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Is *Clostridium difficile* associated with the '4C' antibiotics? A retrospective observational study in diabetic foot ulcer patients

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SUMMARY

Aims: *Clostridium difficile* is an anaerobic cytotoxin-producing bacterium that can cause infectious diarrhoea, pseudomembranous colitis and toxic megacolon. The major risk factors for developing *C. difficile* infection include recent or current antimicrobial use, diabetes, age over 65, proton pump inhibitor use, immunosuppression and previous infection with *C. difficile*. Most diabetic foot ulcers are polymicrobial. **Methods:** As a result guidelines advise treatment with broad spectrum antibiotics which include the '4C's' (clindamycin, cephalosporins, co-amoxiclav and ciprofloxacin) which are associated with a higher risk of *C. difficile* infection. Retrospective observational data (June 2008 to January 2012) for the diabetes foot ulcers were gathered from the Diabetes/Podiatry Clinic database in NHS Ayrshire and Arran and cross-matched with the NHS Ayrshire and Arran Microbiology database. There were 111 patients with mean age 59 years (range 24–94 years), 33 type 1 patients, 78 type 2 patients, mean duration of diabetes 16 years (6 months–37 years) and mean HbA_{1c} 67 mmol/mol (54–108 mmol/mol) [8.3% (7.1–12%)]. **Results:** The total number of days antimicrobials prescribed for all patients was 7938 (mean number of antimicrobial days per patient = 71.5 days). There was one case of *C. difficile* infection of 111 patients giving an incidence of 1.25 cases per 10,000 patient-days of antibiotics/1 case per 209 foot ulcers. **Conclusions:** Large doses, numbers and greater duration of antibiotic therapy all result in a greater degree of normal gut flora depletion. It is possible that the alterations in gut flora in diabetic foot ulcer patients protect them from antibiotic-induced *C. difficile* overgrowth.

What's known

- *Clostridium difficile* is an anaerobic cytotoxin-producing bacterium that can cause infectious diarrhoea, pseudomembranous colitis and toxic megacolon.
- The major risk factors for developing *C. difficile* infection include recent or current antimicrobial use, diabetes, age over 65, proton pump inhibitor use, immunosuppression and previous infection with *C. difficile*.
- Most diabetic foot ulcers are polymicrobial.
- We found one case of *C. difficile* infection of 209 antibiotic-treated infected foot ulcers.

What's new

- Large doses, numbers and greater duration of antibiotic therapy all result in a greater degree of normal gut flora depletion.
- It is possible that the alterations in gut flora in diabetic foot ulcer patients protect them from antibiotic-induced *C. difficile* overgrowth.

Introduction

Clostridium difficile is an anaerobic cytotoxin-producing bacterium described by Hall and O'Toole in 1935 when isolated from the stool of healthy neonates (1). In the 1970s, *C. difficile* was first associated with antibiotic use and specifically clindamycin. Tedesco et al. found that 10% of patients on clindamycin had pseudomembranous colitis (2). In the mid to late 1990s in the United States, the reported incidence of *C. difficile* infection in acute care hospitals was 30–40 per 1,00,000 population (3) By 2005, this had risen significantly to 84 per 1,00,000 population (4). *Clostridium difficile* infection is now recognised as a significant cause of morbidity and mortality *causing infectious diarrhoea, pseudomembranous colitis and toxic megacolon*. However, it is also estimated that up to 3% of the gastrointestinal tracts of healthy adults and up to almost 20% of hospital patients are colonised with *C. difficile* (4).

The major risk factors for developing *C. difficile* infection include recent or current antimicrobial use, age over 65, proton pump inhibitor use, immunosuppression and previous infection with *C. difficile* (5–13). In addition, the greater the number of antibiotics or the greater the length of time on antibiotics, the greater the risk of *C. difficile* (10). Being on two antibiotics compared with one increases the rate of *C. difficile* infection by 2.5-fold (13). A further recent study demonstrated taking an antibiotic for more than 18 days as compared with less than 4 days constitutes an eightfold increased risk of infection (9). Diabetes has also been identified as both a risk factor for the development and the recurrence of *C. difficile* infection (14).

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Patients with diabetes have up to a 25% lifetime chance of developing a foot ulcer and given the increasing incidence of diabetes worldwide, this represents significant morbidity and mortality (15,16). Most diabetic foot ulcers are polymicrobial with up to seven different organisms involved. Guidelines advise treatment with narrow spectrum antibiotics where possible. However, as a result of the diverse polymicrobial nature of foot ulcers treatment with broad spectrum antibiotics such as penicillins, quinolones, cephalosporins and clindamycin which cover the most common organisms is often necessary (17–21). These antibiotics are all part of the '4 C's' (clindamycin, cephalosporins, co-amoxiclav and ciprofloxacin) which are associated with a higher risk of *C. difficile* infection. Often two or more antibiotics are prescribed at the same time and in the case of osteomyelitis for a minimum course of 4–6 weeks (17–21).

