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Laboratory-Based Surveillance of *Clostridium difficile* Infection in Australian Health Care and Community Settings, 2013 to 2018

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ABSTRACT In the early 2000s, a binary toxin (CDT)-producing strain of *Clostridium difficile*, ribotype 027 (RT027), caused extensive outbreaks of diarrheal disease in North America and Europe. This strain has not become established in Australia, and there is a markedly different repertoire of circulating strains there compared to other regions of the world. The *C. difficile* Antimicrobial Resistance Surveillance (CDARS) study is a nationwide longitudinal surveillance study of *C. difficile* infection (CDI) in Australia. Here, we describe the molecular epidemiology of CDI in Australian health care and community settings over the first 5 years of the study, 2013 to 2018. Between 2013 and 2018, 10 diagnostic microbiology laboratories from five states in Australia participated in the CDARS study. From each of five states, one private (representing community) and one public (representing hospitals) laboratory submitted isolates of *C. difficile* or PCR-positive stool samples during two collection periods per year, February-March (summer/autumn) and August-September (winter/spring). *C. difficile* was characterized by toxin gene profiling and ribotyping. A total of 1,523 isolates of *C. difficile* were studied. PCR ribotyping yielded 203 different RTs, the most prevalent being RT014/020 ($n = 449$; 29.5%). The epidemic CDT$^+$ RT027 ($n = 2$) and RT078 ($n = 6$), and the recently described RT251 ($n = 10$) and RT244 ($n = 6$) were not common, while RT126 ($n = 17$) was the most prevalent CDT$^+$ type. A heterogeneous *C. difficile* population was identified. *C. difficile* RT014/020 was the most prevalent type found in humans with CDI. Continued surveillance of CDI in Australia remains critical for the detection of emerging strain lineages.

KEYWORDS *Clostridium difficile*, molecular epidemiology, ribotyping, surveillance

Clostridium (*Clostridioides*) *difficile* is an important cause of infectious diarrhea in health care settings in high-income countries. Prior to the 2000s, *C. difficile* infection (CDI) was underappreciated because few hospitalized patients progressed to fulminant

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disease (1). In the last 2 decades, an increase in the frequency and severity of CDI was noted, initially in Canada, then the United States and Europe, owing to the emergence of an epidemic strain of *C. difficile*, ribotype 027 (RT027) (1). The incidence and severity of CDI continue to impose a significant burden on global health care systems due to substantial costs associated with extended hospital stays and treatment. In their report, "Antibiotic Resistance Threats in the United States," the Centers for Disease Control and Prevention indicated that *C. difficile* remained an urgent threat to public health that required aggressive action (2). In the United States, the national burden of CDI was estimated at 462,199 cases (3), costing the government $1 billion in health care expenditure in 2017 (2).

CDI is not a notifiable disease in Australia; however, monitoring of CDI rates in hospitals has been mandated by the Australian Commission on Safety and Quality in Healthcare for hospital accreditation since 2010 (4). All states and territories in Australia have reported a significant increase in rates of hospital-identified (HI) CDI since mid-2011 (5). HI CDI was defined as CDI diagnosed in a patient attending any area of an acute-care public hospital (i.e., patients admitted to inpatient wards or units, including psychiatry, rehabilitation, and aged care, and those attending emergency and outpatient departments). Increased rates of CDI may reflect more testing or use of more sensitive diagnostic algorithms; however, enhanced surveillance suggested that much of the increase (24%) was attributable to more community-associated (CA) CDI (5).

Despite this increase in the incidence of CDI in Australia (5) and the emergence of new virulent RTs (6, 7), most clinical microbiology laboratories in Australia do not culture or further characterize strains of *C. difficile* causing disease. The *C. difficile* Antimicrobial Resistance Surveillance (CDARS) study was initiated to address this problem with nationwide longitudinal surveillance of CDI in Australia. Here, we describe the molecular epidemiology of CDI in Australian health care and community settings over the first 5 years of the study, 2013 to 2018. We previously reported antimicrobial susceptibility data for 2013 to 2014, including typing of selected isolates (~30%) (8), and we now present complete molecular typing results for all isolates from 2013 to 2018.

