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MICROBIOLOGY

Laboratory-based surveillance of *Clostridium difficile* circulating in Australia, September – November 2010

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Summary

Clostridium difficile rose in prominence in the early 2000s with large-scale outbreaks of a particular binary toxin-positive strain, ribotype 027, in North America and Europe. In Australia outbreaks of the same scale had not and have not been seen. A survey of *C. difficile* across Australia was performed for 1 month in 2010. A collection of 330 *C. difficile* isolates from all States and Territories except Victoria and the Northern Territory was amassed. PCR ribotyping revealed a diverse array of strains. Ribotypes 014/020 (30.0%) and 002 (11.8%) were most common, followed by 054 (4.2%), 056 (3.9%), 070 (3.6%) and 005 (3.3%). The collection also contained few binary toxin positive strains, namely 027 (0.9%), 078 (0.3%), 244 (0.3%), 251 (0.3%) and 127 (0.3%). The survey highlights the need for vigilance for emerging strains in Australia, and gives an overview of the molecular epidemiology of *C. difficile* in Australia prior to an increase in incidence noted from mid-2011.

Key words: Clostridium difficile; ribotype; epidemiology; surveillance; molecular typing; Australia.

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INTRODUCTION

An epidemic strain of *Clostridium difficile* [PCR ribotype (RT) 027] was first identified in Quebec Province, Canada, in 2005, as a cause of hospital outbreaks of severe infection with high mortality rates.[1] Retrospective analyses suggested this strain caused outbreaks across North America dating back to 2000. The organism later spread to Europe and cases have now been described in Asia and Central America.[2] Increased toxin A and B production by *C. difficile* RT 027, as well as the presence of an additional binary toxin (CDT), may be responsible for its increased virulence,[3] however, fluoroquinolone resistance is likely to have contributed to its spread.[4] Infection with this strain leads more often to severe disease, more recurrences and a greater risk of death.[1]

There has been concern in Australia because of the lack of suitable surveillance systems to detect the entry of epidemic *C. difficile* into this country.[5,6] The first infected patient with RT 027 in Australia was reported in 2009 in Western Australia (WA), but the infection was thought to have been acquired in North America.[7] The first case of *C. difficile* RT 027 infection thought to have been acquired in Australia was reported in early 2011 (although detected at the beginning of 2010) in a case from Melbourne, Victoria.[8] The strain was identified after clinicians alerted the laboratory to the severity of the infection and the possibility of a 'hyper-virulent' strain, and molecular strain typing identified *C. difficile* RT 027. Of concern, several other cases were subsequently detected at the same hospital, other hospitals and a nursing home in Melbourne. In late 2010, a cluster of cases of RT 027 infection was discovered in North Sydney, New South Wales (NSW).[9] The outbreaks of RT 027 in Victoria appear to have originated from a single introduction into the country from North America.[10]

Ongoing surveillance, including monitoring of changes in molecular epidemiology, is required to provide information for clinicians and to inform infection prevention interventions. A recommendation from the Australian Commission on Safety and Quality in Healthcare for hospital surveillance programs in all States and Territories to monitor *C. difficile*[11] was approved by Australian Health Ministers in November 2008. All States and Territories have implemented this recommendation. A significant increase in both hospital-acquired CDI (HA-CDI) and community-acquired (CA-CDI) in Australia during 2011–2012 was identified through collation of hospital surveillance data.[12] In this study, we describe the molecular epidemiology of *C. difficile* infection (CDI), and the relative frequency of epidemic strains in Australia in late 2010 prior to the increases in CDI reported for 2011. As such, this analysis provides baseline results for future comparisons.