Anecdotally many diabetologists do not associate diabetic foot ulcers and their treatment with *C. difficile*. There is a lack of an evidence base in the literature as to the real risk of developing *C. difficile* in patients with diabetes taking antibiotics for prolonged periods. The aim of this study was to investigate the risk of *C. difficile* infection in patients with infected diabetic foot ulcers attending the Diabetes/Podiatry Clinic in NHS Ayrshire and Arran, Scotland.

Methods

Retrospective observational data for the Diabetes Foot Ulcers were gathered from the Diabetes/Podiatry Clinic database and cross-matched with the NHS Ayrshire and Arran Microbiology database. The audit was registered with the Clinical Governance Department, NHS Ayrshire and Arran, and Caldicott Guardian approval was obtained. The Diabetes/Podiatry database included all patients attending between June 2008 and January 2012. There were 111 patients with mean age 59 years (range 24–94 years), 33 type 1 patients (19 men/14 women), 78 type 2 patients (51 men/27 women), mean duration of diabetes 16 years (6 months–37 years) and mean HbA_{1c} 67 mmol/mol (54–108 mmol/mol) [8.3% (7.1–12%)]. There were 209 separate infected diabetic foot ulcer episodes. All ulcers treated had evidence of inflammatory change and/or pus. At the Diabetes/Podiatry Clinic, all outpatient antibiotics are prescribed at the Clinic and are listed on a database which contains the names of antibiotics prescribed and the length of antibiotic courses. We did not exclude any patients because of age, previous *C. difficile* infection, use of proton pump inhibitors or immunosuppression. The diagnosis of *C. difficile* infection was based on the presence of *C. difficile* toxin in the stool.

Statistical analysis

To compare our data with results from other studies, the p-values were obtained using the Fisher's exact test.

Results

The total number of days antimicrobials prescribed for all patients was 7938 (Table 1). The mean number of antimicrobial days per patient was 71.5 days with a mean number of antimicrobial days per ulcer episode of 37.9 days. The shortest course of antimicrobial for a patient was 14 days and the longest was 280 days.

Metronidazole was prescribed for 644 days: in combination with another antibiotic for 394 days and on its own for 250 days. Combination prescribing for metronidazole was limited to 5% of all antibiotics prescribed.

In our audit, we did not include any inpatient antibiotics prescribed for the treatment of foot ulcers, antibiotics prescribed as part of our daily OutPatient Antibiotic Therapy service or antibiotics prescribed by the GP in the community for any other reason. These data were difficult to collect accurately and accounted for less than 5% of all antibiotics prescribed. Therefore, our database for '4C' antibiotic prescribing was an underestimate of their real usage.

Clostridium difficile infection

There was only one case of clinically significant *C. difficile* infection of 111 patients (209 separate dia-

Table 1 Antibiotic prescribed and days prescribed

Antimicrobial	Number of days prescribed	Percentage of total number of days prescribed (%)
Co-amoxiclav	3122	39
Clindamycin	2758	35
Ciprofloxacin	868	11
Metronidazole	644	8
Trimethoprim	126	1.5
Erythromycin	112	1.4
Doxycycline	98	1.2
Fluconazole	98	1.2
Rifampicin	84	1.1
Flucloxacillin	14	0.2
Clarithromycin	14	0.2

betic foot ulcer episodes) with the stool being positive for toxin on analysis. Three samples were equivocal but were negative on repeat testing. This related to one case of *C. difficile* during 7938 days of antibiotics prescribed giving an incidence of 1.25 cases per 10,000 patient-days of antibiotics/1 case per 209 foot ulcers. There was no formal follow up of the patients following discharge from the clinic. However, if any of the patients had developed *C. difficile* infection following discharge then it would have been evident from the cross-checking with the NHS Ayrshire and Arran Microbiology database.

Discussion

Diabetic foot ulcers can be classified as neuropathic, ischaemic or neuroischaemic depending on the relative contributions of peripheral neuropathy and peripheral arterial disease. Motor and autonomic deficits, restricted joint mobility thermal injury, poor foot care and foot deformity resulting in bony prominences, also contribute to the risk of ulceration. Neuropathic ulcers are the most common type and result from tissue-damaging mechanical loads applied to an insensate foot. Reduced sensation can substantially impair the patient's perception of touch, deep pressure, temperature and joint position. Peripheral vascular disease in the form of macro- or micro-vascular disease is an important component cause of one-third of foot ulcers and is a risk factor for recurrent ulcers (22–24).

Superadded foot infections are common in patients with diabetic foot ulcers and vary from mild (restricted to involvement of skin and subcutaneous tissue) to severe (accompanied by systemic signs of infection or metabolic instability) (17,18). Commonly in superficial ulcers, the organisms are Gram-positive cocci, i.e. *Staphylococcus aureus*, β-haemolytic streptococci, *Streptococcus pyogenes* or coagulase-negative staphylococci. Deeper ulcers or patients who are not antibiotic-naïve are likely to be infected with enterococci, *Pseudomonas* or anaerobes (17,18). *Clostridium* species have been grown and are found in deeper, chronically infected ulcers but *C. difficile* has not been grown from diabetic ulcers and identified as a pathogenic organism (18). Infection is the most important precipitating factor for lower limb amputation in patients with diabetes (24).

