Risk factors for *Clostridium difficile* colonisation and toxin production

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Abstract

Objectives: to examine risk factors for patients becoming *Clostridium difficile* culture and toxin positive.
Design: prospective cohort study.
Setting: two medicine for the elderly wards.
Participants: patients admitted to the wards over a 17-month period.
Measurement: presence of *Clostridium difficile* on culture of stool specimens and toxins A and/or B. Patient's age, sex, source of admission, antibiotic, laxative, antacid and steroid use, presence/absence of colonic disease, neoplasia, leukaemia and nasogastric or percutaneous endoscopic gastrostomy tube feeding.
Results: 390 of 865 patients admitted provided a total of 1003 faecal specimens. Age (OR 1.04, 95% CI 1.001–1.07 per year), admission from another hospital (OR 2.13, 95% CI 1.29–3.50), non-cephalosporin antibiotics (OR 2.08, 95% CI 1.25–3.46) and cephalosporin use (OR 8.45, 95% CI 2.99–23.9) increased risk of becoming *Clostridium difficile* culture positive. Becoming toxin-positive was associated with antibiotic use only (OR 3.02, 95% CI 1.15–7.92), specifically amoxycillin (OR 8.72, 95% CI 1.66–45.9) and cephalosporins other than ceftriaxone (OR 7.28, 95% CI 1.34–39.6).
Conclusion: different risk factors are important for the two stages leading to *Clostridium difficile* diarrhoea. Age, source of admission and third generation cephalosporins increase risk of becoming culture positive, whilst only antibiotic use is associated with the step of becoming toxin-positive. Understanding these differential risks may aid infection control strategies.

Keywords: *Clostridium difficile, cephalosporins, epidemiology*

Introduction

Clostridium difficile is a major nosocomial infection associated with antibiotic use, especially third-generation cephalosporins [1, 2]. The general aetiological model for the disease hypothesises that antibiotics suppress the normal bowel flora and thus allow colonisation of *C. difficile*, which is relatively resistant to many antibiotics [3]. If adequate numbers of toxin-producing strains colonise the colon, symptoms of diarrhoea ensue. Investigators have found that failure to mount an immune response is associated with both colonisation and toxin-related symptoms [4, 5]. Moreover, the immune responses to *C. difficile* and its toxins appear distinct so that a staged aetiological model is proposed [6]. In everyday clinical practice and much research, the focus is on those patients with symptoms, and there is no attempt to identify patients who are colonised with *C. difficile* but remain symptom free. Hence, putative risk factors, such as old age, are predicated to both stages combined. However, it is possible that conversion from *C. difficile* culture negative (Cdc −ve) to culture positive (Cdc +ve) status is influenced by different factors from the conversion from culture positive toxin negative (Cdt −ve) to culture positive toxin positive (Cdt +ve) status. This is of real importance in *C. difficile* infection control because the number of symptomatic cases does not relate linearly to a change in infectivity rate, but instead threshold effects are likely due to 'herd immunity' [7]. In a two-stage model, the relative infectivity of colonised and symptomatic patients has implications for how likely changes in risk factors are to lead to an outbreak. Moreover, a risk factor that increases colonisation, but not toxin production, may be of little importance in a setting where patients are unlikely to develop diarrhoea. This might be the case for third generation cephalosporin use in orthopaedic wards where there are a substantial number of young, fit patients. However, if the surrounding patients were older and frailer and therefore likely to become toxin-positive once colonised, such as in a geriatric rehabilitation ward, an epidemic would be highly probable.

As a preliminary stage to producing a stochastic model of *C. difficile* infection, we sought to identify important risk factors for each stage of the model [8], and in particular to determine whether those that predisposed to colonisation were also associated with toxin production. We included known risk factors for *C. difficile* [9], such as age, antibiotic use, nasogastric feeding and underlying disease, in a prospective study where we identified asymptomatic colonised patients as well as those with symptoms.

