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Clostridium difficile ribotype 027 is most prevalent among inpatients admitted from long-term care facilities

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SUMMARY

Intestinal inflammation was evaluated using faecal lactoferrin and ribotype in 196 hospitalized adults with *Clostridium difficile* infection to determine the impact of ribotype 027 in long-term care facilities (LTCFs). LTCF residents (n = 28) had greater antibiotic use (P = 0.049) and more ribotype 027 infection [odds ratio (OR): 4.87; 95% confidence interval (CI): 2.02–11.74; P < 0.01], compared to those admitted from home. Patients infected with ribotype 027 strains had worse six-month mortality (OR: 1.90; 95% CI: 1.08–3.34; P = 0.03) and more inflammation (95.26 vs 36.08 μg/mL; P = 0.006), compared to those infected with non-027 strains. This study was not designed to determine acquisition site, but, in this population, suggests that the location from which the patient has been admitted is strongly associated with ribotype 027 and more severe *C. difficile* disease.

Keywords

Clostridium difficile infection; Long-term care residents; Nosocomial diarrhoea; Strain BI/NAP1/027

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Conflict of interest statement

Drs Archbald-Pannone and Guerrant have no relevant conflicts of interest. Mr Boone and Drs Carman and Lyerly are employed by TechLab, Inc.

Introduction

The impact of diarrhoea in long-term care facility (LTCF) residents is under-recognized.[1,2] *Clostridium difficile* is the main cause of infectious diarrhoea in hospitals and LTCFs, and the incidence and severity of *C. difficile* infection (CDI) have recently increased.[3,4] Elderly patients receiving antibiotics, especially those in healthcare settings, are at greatest risk for infection and have the highest mortality rate.[4,5] Previous studies have found that the NAP1/027/BI strain (ribotype 027) is associated with increased prevalence and worse outcomes.[6,7] However, other studies have shown no association of ribotype 027 with worse outcome.[8] Additionally, we have reported that patients infected with fluoroquinolone-resistant strains had more intestinal inflammation.[9] These seemingly incongruent findings suggest that factors previously unaccounted for may be related to the poor outcomes reported in patients infected with ribotype 027.

Methods

Hospitalized cohort

Sequential inpatients with CDI at one US acute, tertiary care, academic hospital were identified in the Clinical Microbiology Laboratory (see 'Stool analysis') between May 2010 and August 2011. Patients were eligible for inclusion (HSR-IRB #13630, waiver of consent obtained) if they were aged ≥18 years, had no chronic diarrhoea (≥4 weeks), and adequate stool volume for analysis was available.

The following parameters were recorded at the time of diagnosis: basic demographics, location prior to admission (LTCF or home), comorbidities using the Charlson comorbidity index, peripheral white blood cell count (WBC), presence of systemic inflammatory response syndrome (SIRS), body mass index (BMI), and serum albumin. Nosocomial CDI was defined when diagnosed three or more days following admission. Patients were contacted and their medical records reviewed at one, six, and 12 months following CDI diagnosis to ascertain all-cause mortality rates.

Stool analysis

Stool testing for CDI was requested based on clinical suspicion by the medical team. The hospital policy did not have specific criteria to limit or guide testing. Samples were tested in the clinical microbiology laboratory using polymerase chain reaction (PCR) for toxin B gene [either BD GeneOhm™ Cdiff Assay (Becton–Dickinson, Franklin Lakes, NJ, USA) or Xpert *C. difficile* (Cepheid, Sunnyvale, CA, USA)] according to manufacturers' instructions. The clinical laboratory used both PCR methods during the time of the study, and choice of test was based on the time at which samples were received and batching protocols. PCR-positive samples were frozen and sent to TechLab, Inc. (Blacksburg, VA, USA) for stool toxin detection using the TOX-B cytotoxin test (TechLab), toxigenic culture, ribotyping, and measurement of intestinal inflammation (quantitative faecal lactoferrin, IBD-SCAN®) using methods previously described.[7] Fluoroquinolone resistance using E-test® (bioMérieux, Durham, NC, USA) was performed according to the manufacturer's instructions.

Statistical analysis

Student's *t*-test and χ^2-tests with non-adjusted odds ratios were used to test significance for parametric variables (SPSS v.20, IBM, Armonk, NT, USA). The Mann–Whitney *U*-test was used to analyse the non-parametric faecal lactoferrin. Significance was defined as $P \leq 0.05$.

