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## Severe *Clostridium difficile* infection in New Zealand associated with an emerging strain, PCR-ribotype 244

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### Abstract

**Aim** To compare disease severity and clinical outcome of *Clostridium difficile* infection (CDI) due to PCR-ribotype (RT) 244 with CDI due to other strains present in Auckland.

**Method** A retrospective, case-control study was conducted. Ten cases with CDI due to RT 244 were compared with 20 controls infected with other *C. difficile* strains. RT 244 isolates were further analysed for antimicrobial susceptibility, binary toxin genes and mutations in the *tcdC* gene.

**Results** Cases were significantly more likely to have severe disease than controls (OR 9.33;  $p=0.015$ ). 50% of cases had community-associated CDI compared with 15% of controls ( $p=0.078$ ). All RT 244 isolates produced binary toxin and had a single-base pair deletion in *tcdC* at position 117.

**Conclusion** *C. difficile* RT 244 is a newly recognised strain in New Zealand. It shares several features that characterise RT 027. Given its propensity to cause severe community-associated disease, a heightened awareness of this strain is needed to ensure early testing in patients admitted from the community with identified risk factors for CDI.

*Clostridium difficile* infection (CDI) is the commonest cause of healthcare-associated diarrhoea. In Europe and North America the incidence and severity of *Clostridium difficile* infection (CDI) has increased over the past decade.<sup>1</sup> This increase has been attributed largely to the emergence of so called “hypervirulent” strains such as PCR-ribotype (RT) 027.<sup>2</sup> However, to date these strains have remained very uncommon in New Zealand.<sup>3,4</sup>

Recently a cluster of “presumptive 027” cases was identified in Melbourne, Australia (personal communication, Dr R. Stuart, Monash Medical Centre, 2012). On further testing the isolates were not confirmed as RT 027 but rather a ribotype new to Australia designated RT 244. Preliminary investigations showed that compared to other strains, infection with RT 244 was associated with severe community-onset disease and higher mortality. In New Zealand a newly recognised strain of *C. difficile*, identified during a national survey in November 2011 was compared to RT 244 and the ribotyping patterns were indistinguishable.

We sought to compare disease severity and clinical outcome of CDI due to RT 244 with CDI due to other *C. difficile* strains circulating during the same time period. We further characterised RT 244 by performing antimicrobial susceptibility testing

against moxifloxacin, clindamycin and metronidazole, and determining the presence of the binary toxin genes and mutations in the *tcdC* gene.

## Methods

A retrospective, matched case-control study was undertaken. The cases were 10 patients with CDI due to RT 244 who were hospitalised in the Auckland region of New Zealand between October 2011 and May 2012. Each case was matched for age ( $\pm 10$  years) and gender with two controls. Controls were drawn from patients within the Auckland region who had *C. difficile* other than RT 244 isolated from stool.

Data collected included: demographics; comorbidities; hospitalisation in the preceding 6 months; location at symptom onset; antibiotic exposure during 4 weeks prior to symptom onset; and exposure to H<sub>2</sub> antagonists, proton-pump inhibitors or chemotherapy. For each patient, the Charlson Co-morbidity Index was calculated.<sup>5</sup> Disease severity, treatment regimen, disease recurrence and all-cause 30-day mortality were also determined.

CDI was defined as the presence of diarrhoea and a positive stool *C. difficile* toxin result. Each patient was classified as having either community or healthcare-associated disease using surveillance definitions.<sup>6</sup> Community-associated (CA-CDI) was defined as CDI symptom onset in the community, or within 48 hours after admission to a healthcare facility, provided symptom onset was more than 12 weeks after the last discharge from a healthcare facility.

Severe CDI was defined as an episode of CDI with one or more signs of severe colitis including: fever (temperature  $>38.5$  °C); rigors; haemodynamic instability; signs of ileus; marked leukocytosis (leukocyte count  $>15 \times 10^9 \times /L$ ); marked left shift (band neutrophils  $>20\%$  of leukocytes); rise in creatinine ( $>50\%$  above baseline); pseudomembranous colitis and abnormal imaging including distension of large intestine; colonic wall thickening including low-attenuation mural thickening; pericolonic fat stranding; and ascites not explained by other causes.<sup>7</sup> These markers could not be attributable to a concomitant disease.

