4)	2 (1)	0.02
Antibiotics in previous 4 weeks	52 (16)	18 (15)	0.001
Gastrointestinal therapy			
any	2 (10)	9 (7)	1.0
PPI	100 (2)	72 (5)	0.69
NSAID	0	14 (1)	
H_2 antagonist	0	14 (1)	
Previous CDI	0	7 (2)	0.53
Hospitalization in last 6 months	45 (14)	23 (18)	0.022
Number of diarrhoeal stools per day			
1–2	17 (4)	14 (9)	0.87
3–4	33 (8)	39 (25)	
>4	50 (12)	47 (30)	
Treatment given for diarrhoea	57 (17)	27 (26)	0.003
Resolution of diarrhoea			
resolved	77 (23)	83 (74)	0.53
continued	13 (4)	12 (11)	
recurrence	10 (3)	5 (4)	
Outcome following diarrhoea			
none	52 (16)	76 (68)	
short-term	26 (8)	14 (12)	
hospitalization	13 (4)	9 (8)	
death	10 (3)	1 (1)	0.023

PPI, proton pump inhibitor; NSAID, non-steroidal anti-inflammatory drug.
[a]Numbers given are for when questions were answered, and therefore these do not add up to the total numbers of cases or controls; blank responses are excluded from the analyses.

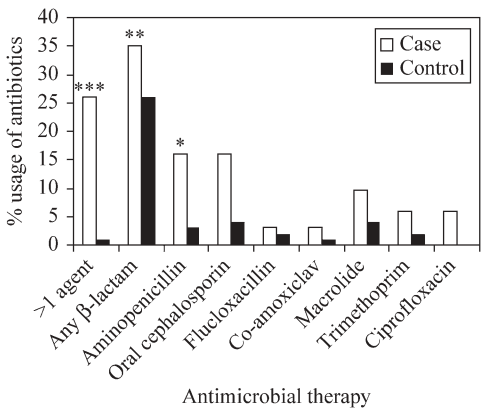

Figure 1. Comparative antibiotic usage in randomly selected community-associated CDI cases and controls. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 1. Prior exposure to any or specific gastrointestinal-acting drugs (proton pump inhibitor, H_2 antagonist or non-steroidal anti-inflammatory) was not significantly more common in cases when compared with controls. A significant excess of cases when compared with controls were reported to be in close contact with infants aged $\leq$2 years (14% versus 2%; $P = 0.02$). No other factors investigated were found to be of significance.

Number of diarrhoeal stools, as a possible proxy indicator of severity of disease, was not significantly greater in cases when compared with controls. However, a greater proportion of CDI case patients received therapy for diarrhoeal symptoms when compared with controls (57% versus 27%; $P = 0.003$). Further analysis of the type of therapy received because of diarrhoea (antibiotic, anti-motility agent, rehydration therapy, cessation of antibiotics and none) did not show significant associations. There was no significant difference between cases and controls in terms of response to therapy. However, outcome in cases versus controls differed significantly; 10% of cases, for whom follow-up data were supplied, died compared with 1% of controls ($P = 0.023$). CDI was implicated as a cause of death in only one of the three cases who died.

C. difficile isolates were recovered from 54/61 patients with community-associated CDI. DNA fingerprinting using RS-PCR showed that 60% and 13% of isolates from urban and semi-rural cases, respectively, were indistinguishable from the UK epidemic strain PCR ribotype 1. Subsequent AP-PCR fingerprinting of PCR ribotype 1 isolates showed that those from the urban cases were all identified as one subtype (AP-PCR type 1a), whereas those from the semi-rural cases were all a different subtype (AP-PCR type 1b) (Figure 2).

Discussion

The definition of community-acquired CDI in previous studies is not standardized (Table 2).[4,5,8–14] Notably, the true place of acquisition of CDI (e.g. hospital, care home, community), as opposed to where the patient became symptomatic, is rarely known. The relatively frequent carriage of *C. difficile* by asymptomatic elderly individuals, some of whom may then develop CDI at a later stage, clouds this issue. We therefore prefer to use the term community-associated CDI to encompass all those cases of symptomatic CDI that occur in patients in the community setting. Community-associated CDI is also poorly characterized because laboratories have infrequently examined faecal samples from community patients for evidence of CDI. Previous studies that have examined community-associated CDI and associated risk factors differ markedly in design, locale and risk factors that were investigated and, crucially, selection criteria used to identify subjects. Selection criteria such as recent antibiotic use,[13] hospital admission[10,14] or identified as *C. difficile*-positive[8] have potentially introduced bias. We used a case–control design to compare potential risk factors for community CDI, with a follow-up questionnaire designed to maximize the GP response rate (86%). We opted also to examine faecal samples from patients with a history of antimicrobial chemotherapy, as this was both consistent with routine laboratory practice in one of the study centres at the time of study initiation, and permitted comparison of the relative value of using specific and non-specific criteria to identify *C. difficile* cytotoxin-positive community-based patients. However, we have presented here