Results

Clostridium difficile strains were isolated and typed from 196 subjects admitted from LTCFs or home; subjects admitted from other institutions were not included in the analysis. Cohort demographics are shown in Table I. The 28 subjects admitted from LTCFs were older [72 years (SD: 14) vs 58 years (SD: 17), $P < 0.01$] and four times more likely to be receiving antibiotics at time of CDI diagnosis (Table I). Fluoroquinolones and penicillins were the most commonly prescribed antibiotics; however, there was no difference in the use of any class or number of antibiotics prior to CDI diagnosis in the two groups. LTCF residents had higher mortality following CDI, with five times the one-month mortality [odds ratio (OR): 5.28; 95% confidence interval (CI): 2.09–13.36; $P = 0.001$] and three times the 12-month mortality (OR: 3.15; 95% CI: 1.39–7.13; $P = 0.008$), compared to those from home. There were no differences between groups for Charlson comorbidity score, BMI, receipt of acid-suppressing agents, SIRS criteria, or nosocomial CDI (Table I).

Overall, 77 (39%) isolates were ribotype 027 and 94 (48%) had high-level fluoroquinolone resistance. Although few of the patients admitted from home were infected with ribotype 027 (34%, $N = 57$), the majority of strains isolated from subjects admitted from LTCF were ribotype 027 (71%, $N = 20$; $P < 0.001$), and 27 (96.4%) of these patients had measurable stool toxin detected by tissue culture. Patients admitted from LTCFs were more than four times more likely to be infected with ribotype 027 (OR: 4.87; 95% CI: 2.02–11.74; $P < 0.01$), compared to those admitted from home (Table I). There were also more fluoroquinolone-resistant strains isolated from subjects from LTCFs compared to those from home [21 (75%) vs 73 (43%); $P = 0.002$]. All of the 120 ribotype 027 and 62 (95.4%) of the non-027 strains were positive by toxigenic culture; three non-027 strains had negative toxigenic culture.

Patients infected with ribotype 027 strains were twice as likely to die within six months of CDI diagnosis (all-cause mortality), compared to those infected with non-027 strains (OR: 2.15; 95% CI: 1.13–4.08; $P = 0.022$). Patients infected with ribotype 027 strains had a higher degree of intestinal inflammation when compared to all other strains (quantitative faecal lactoferrin: 95.26 vs 38.08 μg/mL; $P = 0.006$). Of the 45 distinct strains that were isolated, four ribotypes (027, 014, 106 and 056) were isolated from more than 10 patients. The most prevalent non-027 ribotypes had less intestinal inflammation measured by faecal lactoferrin, compared to ribotype 027 (Table II). Strains that were fluoroquinolone resistant also had more intestinal inflammation when compared to those that were fluoroquinolone sensitive (90.00 vs 36.38 μg/mL; $P = 0.007$). All of the 77 (100%) ribotype 027 and 17 of the 119 (14%) non-027 isolates were fluoroquinolone resistant. Subjects infected with ribotype 027 strains had more intestinal inflammation, even when compared to subjects infected with non-027 strains that were also fluoroquinolone resistant (56.91 vs 38.59

μg/mL; $P = 0.03$). There was no difference in the degree of intestinal inflammation ($P = 0.243$) when comparing location prior to admission.

Discussion

This cohort of hospitalized patients diagnosed with CDI had a 34% 12-month all-cause mortality rate. Patients admitted from LTCFs had a higher all-cause mortality rate, were older and were more likely to be receiving antibiotics at the time of CDI diagnosis. In all, 71% were infected with *C. difficile* ribotype 027 strains and 75% were infected with strains with high-level fluoroquinolone resistance from LTCFs, compared with 34% and 44% respectively for patients admitted from home. Patients infected with ribotype 027 strains had a higher all-cause mortality rate and more intestinal inflammation, as measured by quantitative faecal lactoferrin. Although CDI has a significant impact in LCTFs and previous studies have shown that >60% of healthcare-associated CDI is in LTCFs, the understanding of the prevalence and impact of ribotype 027 in LTCF residents has been limited.[1,2,5]