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METHODS

Study design

This laboratory-based survey was performed for 1 month between September and November, 2010. Isolates of *C. difficile* from patients developing diarrhoea in hospital or presenting with diarrhoea to a hospital or in the community were collected in participating diagnostic laboratories across all States and Territories except the Northern Territory and Victoria. One laboratory participated in the Australian Capital Territory (ACT), five in NSW, three in Queensland (QLD), one in South Australia (SA), one in Tasmania (TAS) and one in WA. Most of these laboratories provided diagnostic services to public hospitals. No change to current testing strategies operating at the participating laboratories was proposed. Participating laboratories routinely performed culture for *C. difficile* or cultured any specimen positive by a screening test for inclusion in the isolate collection. This may have been as part of primary screening or following positive rapid tests. If toxin detection tests were performed on isolates, then both toxin positive and negative isolates were referred. Isolates from duplicate specimens taken within 7 days were excluded. No patient demographic or clinical data were collected.

Clostridium difficile isolates or specimens were transported to a central reference laboratory [PathWest Laboratory Medicine (WA), Nedlands, WA] in either Robertson's cooked meat (RCM) medium, thioglycollate broth or as spore suspensions on swabs in transport medium. Results of ribotyping were reported back to the referring hospitals/laboratories and/or local department of health.

Clostridium difficile culture and molecular analysis

Clostridium difficile isolates or specimens were cultured on cycloserine cefoxitin fructose agar plates (PathWest). All plates were incubated in an anaerobic chamber (Don Whitley Scientific, Australia) for 48 h at 37°C, in an atmosphere containing 80% nitrogen, 10% hydrogen and 10% carbon dioxide. *Clostridium difficile* was identified on the basis of characteristic colony morphology (yellow, ground glass appearance) and odour (horse dung smell). The identity of doubtful isolates was confirmed by Gram stain, latex agglutination test kit (Oxoid, UK)[13] and/or species specific PCR.[14]

PCR ribotyping was performed for all isolates according to the method of O'Neill *et al.*[15] with some modifications. Amplification was performed in a 50 µL reaction volume with 1× reaction buffer, 4 mM $MgCl_2$, 100 µM of each dNTP, 0.4 µM of each primer, 20 mg/mL BSA, 3.75 U AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), and 10 µL of DNA template. PCR was carried out with the following thermal cycler program: an initial denaturation step of 95°C for 1 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, with a final extension step of 72°C for 7 min. PCR products were purified with the MinElute PCR Purification Kit (Qiagen, Germany). Capillary gel electrophoresis of PCR products was performed using a QIAxcel instrument (Qiagen) with 15 bp/1 kb alignment marker and patterns were compared to known RTs of *C. difficile*. Ribotyping profiles were analysed with Bionumerics (version 7.5; Applied Maths, Belgium) and compared with a collection of reference strains. Isolates were also characterised for toxigenic properties using PCR reactions for the *tcdA*, *tcdB*, *cdtA* and *cdtB* genes.[16,17]

RESULTS

CDI incidence rates

The number of viable isolates of *C. difficile* collected and an estimate of the incidence rate per 100,000 population are shown in Table 1. Person time was calculated by dividing the population (per year) for each state by 12 to give person-months. The overall national incidence rate was calculated at 23.8/100,000 person months. The incidence rate was highest in WA at 30.2/100,000 and lowest in SA at 16.8/100,000.

Molecular epidemiology

The distribution of *C. difficile* RTs throughout Australia is shown in Table 2. The 10 most common RTs comprised 67.3% of the total number. More than 60 RTs were represented in the remaining 31.8% of isolates, many with only one representative strain. The two most common RTs were RT 14/020 (30.0%) and RT 002 (11.8%), followed by RT 054 (4.2%), RT 056 (3.9%) and RT 070 (3.6%). Several CDT-positive isolates were detected. These were three RT 027 (0.9%) isolates identified in NSW, one RT 078 (0.3%) isolate in NSW, one RT 127 (0.3%) in NSW, one RT 251 (0.3%) in NSW and one RT 244 (0.3%) isolate in Qld.