CDI recurrence was considered to have occurred if there was reappearance of symptoms after initial improvement, or if there was a further positive toxin result within 8 weeks of the last positive toxin result in combination with a prescription for metronidazole.

Stool specimens were inoculated onto chromogenic *C. difficile* agar (ChromID™ *C. difficile*, BioMérieux, Marcy L'Etoile, France) and colonies with typical appearance were identified by routine laboratory methods. PCR-ribotyping was performed by a standard method using capillary-gel electrophoresis.<sup>8</sup> PCR for binary toxin genes and *tcdC* gene sequencing for detection of *tcdC* gene mutations was performed as previously described.<sup>9,10</sup>

Antimicrobial susceptibility testing of the RT 244 isolates was performed using metronidazole M.I.C.E. strips (Oxoid, Thermo Fisher Scientific Inc, Hampshire, United Kingdom) and moxifloxacin, clindamycin and vancomycin Etests (BioMérieux, Marcy L'Etoile, France).

The study was assessed by the Northern Region Ethics Committee and formal approval was not considered necessary.

**Statistical analysis**—Continuous and categorical variables were compared using the Student *t* test and Fisher exact test, respectively.  $P < 0.05$  was considered significant.

## Results

Compared with controls, cases with RT 244 causing CDI were significantly more likely to have severe disease (OR 9.33; 95% confidence interval 1.27-82.59;  $p=0.015$ ). In addition, 50% of cases had CA-CDI compared with 15% of controls, although this difference was not significant ( $p=0.078$ ). There were no significant differences between the cases and controls in comorbidities, antibiotic exposure in the previous 4 weeks, the rate of disease recurrence and 30-day all-cause mortality (Table 1).

**Table 1. Comparison of cases with *Clostridium difficile* PCR-ribotype 244 infection and controls infected with other *C. difficile* strains**

Variables	Cases with RT-244 (n=10)	Controls <sup>a</sup> (n=20)	OR (95% CI)	P value
Mean CCI	3.5	5.45		0.095
<b>Risk factors</b>				
Antibiotic therapy <sup>b</sup>	7 (70%)	19 (95%)	0.123 (0.004–1.76)	0.095
PPI/H2 antagonist	5 (50%)	16 (80%)	0.25 (0.03–1.70)	0.115
Chemotherapy	0	1 (5%)	–	1.000
<b>CA-CDI</b>	5 (50%)	3 (15%)	5.67 (0.76–48.23)	0.078
<b>Outcome</b>				
Severe disease	7 (70%)	4 (20%)	9.33 (1.27–82.59)	0.015
Recurrence	4 (40%)	3 (15%)	3.78 (0.49–31.85)	0.181
30-day all-cause mortality	1 (10%)	3 (15%)	0.63 (0.02–8.9)	1.000

**Abbreviations:** OR, odds ratio; CI, confidence interval; CCI, Charlson comorbidity index; PPI, proton-pump inhibitor; CA-CDI, community-associated *C. difficile* infection.

<sup>a</sup> PCR-ribotypes among controls included 002 (3), 014 (5), 020 (1), 070 (3), NZ 11/12 (1), NZ 11/14 (1), NZ 11/16 (1), NZ 11/17 (2), NZ 11/24 (1), NZ 11/25 (1) and NZ 11/30 (1). (Strains that did not match the European Centre for Disease Prevention and Control's set of reference strains were assigned New Zealand PCR-ribotype strain number prefixed with 'NZ11/').

<sup>b</sup> Antibiotic therapy in 4 weeks prior to symptom onset.

The three cases that underwent endoscopy all had macroscopic evidence of pseudomembranous colitis. In comparison, the only control patient that underwent endoscopy did not have macroscopic evidence of pseudomembranous colitis although histological evidence of pseudomembranous colitis was apparent following colectomy for toxic megacolon.