MATERIALS AND METHODS

Sample and data collection. Between 2013 and 2018, 10 diagnostic microbiology laboratories participated in the CDARS study. The laboratories comprised one private (representing community) and one public (representing hospitals) site from each of five states of Australia (Western Australia [WA], New South Wales [NSW], Victoria [VIC], South Australia [SA], and Queensland [QLD]). There were up to two collection phases per calendar year, in February-March and August-September, representing the summer-autumn and winter-spring seasons, respectively. During each phase, sites were asked to save up to 15 nonduplicate isolates of *C. difficile* or stool samples based on the criteria previously described (8). All samples and isolates were stored at −70°C at participating sites, shipped to the reference laboratory (PathWest Laboratory Medicine, Nedlands, WA) on transport swabs under ambient conditions, and processed within 24 h of arrival.

Specimens from private laboratories largely represented CA CDIs, as these facilities served patients from general practitioners (40 to 50%), aged-care facilities (1 to 3%), and private (community) hospitals (50 to 60%), some of which are large tertiary facilities with intensive care units. Conversely, specimens from public laboratories that were based in large tertiary-care medical centers (public hospital sites) represented HI CDIs that, by definition (5), may have included some CA CDI cases also. Basic demographic data (gender and age) were collected, and children <1 year of age were excluded from the analysis. Chi-square tests of significance between data sets were performed in IBM SPSS Statistics version 26.

***C. difficile* culture and epidemiological typing.** Stool samples and isolates of *C. difficile* that were PCR positive for a toxin gene(s) were cultured, and DNA was extracted using methods previously described (8). All recovered *C. difficile* strains underwent toxin gene profiling and PCR ribotyping. The toxin genes *tcdA* (toxin A), *tcdB* (toxin B), *cdtA*, and *cdtB* (CDT) were amplified by PCR as previously described (8). *C. difficile* RTs were determined by amplification of the 16S-23S rRNA intergenic spacer region, and PCR products were separated on the QIAxcel capillary electrophoresis platform (Qiagen GmbH, Hilden, Germany). The BioNumerics software package v.6.5 (Applied Maths, Saint-Martens-Latem, Belgium) was used for dendrogram and cluster analysis of PCR ribotyping band patterns. Isolates that could not be identified with the available reference library were designated with internal nomenclature, prefixed with QX.

TABLE 1 Summary of sample collection and *C. difficile* recovery

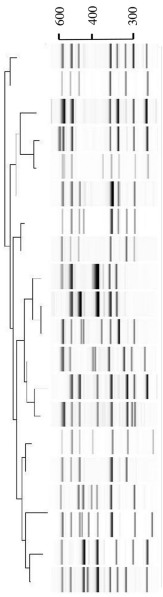
Phase	Site type	No. of specimens or strains received[a]						*C. difficile* recovery	
		NSW	QLD	SA	VIC	WA	Total	*n*	%
1	Private	19	21	13	15	8	76	66	86.8
	Public	31	5	21	22	20	99	87	87.9
	Total	50	26	34	37	28	175	153	87.4
2	Private	15	10	14	15	14	68	67	98.5
	Public	15	6	15	15	15	66	62	93.9
	Total	30	16	29	30	29	134	129	96.3
3	Private	20	24	8	17	13	82	71	86.6
	Public	23	4	17	22	17	83	78	94.0
	Total	43	28	25	39	30	165	149	90.3
4	Private	15	15	8	12	15	65	61	93.8
	Public	15	21	15	15	15	81	76	93.8
	Total	30	36	23	27	30	146	137	93.8
5	Private	19	15	15	17	10	76	66	86.8
	Public	15	15	16	19	20	85	79	92.9
	Total	34	30	31	36	30	161	145	90.1
6	Private	19	26	10	20	5	80	75	93.8
	Public	20	23	12	19	25	99	90	90.9
	Total	39	49	22	39	30	179	165	92.2
7	Private	20	23	15	20	1	79	76	96.2
	Public	20	22	15	19	20	96	94	97.9
	Total	40	45	30	39	21	175	170	97.1
8	Private	19	26	12	23	4	84	74	88.1
	Public	18	24	23	16	22	103	97	94.2
	Total	37	50	35	39	26	187	171	91.4
9	Private	16	20	15	27	4	82	71	86.6
	Public	20	16		19	15	70	59	84.3
	Total	36	36	15	46	19	152	130	85.5
10	Private	25	26	13	15	7	86	74	86.0
	Public	22	23	27	19	24	115	100	87.0
	Total	47	49	40	34	31	201	174	86.6
Total		386	365	284	366	274	1,675	1,523	90.9

[a]NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

RESULTS

Isolate collection. A total of 1,675 eligible samples (stool or isolates) were received during the 10 collection phases, of which 46% ($n = 778$) were submitted by private laboratories. From these samples, 1,523 isolates of *C. difficile* (90.9%) were recovered (Table 1). For the entire study, 60.6% of cases were female, the median age was 69 years (interquartile range, 50 to 81 years), and the majority (62%) of patients were ≥65 years old.