Antimicrobial drug use is the single most important risk factor for *C. difficile* infections (12). Large doses and numbers plus greater duration of antibiotic therapy all result in a greater degree of normal gut flora depletion relative to shorter courses of fewer antibiotics leading to smaller overall doses. The degree of depletion depends on the concentration of the drug achieved, duration of exposure and susceptibility of the microorganisms of the intestine along with the ability of *C. difficile* to overgrow and to cause disease (7,9). All antibiotic exposure increases the likelihood of *C. difficile* infection and in different studies exposure odds ratios vary considerably (11). Clindamycin is reported to increase the chance of acquiring *C. difficile* by 2.12–42. Cephalosporin exposure at 1 week doubles the chance of infection, and in studies for third generation cephalosporins the risk varied between 3.84 and 26 (11). Coamoxiclav increased the relative risk of *C. difficile* at 1–2 weeks by around fourfold (10,11). Ciprofloxacin, which is another antibiotic strongly associated with *C. difficile* infection (specifically the NAP-1/027 strain), makes up the '4C' (9). Previous studies have shown that these factors as well as diabetes itself significantly increase the risk of *C. difficile*.

In our study, we found 1 case out of 111 patients which amounted to 1.25 cases of *C. difficile* per 10,000 days of antibiotics. An audit undertaken in Ayrshire and Arran in 2010 investigating in patients aged over 65 found 8.6 cases per 10,000 inpatient-days (8). The decreased immune responsiveness commonly observed in patients over 65 years of age may in part account for the increased risk of *C. difficile* seen in this older group (4).

Why the rate of *C. difficile* infection in our study should be so low is not clear. The *C. difficile* infection incidence per ulcer patient in our study was 0.48% compared with the patient incidence of 2.2% in Loo et al. (2p = 0.1) (9) and 3.1% in Stevens et al. (2p = 0.02) (7). These studies investigated general inpatient medical and surgical populations while our study audited the incidence in patients with diabetes, itself a risk factor for the development of *C. difficile* infection. All of our patients were taking one of the '4Cs', often more than one and for many days or weeks. Specifically, previous studies have shown clindamycin to be particularly high risk: clindamycin was the second most prescribed antibiotic in our audit (2758 days). All the patients audited in our study were outpatients which may have been a protective factor. However, over a third of our patients had an inpatient period of 7 days or more. We also wondered if the co-prescription of metronidazole could have been a protective factor. As metronidazole was only co-prescribed in 5% of all the diabetic foot ulcers, this is unlikely to have been a significant factor.

The data in our study were collected retrospectively. Antibiotics prescribed for inpatients or antibiotics prescribed by General Practitioners were not included in the audit. However, as already stated this strengthens our findings as this short-fall in data

demonstrates that our risk estimate of *C. difficile* development is clearly an underestimate. The strengths of the study are the accurate recording of the antibiotics for diabetic foot disease prescribed at the Diabetic/Podiatry clinic on the Podiatry database, and the robust recording and cross-matching of Diabetes/Podiatry Clinic database and the NHS Ayrshire and Arran Microbiology database. Although there was no formal follow up of the patients, if a patient had developed *C. difficile* infection following discharge from the Diabetic/Podiatry clinic then that would have been evident from the cross-checking with the Microbiology database. Indeed, there are more community cases of *C. difficile* in Ayrshire and Arran than hospital cases. This shift in the epidemiology is a result of enhanced infection control measures and changes to the hospital antibiotic policy over the last 2 years (8). Thus, it is very unlikely that patients with diabetes would not be captured in this process.

Despite the findings in this study, we are not advising that the '4C' antibiotics be used with less caution. Long courses and combinations of antibiotics are in multiple studies significantly associated with *C. difficile* (Table 2). However, it is the case that in this study that the incidence of *C. difficile* was lower than would be expected and the reasons behind this remain unclear. In type 1 diabetes, the gut has been recognised as a regulator of early inflammation (25–27). In type 2 diabetes, intestinal microbiota appears to play a part in converting nutrients into energy and possibly contributing to the development of the Metabolic Syndrome (28,29). Gut flora is also recognised to change in the presence of autonomic neuropathy (30–32). It is reasonable to speculate that the alterations in gut flora in these patients with diabetic foot ulcers protected them from antibiotic-induced *C. difficile* overgrowth. Further research into the gastrointestinal tract and gut microbiota in diabetes may further our understanding of *C. difficile* infection, the '4Cs' and diabetes foot disease.

Table 2 Days of antibiotics prescribed in combination

	Days prescribed	Days prescribed (Percentage of total)
One antimicrobial	5460	69
Two antimicrobials	2198	28
Three antimicrobials	280	3

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