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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.