Epidemiological typing and toxin profiling. There were 1,523 *C. difficile* isolates recovered, and PCR ribotyping yielded 203 unique RTs. Of these isolates, 1,197 (78.6%) were assigned to one of 51 internationally recognized RTs or were given an internal QX number ($n = 275$; 18.1%). A small number of isolates were singleton strains unique to our laboratory and could not be identified with the available reference library ($n = 51$; 3.3%). *C. difficile* RT014 and RT020 and *C. difficile* RT297 and RT310 were grouped as RT014/020 and RT297/310, respectively, due to similarities in their banding patterns. The 20 most prevalent RTs of *C. difficile*, which comprised 76.1% of all isolates ($n = 1,159$), along with their distribution between states and laboratory type, are shown



RT	Toxin gene profile			RT distribution							N	%
				Site type		State						
	tcdA	*tcdB*	*cdtA/cdtB*	Private	Public	NSW	QLD	SA	VIC	WA		
RT043	+	+	–	7	11	4	2	4	7	1	18	1.2
RT046	+	+	–	11	15	7	8	2	3	6	26	1.7
RT297/310	+	+	–	10	19	5	7	13	3	1	29	1.9
RT014/020	+	+	–	195	254*	79	92	100	83	95	449	29.5
QX013	+	+	–	5	7	3	5	1	3		12	0.8
RT137	+	+	–	10	7	3		5	4	5	17	1.1
RT005	+	+	–	9	16	6	2	6	4	7	25	1.6
RT106	+	+	–	12	9	6	7	1	4	3	21	1.4
RT070	+	+	–	19	25	8	8	14*	7	7	44	2.9
RT126	+	+	+	6	10	1	1	4	11		17	1.1
RT012/QX158	+	+	–	22	26	13	4	6	11	14	48	3.2
RT056	+	+	–	35	47	15	17	8	22	20	82	5.4
RT103	+	+	–	20	16	9	9	3	8	7	36	2.4
RT018	+	+	–	8	7	2	4	5	2	2	15	1.0
RT010	–	–	–	12	3	4		2	5	4	15	1.0
RT002	+	+	–	82	97	76*	43	24	19	17	179	11.8
RT054	+	+	–	18	15	10	7	6	6	4	33	2.2
QX076	+	+	–	12	12	3	5	4	8	4	24	1.6
RT017	–	+	–	9	12	4	6	4	2	5	21	1.4
RT039	–	–	–	10	1				11		11	0.7

FIG 1 Dendrogram summary, toxin profiles, and distributions of the 20 most prevalent *C. difficile* PCR RTs. PCR ribotyping pattern cluster analysis using both the Dice coefficient and the neighbor-joining method was used. Proportions were compared by χ^2 test. *, $P < 0.05$. NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

in Fig. 1. Overall, *C. difficile* RT014/020 ($n = 449$; 29.5%) was the most prevalent, followed by RT002 ($n = 179$; 11.8%), a majority of which was found in a public laboratory in NSW ($n = 48$; 26.8%; $P < 0.05$), and RT056 ($n = 82$; 5.4%). The distribution of the 12 most common RTs was consistent throughout the 5-year period with only slight variations between laboratory types (Fig. 2). The epidemic *C. difficile* RT027 ($n = 2$) and RT078 ($n = 6$) and the recently described RT251 ($n = 10$) and RT244 ($n = 6$) were found in low numbers.