All 10 *C. difficile* RT 244 isolates were susceptible to moxifloxacin (minimum inhibitory concentration (MIC) 1 mg/L) and metronidazole (MIC 0.25-0.5 mg/L) by CLSI criteria.<sup>11</sup> The clindamycin MICs ranged from 2 to 4 mg/L (the breakpoint for resistance is  $\geq 8$  mg/L). All isolates were susceptible to vancomycin (MIC 1 mg/L) by EUCAST criteria.<sup>12</sup> All 10 isolates carried the binary toxin genes and had a 1-base pair deletion in the *tcdC* regulatory gene at position 117. No additional deletions or insertions were found within the *tcdC* gene.

## Discussion

This study reports on the characteristics of *C. difficile* infection caused by RT 244 strains. This is a newly recognised strain in New Zealand. In our study, patients infected with RT 244 were more likely to present with severe disease ( $p=0.015$ ) and there was a trend towards community-associated disease ( $p=0.078$ ) similar to the findings in the Australian cohort.

Traditionally, CDI has been considered a hospital-acquired infection and testing of patients presenting with community-onset diarrhoea for CDI is not standard practice in New Zealand. These findings provide further support to suggestions that CDI should be considered in the differential diagnosis of adult patients presenting with community-onset diarrhoea, in whom routine enteric pathogens have been excluded and, in particular, for patients with severe diarrhoea and who have had a recent course of antibiotics.

This strain has features similar to *C. difficile* RT 027, in particular, the presence of binary toxin and a 1-base pair deletion at position 117 in the *tcdC* gene. Indeed, whole genome sequencing undertaken at Oxford University suggests that these two strains have descended from a common ancestor (Prof TV Riley et al., unpublished data, 2012). However, RT 244 lacks the 18-base pair *tcdC* deletion characteristic of RT 027. Another major difference between the recent epidemic RT 027 and RT 244 is susceptibility to moxifloxacin; epidemic RT 027 isolates are typically resistant whereas RT 244 isolates are susceptible.

Testing algorithms for CDI differ between hospitals in New Zealand.<sup>13</sup> Laboratories that use the GeneXpert *C. difficile* PCR assay are likely to detect RT 244 as the assay offers presumptive identification of RT 027 based on the presence of binary toxin genes and a 1-base pair deletion in the *tcdC* gene at position 117. However, most testing algorithms limit the use of the GeneXpert assay to testing of samples that have glutamate dehydrogenase, but not toxin, detected by an enzyme immunoassay. With this algorithm, infections caused by the RT 244 strain may go undetected, unless other circumstances, such as severe disease, trigger further testing.

This study has a number of limitations. It was retrospective and the quality of documentation in the clinical records was variable. There were only 10 cases and this may have limited our ability to show statistical difference between the cases and the controls. Regardless, our findings highlight the need for an increased awareness of *C. difficile* RT 244 and its potential risks, given its similarity to RT 027, and support calls for enhanced measures for its diagnosis in our patient population.

The source of this newly emerging strain is not known. It belongs to the same clonal lineage as RT 027 and has the potential to cause severe disease with significant morbidity and mortality in patients with and without comorbidities.<sup>14,15</sup> Severe disease can be defined in a number of ways.

A recent study in which severe disease was defined as intensive care admission, interventional surgery or death within 30 days of diagnosis, failed to show an association between specific ribotypes and severe infection.<sup>16</sup> White cell count and albumin level at presentation were the most clinically relevant predictors of severe disease in that study.

We used a definition for severe disease based on clinical and laboratory findings which may explain the association between infection with RT 244 and disease severity. Also, there was a trend towards community-associated disease suggesting that our patients were less likely to have some of the risk factors traditionally associated with CDI.

Further work needs to be done to determine the reservoirs of this strain. It is important that we focus on infection prevention and control measures that should reduce the introduction and transmission of this strain at least in healthcare settings.

**Competing interests:** None identified.

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