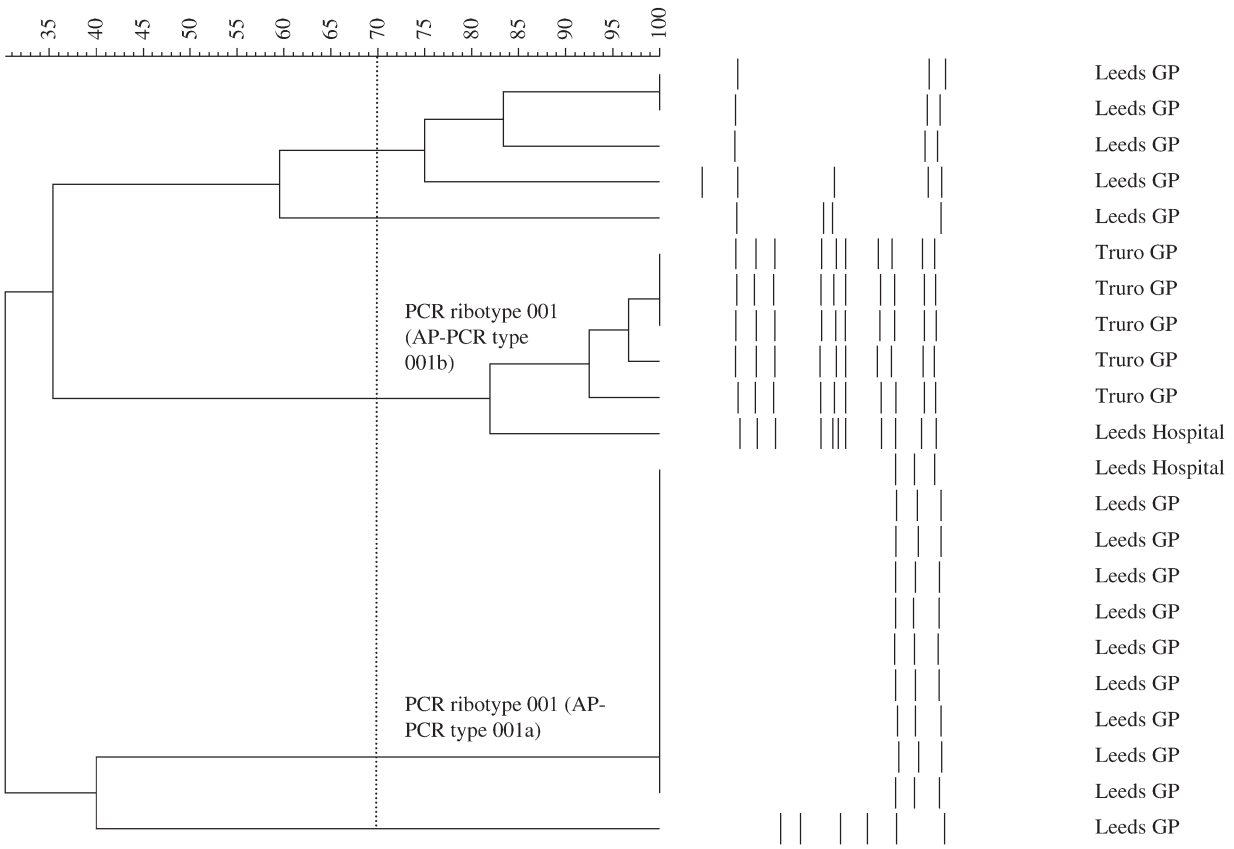

Figure 2. Analysis of AP-PCR profiles of *C. difficile* isolates from cytotoxin-positive faecal samples from patients with community-associated CDI. DNA profiles were analysed by using BioNumerics software (Applied Maths, BioSystematica). Dendrograms were constructed by the unweighted pair group method with arithmetic mean clustering using the Dice correlation coefficient. The percentage level (70%) of similarity used for distinct type determination is indicated by the vertical dashed line.

questionnaire-generated risk factor data for the randomly selected cases and their controls to avoid potential bias related to prior antibiotic exposure.