In clinical practice, the infecting *C. difficile* strain is not routinely determined, nor is it clear whether this identification is clinically useful.[6–8] In addition, there are multiple diagnostic approaches available resulting in diversity in patient populations. During the study period, the diagnostic procedure at our hospital included the combination of clinical symptoms and a positive PCR test. This approach is routine for many hospitals in the USA, but raises issues with respect to differentiating between infected patients and those that are colonized with toxigenic *C. difficile*. In this study, we performed additional stool testing that confirmed that most of the LTFC patients also had measurable stool toxin and elevated faecal lactoferrin. In a previous study, we have found that patients infected with ribotype 027 who also have detectable stool toxin suffer worse clinical outcomes.[7] In our cohort, admission from LTCF is a potential risk factor for CDI with ribotype 027 and of more severe disease. If validated in other populations, this demographic information could assist clinicians in identifying patients at risk of complicated CDI and targeting of optimized treatment.

Although the relevance of any particular strain of *C. difficile* is not clear, our study illustrates that hospitalized residents from LTCFs have worse outcomes after CDI diagnosis. In our study, there was no significant difference measured in comorbidity (Charlson comorbidity index), systemic inflammatory response (WBC, SIRS), frailty (serum albumin, BMI), high-risk medication use (current antibiotic, fluoroquinolones, gastric acid-suppressing medications), or nosocomial CDI diagnosis between patients from LTCFs and home; however, there was a significantly different distribution of infecting strains.

There are several potential limitations of this study. First, any underlying functional deficits in the LTCF population that may contribute to the impact of infection on mortality have not been fully accounted. Second, only 13% of our cohort was admitted from LTCF; a larger study would allow a better understanding of the applicability of the findings to the general hospitalized population. Third, our study design did not enable us to determine site of *C.*

difficile acquisition. Definitively assessing hospital acquisition would require screening on admission, which is not currently recommended or in routine clinical practice.

Although we have not demonstrated the causality of infection with fluoroquinolone resistant ribotype 027 on increased mortality, the data presented suggest that the impact of infecting *C. difficile* strain on the extremely vulnerable LTCF population may be able to predict risk of poor outcome when also evaluating function, frailty, and clinical decline.

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Table I

Demographic differences among subjects admitted from long-term care facility (LTCF) and home

	Total (*N* = 196)	**Admitted from home [*n* = 168 (76%)]**	**Admitted from LTCF [*n* = 28 (13%)]**	**OR**	**95% CI**	***P*-value**
Age ≥60 years	105 (54)	80 (48)	25 (89)	9.17	2.67–31.53	<0.001
Comorbidity score						
≥3	148 (76)	125 (74)	23 (82)	1.58	0.57–4.42	0.480
≥4	122 (62)	105 (63)	17 (61)	0.93	0.41–2.11	0.837
≥5	97 (50)	83 (49)	14 (50)	1.02	0.46–2.28	1.000
BMI ≤18.5 kg/m^2	13 (7)	9 (6)	4 (17)	3.65	1.02–13.0	0.058
SIRS score ≥2	115 (59)	100 (60)	15 (54)	0.79	0.35–1.75	0.679
Nosocomial CDI	83 (42)	75 (45)	8 (29)	2.02	0.84–4.83	0.148
Current antibiotic use	154 (79)	128 (76)	26 (93)	4.06	0.92–17.87	0.049
Multiple antibiotics	125 (64)	102 (62)	21 (75)	1.85	0.74–4.59	0.208
Acid suppression	155 (79)	133 (79)	22 (79)	0.97	0.36–2.56	1.000
Ribotype 027	77 (39)	57 (34)	20 (71)	4.87	2.02–11.74	<0.001
Fluoroquinolone resistance	94 (48)	73 (44)	21 (75)	3.90	1.57–9.68	0.002

OR, odds ratio; CI, confidence interval; BMI, body mass index; SIRS, systemic inflammatory response syndrome; CDI, *Clostridium difficile* infection.

Table II

Intestinal inflammation (faecal lactoferrin) according to ribotype

***C. difficile* strain**	**Quantitative faecal lactoferrin (μg/mL)**
Ribotype 027 (*n* = 77)	95.26[a]
Ribotype 014 (*n* = 21)	36.98
Ribotype 106 (*n* = 14)	41.58
Ribotype 056 (*n* = 13)	20.85
Ribotype ≤10 isolates (*n* = 60)	68.59
Unable to isolate organism	53.64

[a] Significantly more intestinal inflammation than patients infected with any other strain (*P* = 0.006).