The vast majority of *C. difficile* strains (93.4%; $n = 1,423$) were positive for the major toxin genes *tcdA* and *tcdB* (A^+B^+), and 4.1% ($n = 63$) also contained *cdtA* and *cdtB* (CDT^+) genes. Twenty-two strains had a variant toxin profile, $A^-B^+CDT^-$ (RT017, $n = 21$; QX134, $n = 1$), while five were positive for *tcdB* and *cdtA* and *cdtB*, resulting in the rare toxin profile $A^-B^+CDT^+$. The overall prevalence of CDT^+ *C. difficile* strains was 4.5% ($n = 69$). One strain of *C. difficile* RT033 was positive for *cdtA* and *cdtB* genes only. Interestingly, *C. difficile* RT126 was the most prevalent CDT^+ type in Australia ($n = 17$), with 64.7% ($n = 11$) of isolates originating from Victoria during winter-spring of 2016 and the majority (72.7%; $n = 8$; $P < 0.05$) coming from the public laboratory. The six *C. difficile* RT078 strains were mainly from NSW ($n = 4$), with no obvious temporal clustering. Notably, the majority of CDT^+ *C. difficile* strains were isolated from public laboratories. In contrast, 71 nontoxigenic *C. difficile* strains were submitted, and 77.5% ($n = 55$) were from private laboratories. Two nontoxigenic strains, RT010 and RT039 ($n = 12$ and $n = 10$, respectively), ranked in the top 20 most prevalent *C. difficile* strains in Australia (Fig. 1). There was no evidence of seasonality in RT distribution (Fig. 3).

DISCUSSION

Here, we report the results of nationwide longitudinal laboratory-based surveillance of *C. difficile* in health care and community settings in Australia. The study was performed on a collection of 1,675 specimens and isolates submitted by 10 laboratories in five states of Australia from 2013 to 2018, yielding 1,523 strains of *C. difficile*. Between 2011 and 2016, the average rate of CDI across Australia was 4 cases/10,000 patient bed-days, corresponding to approximately 10,000 cases of CDI per year (9). Thus, for every year of our study, approximately 3% of strains of *C. difficile* recovered from these