DISCUSSION

In this study, we found a significant number of confirmed cases of CDI from participating laboratories. The crude incidence rate of CDI at 23.8/100,000 (Table 1) represents an underestimate of the true population rate, as not all laboratories in participating States and Territories referred isolates for the survey. Rates also varied between jurisdictions, due in part to variation in numbers of participating laboratories. However, in the ACT and TAS, where the participating laboratories service the entire population of these jurisdictions, ascertainment is likely to be close to complete. A US study estimated the national incidence rate in 2011 to be 51.9/100,000 population for CA-CDI and 95.3/100,000 for HA-CDI.[18] In Canada, incidence rates increased from a baseline rate of 35.6/100,000 population in 1991 to 156.3/100,000 in 2003[19] and in Germany rates increased from 1.7–3.8/100,000 population in 2003 to 14.8/100,000 in 2006.[20] While the Australian incidence rate appeared to be lower than those identified in North America, following this study the nationwide incidence of hospital-identified CDI in Australia increased from 3.25/10,000 patient days (PD) in 2011 to 4.03/10,000 PD in 2012.[12]

Table 1 Number of isolates of *C. difficile* received from participating State or Territory

Jurisdiction	Population	Person months of surveillance	Number of isolates	Rate per 100,000 population
NSW	7,253,400	604,450	154	25.5
QLD	4,532,300	377,692	76	20.1
WA	2,306,200	192,183	58	30.2
SA	1,647,800	137,317	23	16.8
TAS[a]	508,500	42,375	10	23.6
ACT[a]	359,700	29,975	9	30.0
Australia	16,607,900	1,383,992	330	23.8

[a] Jurisdictions with complete ascertainment.

Table 2 Distribution of *C. difficile* ribotypes in participating States and Territories

Ribotype	State/Territory n (%)					Australia
	NSW	QLD	WA	SA	TAS/ACT	n (%)
014/020	44 (28.6)	18 (23.7)	26 (44.8)	6 (26.1)	5 (26.3)	99 (30.0)
002	24 (15.6)	7 (9.2)	5 (8.6)	2 (8.7)	1 (5.3)	39 (11.8)
054	7 (4.5)	1 (1.3)	1 (1.7)	3 (13.0)	2 (10.5)	14 (4.2)
056	4 (2.6)	7 (9.2)	1 (1.7)	0	1 (5.3)	13 (3.9)
070	5 (3.2)	3 (3.9)	1 (1.7)	2 (8.7)	1 (5.3)	12 (3.6)
005	2 (1.3)	1 (1.3)	5 (8.6)	2 (8.7)	1 (5.3)	11 (3.3)
012	7 (4.5)	1 (1.3)	1 (1.7)	0	0	9 (2.7)
018	8 (5.2)	1 (1.3)	0	0	0	9 (2.7)
046	5 (3.2)	3 (1.3)	0	0	0	8 (2.4)
103	2 (1.3)	2 (2.6)	1 (1.7)	2 (8.7)	1 (5.3)	8 (2.4)
Other	45 (29.2)	31 (40.8)	17 (29.3)	5 (21.7)	7 (36.8)	105 (31.8)
NT	1 (0.6)	1 (1.3)	0	1 (4.3)	0	3 (0.9)
Total	154	76	58	23	19	330

NT, not typeable.

This is the first systematic study of the molecular epidemiology of CDI across Australia. Previously, the scope and prevalence of epidemic strains of *C. difficile* in Australian hospitals were not known. The survey revealed that RTs 014/020 and 002 were most common, similar to European and North American isolate collections.[21,22] RT 014 is the most common RT of *C. difficile* in most typing studies worldwide. As part of a study that evaluated a new antimicrobial treatment for CDI, Cheknis *et al.* typed 24 Australian *C. difficile* isolates recovered in the mid-2000s by restriction endonuclease analysis (REA) and found that two-thirds of them were uncommon types (compared to isolates from North America and Europe). However, a quarter of the isolates belonged to REA group Y, a group that corresponds to PCR RTs 014 and 020.[23]

The isolation of eight RT 018 strains in NSW is notable. RT 018 is highly prevalent in Asia, particularly in Japan.[24] It appeared in Korea in the early 2000s[25] and also emerged in Italy at around the same time and caused widespread severe disease and a number of outbreaks.[26] A recent study of Italian *C. difficile* strains showed RT 018 was the most prevalent RT overall and was highly transmissible, accounting for 95.7% of secondary CDI cases within hospitals.[27] This apparently high transmissibility may be related to enhanced virulence, and surveillance for this strain in Australia appears warranted to guard against increasing RT 018 infection rates in the future. Another common strain in Asia, RT 017, was isolated only once in our collection (0.3%). Given the high frequency of travel between Australia and its close neighbours in Asia, the rarity of RT 017 among the Australian collection was unexpected.