Participants and methods

As part of hospital infection control policy, all patients admitted to two medicine for the elderly wards who were willing and able provided stool specimens at weekly intervals. The wards admitted patients directly from the community, including a small number from nursing homes, and also took patients transferred from other wards for ongoing rehabilitation, such as following orthopaedic surgery. The wards were mirror images of each other, with four six-bedded bays and six side-rooms each. Patients who developed *C. difficile* diarrhoea were isolated in side-rooms. Case-mix was similar on both wards except that one took in a small number of patients requiring hospital respite who, although frail, were less likely to be treated with antibiotics. Patient's age, sex, source of admission (community, hospital, nursing home), antibiotic (for the 2 weeks prior to sample), laxative, antacid and steroid use, presence/absence of colonic disease, neoplasia, leukaemia and nasogastric or percutaneous endoscopic gastrostomy tube (NG/PEG) feeding noted. *C. difficile* was cultured in the Scottish national specialist laboratory using cycloserine-cefoxitin egg-yolk selective agar [10]. Toxins A and/or B were detected directly using the Techlab ELISA test kit. Strain phenotyping was performed by identifying surface-layer proteins and confirmed the presence of a predominant 'epidemic' strain, which is identical to PCR ribotype 1 [11]. Statistical analysis was performed using the SPSS 10.0 statistical package.

Results

Eight hundred and sixty-five patients (mean age 82.4, standard deviation 7.6 years, 62.4% female, 39% transferred from another hospital ward) were admitted to the two wards over a 17-month period. One thousand and three faecal specimens were obtained from 390 (45%) of the patients (mean age 82.5, standard deviation 7.3 years, 64.9% female, 43.6% transferred from another hospital ward). Two hundred and ninety patients were Cdc −ve, of whom 17 were toxin positive. One hundred patients were Cdc +ve of whom 34 were Cdt +ve. Of the 339 patients who were Cdt −ve, 53 (19.1%) of the 273 who were Cdc −ve had an episode of diarrhoea within one week after testing compared with 21 (31.8%) of the 66 Cdc +ve. Of the 51 Cdt +ve patients 9 (52.9%) of the 17 who were Cdc −ve had an episode of diarrhoea within one week after testing compared with 29 (85.3%) of the 34 Cdc +ve. We excluded the 17 Cdc −ve, toxin positive patients from the analysis because we were unsure how to categorise them in the staged model [6]; they may represent a group where *C. difficile* load was too small to detect by culture.

Table 1. *Clostridium difficile* status for 373 patients according to antibiotic treatment (*$P < 0.05$, **$P < 0.01$). Note that patients may have received more than one antibiotic

Antibiotic	Number of patients	Number culture positive (%)	Number toxin positive (%)
None	160	36** (22.5%)	7* (4.4%)
Amoxycillin	19	9 (47.4%)	7** (36.8%)
Co-amoxyclav	104	36* (34.6%)	16 (15.4%)
Other penicillin	18	6 (33.3%)	1 (5.6%)
Ceftriaxone	11	9** (81.8%)	4 (36.4%)
Other cephalosporin	14	8* (57.1%)	6* (42.9%)
Macrolide	30	15** (50.0%)	5 (16.7%)
Quinolone	39	13 (33.3%)	6 (15.4%)
Trimethoprim	33	7 (21.2%)	3 (9.1%)
Other	21	9 (42.9%)	6 (28.6%)

There was no significant difference in risk of becoming Cdc +ve ($P = 0.14$) or Cdt +ve ($P = 0.11$) between wards. There was a trend ($P = 0.073$) for Cdc +ve patients to be older than Cdc −ve patients, and Cdt +ve were significantly older than Cdt −ve patients (mean age 84.6 *versus* 82.1 years, $P = 0.021$). Several antibiotics had univariate significant associations with conversion (Table 1), but many patients received more than one antibiotic. Of the other risk factors, only being admitted from another hospital ward, NG/PEG feeding or not receiving laxatives were significant risk factors in univariate analyses (Table 2). Forward conditional logistic regression identified age (OR 1.04, 95% CI 1.001–1.07 per year), admission from another hospital (OR 2.13, 95% CI 1.29–3.50), non-cephalosporin antibiotics (OR 2.08, 95% CI 1.25–3.46) and cephalosporin use (OR 8.45, 95% CI 2.99–23.9) to increase conversion to Cdc +ve risk; no other variables reached significance. The only specific antibiotic identified as increasing risk was ceftriaxone (OR 18.3, 95% CI 3.24–103.0).

For those Cdc +ve, conversion to Cdt +ve status was associated with antibiotic use only (OR 3.02, 95% CI 1.15–7.92). None of the other risk factors were significant in univariate analyses (Table 2). Specific antibiotics conferring risk were amoxycillin (OR 8.72, 95% CI 1.66–45.9) and cephalosporins other than ceftriaxone (OR 7.28, 95% CI 1.34–39.6). Considering the overall conversion from Cdc −ve to Cdt +ve status, age (OR 1.06, 95% CI 1.006–1.11 per year), both non-cephalosporin antibiotics (OR 4.12, 95% CI 1.70–10.0) and cephalosporins (OR 11.7, 95% CI 3.39–40.6) increased risk. Specific antibiotics identified were amoxycillin and cephalosporins other than ceftriaxone, as with the Cdc +ve to Cdt +ve stage.