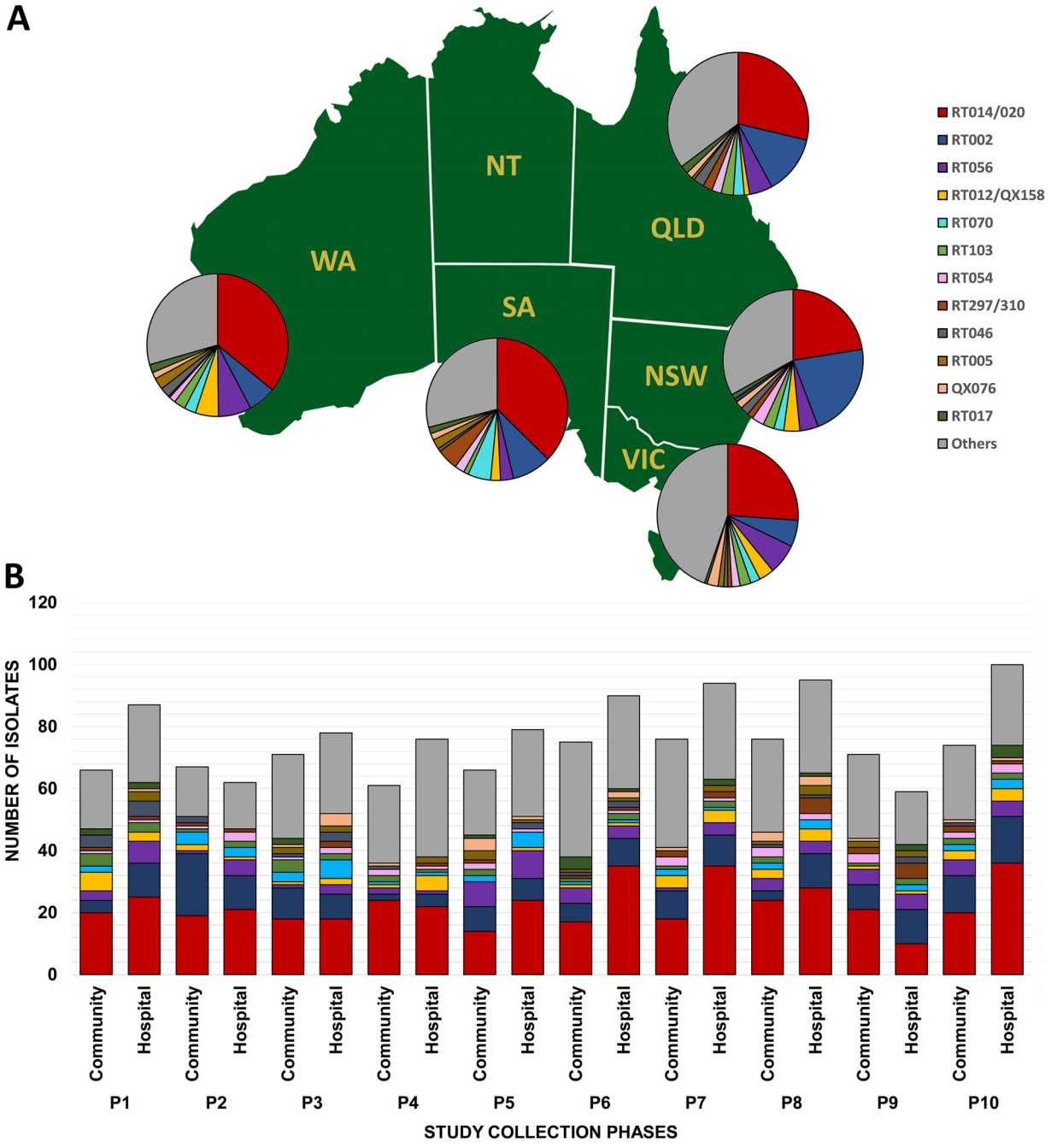


FIG 2 Molecular epidemiology of CDI in Australia. Distribution of *C. difficile* RTs by Australian state (A) and by private or public collection site (B) over 10 collection phases, 2013 to 2018 (n = 1,523). NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia. (The map was created with mapchart.net and is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License [https://creativecommons.org/licenses/by-sa/4.0/].)

cases were analyzed. A heterogeneous *C. difficile* population was identified with an RT distribution similar to those reported in 2013-2014 (8) and our earlier studies in 2010 (10) and 2012 (11). Our findings in the current study suggest that while temporal shifts occurred in some circulating strains of *C. difficile*, the most common RTs in Australia did not change. However, understanding these temporal shifts is important, as is indicated by RT244 emerging as a cause of severe community-associated infection and subsequently becoming the third most common RT detected in Australia in 2012 (6, 12).

C. difficile RT014/020 has remained the most common type causing CDI in humans

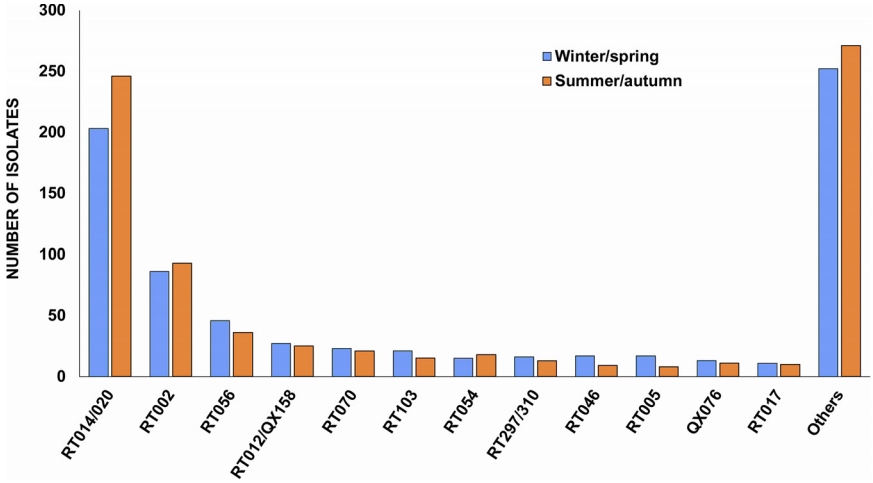


FIG 3 Seasonal distribution for the top 12 most prevalent RTs in Australia, 2013 to 2018.

in Australia since it was first reported as such in 2010, with a prevalence of 29.5%, similar to previous reports (8, 10, 11). Moreover, the proportions of RT014/020 isolated from private and public laboratories were comparable (43.4% and 56.6%, respectively), although significantly more strains were isolated from public laboratories based in tertiary hospitals (Fig. 1). *C. difficile* RT014 was reported as the most prevalent RT in pig herds in Australia (13), and more recently, genome analysis of the RT014 lineage in piglets and humans showed close genetic relatedness by core genome single-nucleotide polymorphism (cgSNP) analysis, suggesting long-range interspecies transmission and implying that CDI was a zoonoses (14).

