Molecular Epidemiology and Susceptibility Profiles of *Clostridium difficile* in New Zealand, 2009

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Background

- Over the last decade *Clostridium difficile*-associated disease (CDAD) has increased in frequency and severity throughout North America and Europe.
- This change in disease spectrum is largely due to the emergence of a newly recognised hypervirulent strain of *C. difficile*, termed PCR ribotype 027 or NAP 1. This strain's increased virulence is due to the hyperproduction of toxins A and B, the production of a binary toxin and resistance to fluoroquinolones.
- Little is known about the *C. difficile* strains that are currently circulating in New Zealand or whether the epidemic hypervirulent strain, PCR ribotype 027, is present.
- *C. difficile* PCR ribotype 027 infection was recently reported for the first time in Australia.[1]

Aim

- To describe the molecular epidemiology of approximately 100 consecutive clinical isolates of *C. difficile* collected from patients throughout New Zealand.

Method

- Eight laboratories in five regions throughout New Zealand forwarded faecal specimens between February and June 2009 that were *C. difficile* toxin positive by EIA assay to LabPLUS for culture.
- Faecal specimens were cultured on CCF (cycloserine, cefoxitin and fructose) agar and isolates were identified by their colonial appearance and typical biochemical profile.
- Susceptibility testing was carried out using the agar dilution MIC method and, where available, CLSI interpretive criteria were applied.[2] The antimicrobial agents tested were penicillin, piperacillin-tazobactam, vancomycin, ciprofloxacin, moxifloxacin, clindamycin, clarithromycin, meropenem and metronidazole.
- PCR ribotyping was performed at ESR according to the method used by the Anaerobe Reference Unit, National Public Health Service, Cardiff, Wales.[3]

Results

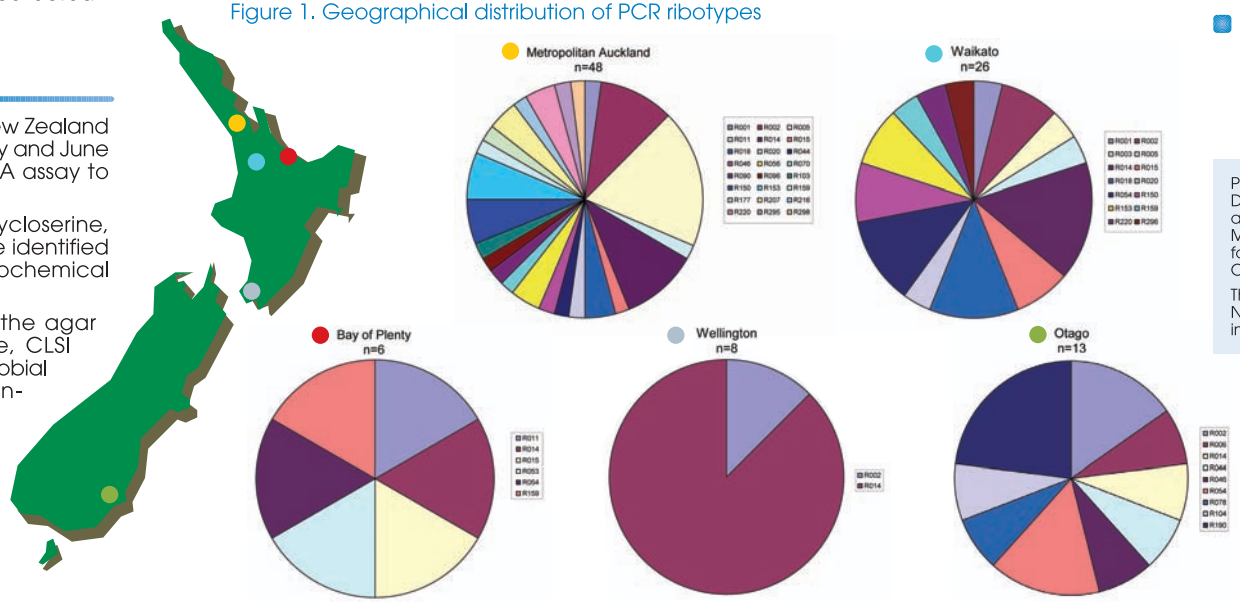
- 159 faecal specimens that tested positive for *C. difficile* toxin were submitted.
- *C. difficile* was isolated from 108 specimens.
- Four patients had the same strain isolated from ≥2 faecal specimens and four patients each had two distinct strains.
- Susceptibility and PCR ribotyping results from 101 non-duplicate isolates obtained from 97 patients are reported:
  - Susceptibility results; table 1
  - PCR ribotyping results; figure 1

Table 1. Susceptibility of 101 isolates to a range of antimicrobial agents.

Antimicrobial	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	% susceptible
Penicillin	1	2	1 - 4	0
Piperacillin-tazobactam	8	8	8 - 16	100
Vancomycin	0.5	1	0.5 - 2	100
Ciprofloxacin	8	16	8 - 128	*
Moxifloxacin	2	2	1 - 16	98
Clindamycin	8	8	1 - >128	39#
Clarithromycin	1	1	1 - >128	95
Meropenem	2	2	1 - 4	100
Metronidazole	0.25	0.5	0.25 – 0.5	100

* Interpretive criteria are not available for ciprofloxacin.
\# Only six isolates had MICs of >8 mg/L. 5 isolates had a MIC ≥ 32 mg/L. Epidemiological studies have shown MICs of clindamycin for epidemic strains of *C. difficile* to be ≥ 32 mg/L.

Figure 1. Geographical distribution of PCR ribotypes



Conclusions

- There is a wide range of *C. difficile* PCR ribotypes in New Zealand.
- The most common PCR ribotypes were 014 (18 isolates), 002 (11 isolates) and 005 (10 isolates). Three novel PCR ribotypes (295, 296 and 298) were identified.
- No PCR ribotype 027 isolates were identified, but one isolate of another hypervirulent strain, PCR ribotype 078, was identified.
- Most isolates were fully susceptible to the range of antimicrobial agents tested.
- Monoresistance to macrolides, clindamycin and fluoroquinolones was seen.
- Active laboratory-based surveillance is required to detect the silent introduction of *C. difficile* hypervirulent strains into New Zealand.

Participating laboratories were from the following District Health Boards (DHB): Waitemata DHB, LabPLUS and Diagnostic Medlab for Auckland DHB, Counties Manukau DHB, Waikato DHB, MedLab Bay of Plenty for Bay of Plenty DHB, Capital Coast DHB and Southern Community Laboratory for Otago DHB.

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References

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