Table 2. *Clostridium difficile* status for 390 patients according to risk factors (*$P<0.05$, **$P<0.01$). Note that patients may have more than one risk factor

Risk factor	Number of patients with risk factor	Number culture positive	Number toxin positive
Gender			
Male	137	40	11
Female	253	60	23
Source			
Community	200	39	13
Nursing home	20	1	1
Hospital	170	60**	20
Colonic disease	42	7	1
Neoplasia	36	7	1
Leukaemia	4	2	0
NG/PEG feeding	6	4*	2
Antacids	101	32	13
Laxatives	178	36*	11
Steroids	25	7	4

Discussion

This study corroborates others identifying cephalosporins as conferring a greater risk of *C. difficile* diarrhoea compared with other antibiotics [1, 12, 13]. This effect was detected despite relatively small numbers exposed. The implication of amoxycillin, but not co-amoxiclav, in the Cdc +ve to Cdt +ve stage may imply a protective effect of co-amoxiclav against anaerobe infections. Age and source of admission are important in increasing the pool of Cdc +ve patients who can then become Cdt +ve with further antibiotic exposure. Ceftriaxone may also play a specific role in Cdc −ve to Cdc +ve conversion.

A previous study identified nursing homes as a source of patients at high risk of *C. difficile* diarrhoea [12]. Only a small number of admissions in this study were from nursing homes, but patients transferred from other wards were at greater risk of colonisation. This risk was specifically at the colonisation rather than toxin-production stage, and source did not increase the overall risk of symptoms. Since many wards were potential sources for these patients, we cannot comment on possible predisposing factors prior to transfer, though transfer itself could be a marker for increased frailty and debility. One study suggests that a proportion of symptomless, colonised patients are at relatively low risk whether or not they carry a toxigenic strain [14]. Nevertheless, they may still represent a risk of infection to others on the ward, especially if the case-mix includes a high proportion of susceptible patients [15]. A failure to find other factors significantly associated with either stage may reflect small numbers for some variables, such as NG/PEG feeding, but is consistent with other prospective studies [13]. Whilst patients with several of these secondary risk factors may be at increased risk [13], such patients were relatively uncommon in our sample, and it may be that both multiple pathology and age are proxies for underlying frailty that is more difficult to quantify reliably on a single measure.

Despite our best efforts, we failed to sample 55% of the patients, all without diarrhoea. Sample bias is therefore quite possible. Those sampled were, however, representative in terms of age and sex, and fairly representative in terms of the proportion of patients transferred from another hospital ward rather than admitted direct from the community. Our *C. difficile* diarrhoea rate of 3.9% for all 865 patients admitted lies in the middle of the range found prospectively in five Scandinavian centres [13]. Taking the entire 865 patients as the denominator would give a colonisation rate of 11.6%, not that much higher than the 7.6% found in healthy Japanese adults who had no recent exposure to antibiotics [16]. Hospital studies suggest a colonisation rate of about 24% [14], which is close to our rate of 25.6% if only those patients who provided stool specimens are included as the denominator. It is therefore likely that we failed to detect a considerable number of asymptomatic *C. difficile* carriers. Since most of the Cdc +ve patients remained asymptomatic, our estimates for risk factors for the Cdc −ve to Cdc +ve stage are likely to be fairly good. However, our estimates for the Cdc +ve to Cdt +ve stage are more questionable. A further difficulty is that *C. difficile* infection has clear spatio-temporal characteristics. Hence, patients exposed to major risk, for example third generation cephalosporin treatment, may not develop diarrhoea if there are no other cases on the ward at that time, assuming that patients with *C. difficile* diarrhoea are far more infectious than asymptomatic carriers. It is for these reasons of missing data, with the associated possible sample bias, and spatio-temporal variation, that stochastic inferential modelling is needed to better define *C. difficile* epidemiology [8]. Nevertheless, initial estimates of risk are essential as starting points for more complex modelling that cannot take into account all possible risk factors in a single model. Moreover, these data confirm the need for a multi-stage model of *C. difficile* infection in which risk factors may be specific to particular steps.