The prevalence of *C. difficile* cytotoxin positivity was similar in our randomly selected cohorts in the urban and semi-rural settings (2.1% and 2.1%, respectively). Using local population data, we estimated that the annual incidence of community-associated CDI in Leeds was ~50% higher than in Truro (29.5 versus 20.2, per 100 000, respectively). Karlström *et al.*[5] described 6-fold differences in overall CDI incidence between different counties in Sweden. Our estimated annual incidences of CDI are consistent with those reported in other prospective studies.[5,8–10] Crucially, however, underestimation of community-onset infectious IID burden is common, because not all affected individuals seek medical help, and due to differences in specimen submission, investigation and result reporting. Using reporting pyramid analysis, Wheeler *et al.*[7] estimated that for every case of IID detected by national laboratory surveillance, there are 136 in the community. The authors calculated that for every case of CDI presenting to GPs, there are eight more cases in the community. The median age of our CDI cases randomly detected in the semi-rural population was significantly lower than that of cases in the urban patients (45 versus 73 years). Therefore, many community-associated CDI cases would be missed if an age threshold (e.g. 65 years) for surveillance is followed.[6,16] Interestingly, the median age (78 years) of the semi-rural cases, selected on the basis of a history of recent antibiotic use, was similar to that of the urban cases. It is also clear from our data that a laboratory testing policy based solely on a history of antibiotic use would miss a significant proportion of community-associated CDI cases.

Recent antibiotic exposure was significantly associated with community-associated CDI. We found that while CDI cases (as detected by random sampling) were twice as likely as controls to have been hospitalized in the preceding 6 months (45% versus 23%, respectively; $P = 0.022$), the majority (55%) of *C. difficile* toxin positive individuals had no such history. Karlström *et al.*[5] found that 28% of all cases of CDI involved no recent (previous 4 weeks) hospitalization and thus defined these as community-acquired, although a much larger proportion of these patients received prior antimicrobial therapy (93%) in comparison to the present study (52% of the randomly selected cohort). Similarly, Svenungsson *et al.*,[14] investigating the epidemiology of hospitalized *C. difficile*-positive patients, observed that 28% were in fact community-associated, as did Norén *et al.*[10] (22%). We found a very low proportion (8%) of CDI cases in individuals from nursing/residential homes, and thus all non-hospital-associated CDI were assumed to be of

Table 2. Summary of previous studies on community-associated CDI

Author	Study design	Duration	Definition of community acquisition	Incidence of community CDI[a]	Antibiotic exposure determination	Observations	Molecular epidemiology
Norén *et al.*[10]	retrospective cohort—hospital and community in a Swedish county	1 year	no hospitalization during study period	25	whole antibiotic consumption [daily defined dose (DDD)]	hospital CDI: 477 DDD/1000 bed days versus community: 13 DDD/1000 inhabitants	PCR ribotyping: 1 serotype responsible for 6% community isolates and 20% of hospital isolates
Karlström *et al.*[5]	retrospective CDI survey in Sweden: community cases analysed: retrospective review	1 year	no hospitalization in previous month	20	overall prior antibiotic exposure	88% community cases had prior antibiotic exposure	NA
Hirschhorn *et al.*[8]	retrospective cohort study of members of Health Maintenance Organization in ambulatory setting	2.5 years	CDI with onset ≥42 days post-hospitalization	7.7	antibiotic-specific attack rates for each risk period for CDI = 2–42 days post-prescription	65% cases developed CDI post oral antibiotic exposure. Increased risk associated with: β-lactam inhibitor combinations plus oral cephalosporin, or oral cephalosporin alone	NA
Levy *et al.*[9]	retrospective cohort with nested case–control analysis: ambulatory care patients (USA)	2 years	ambulatory patient	12	pre-risk period: 6 months prior to prescription. Antibiotic risk period: interval of 2–42 days post-prescription. Single antibiotic exposures only	Amoxicillin = highest number risk periods, cases of diarrhoea and *C. difficile* tests. Single exposure: cefalexin and cefixime = greatest frequencies of CDI cases	NA
Riley *et al.*[4]	prospective survey: *C. difficile* isolation rate in community patients (W. Australia)	14 months	patients attending GPs or community healthcentre	NA	prior exposure to antibiotics	isolation rate = 4.7% increasing to 15.9% in patients with prior antibiotic exposure	NA