RT 056 was among the five most common strains in this collection. It was also isolated frequently in the European survey of *C. difficile* in hospital patients,[21] where it was associated with complicated disease outcomes. A recent study found RT 056 was commonly isolated from the gastrointestinal tracts of Australian veal calves at slaughter,[28] meaning it may have potential to enter the food chain in Australia, and possibly be exported to other countries as well. The relationship between human RT 056 strains and animal RT 056 strains is currently being explored with whole genome sequencing.

The prevalence of CDT positive strains, particularly RTs 027 and 078, in this collection was low. Nonetheless, the study highlighted the need for vigilance for the appearance of virulent or 'hyper-virulent' strains. Three RT 027 strains were detected, all from NSW, and all from Sydney. Although Victorian laboratories did not participate in the study, small numbers of cases of infection due to RT 027 associated with hospitals and nursing homes were reported in 2010.[8] Also of some concern was the detection of two RT 078 strains in NSW. RT 078 is commonly found in production animals outside Australia. Many pig and cattle herds in the USA and Europe (up to 95%) are infected with *C. difficile* RT 078.[29] Most animal isolates of *C. difficile* produce CDT and RT 078 is a strain that, like RT 027, also produces more toxins A and B, as well as CDT. RT 078 was the third most common RT of *C. difficile* isolated from human infections in Europe when our study was performed.[21] This is a strain that requires monitoring in Australia. Despite the fact that Australian production animals do not appear to harbour this RT,[28] infections are still occurring in humans Australia-wide (T. V. Riley, unpublished) suggesting, possibly, infection from a source emanating from outside Australia.

Interestingly, this survey includes the earliest known detection of RT 244 in Australia. In 2011 and later, RT 244 went on to cause outbreaks of disease across Australia and New Zealand.[30–32] In a similar snapshot survey of CDI in Qld in 2012, it was the third most common RT in circulation after 002 and 014/020.[33] RT 244 has been associated with higher mortality rates and increased severity of disease.[32] If more virulent strains were identified more quickly, infection control measures could be re-enforced to reduce the spread of these strains of *C. difficile*.

There are several limitations to this study. Systematic bias may result in underestimation of the true incidence of disease due to under-recognition of disease by clinicians, under-testing by laboratories, particularly of community specimens, the use of diagnostics with imperfect sensitivity and a small proportion of non-viable isolates. Estimates of incidence rates per population are approximate as the majority of the isolates came from hospital-identified CDI. We were unable to access details of hospitalisations or patients' length of stay as we did not receive details of source hospitals with

isolates in the study. Also we could not collect clinical or demographic data, and thus were unable to comment on the clinical significance of these strains. This, and previously published work, suggests that there is only a low prevalence of the epidemic strains RTs 027 and 078 in Australia. However, ongoing surveillance is required to monitor the incidence of CDI in the country. Further studies are needed to define the clinical profile, risk factors (including antibiotics, animal reservoirs and long term care facilities), and optimal infection control measures in hospitals and other healthcare facilities.

This study and the subsequent emergence of RT 244[30–33] have highlighted the need to raise awareness of *C. difficile* in Australia. Continued evaluation of the molecular epidemiology of CDI will have implications for antibiotic stewardship programs (particularly use of quinolone antibiotics), isolation and infection control programs, and the need for and scope of ongoing surveillance of severe CDI. This systematic study of the molecular epidemiology of CDI in Australia provides a baseline to evaluate future changes in strain distribution.

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