Effective infection control in hospitals is aided by an understanding of the different stages that lead to *C. difficile* diarrhoea. Restriction of antibiotic use, although a primary prevention strategy [17], is not always easy to implement. In this study ceftriaxone, a third generation cephalosporin, was especially associated with increased *C. difficile* colonisation which, in turn, increases the incidence of toxin-positive cases. Our data suggest that a secondary strategy of substituting co-amoxiclav for amoxycillin and cephalosporins may be beneficial. Intervention studies to evaluate the effectiveness of this measure are needed. Patients who move between wards were at increased risk and this may be because they stay longer in hospital, and therefore at greater risk of exposure, and because they may be more frail since otherwise they would have been discharged before transfer to a geriatric ward was necessary. Particular attention should be paid to this group in terms of preventive measures, such as careful hand washing and antibiotic use. In view of the mortality, morbidity and extra costs to the health service associated with *C. difficile* infection, the improved stratification of risk facilitated by the two-stage model should prove highly useful in a disease where prevention is so clearly much better than cure.

Key points

- There are two stages that lead to *Clostridium difficile* toxin-positive diarrhoea: first becoming *C. difficile* culture positive and then toxin production.
- Age, source of admission and third generation cephalosporins increase risk of becoming culture positive.
- Antibiotic use is associated with the step of becoming toxin-positive.
- Knowledge of those risk factors that are associated with culture positive status aids identification of those patients at risk of progressing to toxin-positive diarrhoea.

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References

1. Impallomeni M, Galletly NP, Wort SJ, Starr JM, Rogers TR. Increased risk of *Clostridium difficile* diarrhoea in elderly patients receiving cefotaxime. Br Med J 1995; 311: 1345–6.

2. McFarland LV, Surawicz CM, Stamm WE. Risk factors for *Clostridium difficile* carriage and *C. difficile*-associated diarrhoea in a cohort of hospitalized patients. J Infect Dis 1990; 162: 678–84.

3. Noren T, Tang-Feldman YJ, Cohen SH, Silva J, Olcen P. Clindamycin resistant strains of *Clostridium difficile* isolated from cases of *C. difficile* associated diarrhoea (CDAD) in a hospital in Sweden. *Diag Microbiol Infect Dis* 2002; 42: 149–51.

4. Mulligan ME, Miller SD, McFarland LV, Fung HC, Kwok RY. Elevated levels of serum immunoglobulins in asymptomatic carriers of *Clostridium difficile*. Clin Infect Dis 1993; 16 (Suppl 4): S239–44.

5. Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. Lancet 2001; 357: 189–93.

6. Wilcox M, Minton J. Role of antibody response in antibiotic-associated diarrhoea. Lancet 2001; 357: 158–9.

7. Starr JM, Rogers TR, Impallomeni M. Hospital acquired *Clostridium difficile* diarrhoea and herd immunity. Lancet 1997; 349: 426–8.

8. Starr JM, Campbell A. Mathematical modelling of *Clostridium difficile* infection. Clin Microbiol Infect 2001; 7: 432–7.

9. Starr JM, Impallomeni M. Risk of diarrhoea, *Clostridium difficile* and cefotaxime in the elderly. Biomed Pharmacother 1997; 51: 63–7.

10. Brazier JS. Role of the laboratory in investigations of *Clostridium difficile* diarrhoea. Clin Infect Dis 1993; 16: 5228–33.

11. McCoubrey J. The Epidemiology of *Clostridium difficile* in a Geriatric Unit. University of Edinburgh, PhD thesis, 2002.

12. Al-Eidan FA, McElnay JC, Scott MG, Kearney MP. *Clostridium difficile*-associated diarrhoea in hospitalised patients. J Clin Pharm Ther 2000; 25: 101–9.

13. Wistrom J, Norrby SR, Myhre EB *et al.* Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. J Antimicrob Chemother 2001; 47: 43–50.

14. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. Lancet 1998; 351: 633–6.

15. Borriello SP. 12th C. L. Oakley lecture. Pathogenesis of *Clostridium difficile* infection of the gut. J Med Microbiol 1990; 33: 207–15.

16. Kato H, Kita H, Karasawa T *et al.* Colonisation and transmission of *Clostridium difficile* in healthy individuals examined by PCR ribotyping and pulsed-field gel electrophoresis. J Med Microbiol 2001; 50: 720–7.

17. Barbut F, Petit JC. Epidemiology of *Clostridium difficile*-associated infections. Clin Microbiol Infect 2001; 7: 405–10.

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