Beaugerie *et al.*[13]	prospective cohort: adult outpatients prescribed 5–10 days antibiotic therapy (Paris, France)	8 months	no hospitalization in previous 6 months, no antimicrobial therapy in previous 2 months	NA	overall exposures to antibiotics, number of agents, type of agent	duration of antibiotic treatment and co-amoxiclav associated with diarrhoea. Estimated acquisition rate CDI: 2700 cases /100 000 exposures	NA
Svenungsson *et al.*[14]	prospective epidemiology of nosocomial CDI (Swedish Teaching Hospital)	1 year	CDI within 72 h admission plus no recent history of hospitalization	NA	overall antibiotic exposure of both hospital and community patients in previous 2 months	28% CDI cases were community-acquired. 80% of all cases (hospital and community) had prior antibiotic exposure	PCR ribotyping: 3 dominating ribotypes accounted for 30% and 34% of hospital- and community-associated CDI, respectively
Riley *et al.*[12]	prospective 2-phase survey: pathology laboratory Western Australia	NA	all stool samples submitted via GPs	NA	GP questionnaire re: antibiotic exposure for CDI cases	*C. difficile* isolation rate: Phase 1 = 2.6% (all samples) Phase 2 = 10.7% (specific request/history antibiotics) β-lactams = most common (58%) of recently prescribed antibiotics	NA
Riley *et al.*[11]	prospective survey: stool samples from GP patients	4 months	all stool samples submitted via GPs	NA	GP questionnaire re: antibiotic exposure for CDI cases	*C. difficile* isolation rate = 5.5% 69% CDI cases exposed to antibiotics in previous 3 months; tetracyclines and amoxicillin accounting for >50% exposures	NA

[a]Annual incidence per 100 000 individuals.
NA, not available.

community-onset. Nonetheless, *C. difficile* colonization and infection is a common occurrence in elderly residents of long-term care facilities, with colonization rates of 9% in nursing home patients being recently reported.[17,18]

Exposure to antibiotics is the most significant and frequently reported predisposing risk factor for CDI among both hospitalized and community patients (Table 2).[4,5,8–14] As shown previously, we found that individuals in receipt of more than one antibiotic were significantly more likely to have CDI than controls (26% versus 1%; $P = 0.0006$).[8] A strength of our study is that previous exposure to antimicrobials was determined for each agent in every case and control, whereas some other community-based studies have examined overall population-based antibiotic prescribing.[5,10,13,14] We found that β-lactam use, in particular oral aminopenicillins or cephalosporins, was significantly associated with community-associated CDI. These findings confirm those of previous studies.[8,9,12] Neither flucloxacillin nor co-amoxiclav were significantly associated with CDI in our randomly selected cohorts. Beaugerie *et al.*[13] found a significant association between co-amoxiclav and *C. difficile* diarrhoea in adult outpatients. Although Hirschhorn *et al.*[8] reported that co-amoxiclav plus cefuroxime or cefaclor were significantly associated with increased risk of community-associated CDI, this was likely due to cephalosporin exposure. We found an excess of CDI cases received ciprofloxacin (6 versus 0 controls), which was of borderline significance ($P = 0.06$). Dhalla *et al.*[19] found no increased risk of CDI requiring hospitalization among patients prescribed gatifloxacin or moxifloxacin when compared with levofloxacin. In a study of ambulatory cancer patients, the multivariate regression analysis showed that each additional day of exposure to clindamycin or third-generation cephalosporin, but not to fluoroquinolones, was associated with significant excess risk of CDI.[20] Fluoroquinolone use has been implicated as a risk factor for CDI in the hospital setting,[21,22] although confounding factors may be important here.[23,24]

Surprisingly, there was no history of recent antibiotic exposure in almost half of our randomly detected CDI cases. Furthermore, approximately one-third of CDI cases neither had recent exposure to antibiotics nor to hospitals. Similarly, 63% and 45% of patients had no recent (90 day) exposure to antimicrobial therapy in two previous studies.[25,26] The only other significant risk factor we found that may account for some of these cases is contact with an infant under 2 years old. It has been consistently demonstrated that a significant proportion of infants may carry *C. difficile*.[27–29] Despite these high carriage rates, there are no reports, to our knowledge, of contact with infants as being a risk factor for CDI. This may be because previous CDI risk factors studies have concentrated on hospitalized cases or have not considered infant carriage as potentially important. Sampling of infant contacts to establish whether strains match those recovered from cases could be a valuable research goal to investigate further the association that we have described here. We did not perform a multivariate analysis, because of limited numbers of replies to some questions, and thus cannot exclude the possibility of confounding by other risk factors such as antibiotic use. Further data on this potential risk factor for *C. difficile* acquisition or infection are required. There is an unresolved controversy concerning proton pump inhibitors as a potential risk factor in CDI. Two retrospective studies by Dial *et al.*[25,26] suggested that community-associated CDI in England was associated with use of prior proton pump inhibitors. A hospital-based case–control study in Wales also found that CDI was independently associated with antibiotic use, acid suppression therapy and female sex.[30] However, two recent large series and our present study have failed to demonstrate such an association.[31,32] Data confounding, which is inherent in retrospective studies, is likely to affect risk factor analyses, and prospective studies are required to resolve this issue.

It is possible that CDI may not in fact have been the cause of the diarrhoeal symptoms that prompted submission of some cytotoxin-positive faecal samples. We tested all samples for evidence of conventional enteropathogens, but did not examine for gastroenteritis viruses or for other potential pathogens such as *Aeromonas* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus* spp. and *Vibrio* spp. However, a recent major study on IID in community patients found that collectively these 'other' pathogens were rarely (6%) implicated.[7] Cases were significantly more likely to be treated for their symptoms when compared with controls (57% versus 27%; $P = 0.003$), implying a difference in severity of diarrhoea or that an infective aetiology was considered more likely in these individuals. However, there was no difference in the number of diarrhoeal stools reported per day by cases and controls. Despite fewer controls receiving active treatment, there was no significant difference in the rate of resolution of diarrhoea, implying that true infection was not present in controls. Also, it was considered that the diarrhoeal symptoms affected patient progress for approximately half of the cases but only a quarter of the controls. Furthermore, there was a significantly higher mortality rate reported in CDI cases when compared with controls (10% versus 1%; $P = 0.023$), although this infection was not recorded as a primary cause of death in any of these patients. This observation is likely reflective of frailty or co-morbidities in elderly CDI cases as has been noted elsewhere, and we recognize the difficulty of determining the relationship of CDI to causes of death.[1] We acknowledge that despite the high response rate to our risk factor questionnaire, we cannot exclude the possibility of recall bias.

The molecular fingerprinting results show variation in the prevalence of *C. difficile* strains in the two study regions. It is well documented that *C. difficile* ribotype 001 is widely distributed, although this strain has decreased in prevalence in the UK since this study was carried out.[33] The community prevalence of PCR ribotype 001 and indeed other ribotypes has not been specifically investigated, making interpretation of these data difficult. We previously established a high hospital prevalence of a clindamycin-susceptible ribotype 001 clone in Leeds (AP-PCR type 001a).[16] The presence of this strain in the population served by this hospital is therefore not surprising. We also identified a much less common genotypically distinct clindamycin-resistant PCR ribotype 001 subtype *C. difficile* strain (AP-PCR type 001b).[34] Interestingly, in the semi-rural cohort, 5 of 39 (13%) isolates were indistinguishable from AP-PCR type 001b. We speculate that AP-PCR type 001b was present in hospitals within the semi-rural area examined in this study. Norén *et al.*[10] identified nine major ribotypes associated with the majority of hospital- (67%) and community-acquired (59%) cases. These observations are consistent with a close interplay between the hospital and community settings, which is likely to strengthen with earlier hospital discharges and more frequent episodes of community-based care.

In conclusion, CDI in the community is almost certainly under-diagnosed. Reliance on antibiotic history and age (>65

years) will contribute to missed diagnoses. Although recent antibiotics (particularly β-lactams) and hospitalization are significantly associated with CDI presenting in the community, about one-third of the cases has neither of these risk factors. We identified close contact with infants under the age of 2 years as a potential risk factor. This and other risk factors for community-onset CDI should be explored further. Finally, the increased incidence of CDI and emergence of *C. difficile* ribotype 027 since this study was performed emphasize the need to delineate the epidemiology and aetiology of community-onset CDI in different settings.

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