C. difficile RT297/310 emerged as a group that may have been underestimated or overlooked in previous studies in Australia. The banding patterns of RT297/310 and RT014/020 differ only in the size of their largest amplicon (~550 to 575 bp, RT014/020; ~650 to 675 bp, RT297/310) (see Fig. S1 in the supplemental material). These small differences demonstrate the difficulty in distinguishing between potentially closely related *C. difficile* strains using conventional band-based typing methods and highlight the need to use sequenced-based methods to reliably determine strain relatedness.

In Europe, RT014/020 was the most common type isolated in 2008 (15); however, epidemic *C. difficile* RT027 emerged to become the most prevalent RT from mid-2011 to 2014 (16). Only two isolates of *C. difficile* RT027 were recovered from specimens submitted from public laboratories in this study over a 5-year period, one each from NSW and QLD. *C. difficile* RT027 has been reported rarely in Australia (17), and it has been postulated that the restricted use of fluoroquinolones in Australia has not favored the establishment of RT027 (18). Also, the geographic isolation of Australia may have contributed to the delayed appearance of this type. However, other CDT^+ RTs of *C. difficile*, RT244 and RT251, were detected in the present study at low prevalences of 0.5% ($n = 7$) and 0.7% ($n = 10$), respectively. *C. difficile* RT244 emerged in Australia and New Zealand in 2011 and was first identified as a "presumptive RT027 strain" (19, 20). CDI caused by RT244 was associated with severe disease and a high mortality rate and predominantly occurred in the community (6). Similarly, *C. difficile* RT251 strains were detected around the same time as RT244 in Australia, and severe disease and death in younger patients have been described (7). Genomic analyses revealed that *C. difficile* RT244, RT251, and RT027 were genetically distinct but all belonged in the same phylogenetic multilocus sequence type (MLST) clade 2, suggesting a recent shared evolutionary ancestry (6, 7, 19).

C. difficile RT126 was the most prevalent CDT^+ type recovered in this study, and the majority of isolates were isolated in VIC in 2016. This RT has been isolated from humans with CDI as well as from pigs and cattle, a trait shared with other RTs belonging to the

same ST11 lineage (21). ST11 strains infect and colonize both humans and animals, with strong evidence of long-range inter- and intraspecies transmission (13). Although the ST11 type RT078 is commonly isolated from humans and animals in Europe and the United States (22, 23), only six isolates were recovered in this study. These cases were sporadic and mostly from NSW. The finding of low numbers of RT078 isolates circulating in NSW was consistent with our previous reports (8, 10, 11). Furthermore, RT078 and RT126 give very similar banding patterns (21) and are sometimes reported together as RT078/126 or not differentiated.

The prevalence of RT056 increased in the current study (8). *C. difficile* RT056 is well established in both humans and livestock in Australia (10, 24). It is also one of the most common RTs in Australian food (organic potatoes, and carrots) and the environment (compost and roll-out lawns) (25, 26). More recently, cgSNP analysis of *C. difficile* RT056 strains from humans, food, and compost demonstrated a clonal relationship between these strains consistent with recent transmission events (S. C. Lim, unpublished data).

The overall prevalence of *C. difficile* RT002 in this study was lower than previously reported in Australia (8). Almost half the RT002 strains reported in this study were isolated in NSW ($n = 76$), the majority (63.2%) from the laboratories based in a tertiary hospital; however, there was no temporal clustering over the 5-year period ($P > 0.05$). This RT was reported as the predominant clone recovered from patients with CDI in Hong Kong and was associated with high morbidity and mortality (27). *C. difficile* RT002 appeared to have a higher sporulation rate and higher rates of fluoroquinolone resistance, similar to epidemic RT027 (27). This type is among the most common *C. difficile* RTs in Europe and the United States (16, 28); however, its significance in Australia is unknown.

A small group of *C. difficile* RT017 strains was identified. This type has a variant toxin profile ($A^-B^+CDT^-$) and is a major cause of CDI across Asia (29). Notably, two of the top 20 most prevalent *C. difficile* strains in Australia, RT010 and RT039, were nontoxigenic. These RTs were predominately from private laboratories and from patients with symptoms of CDI, suggesting that they may have been simultaneously colonized with toxigenic strains. Coinfection with and carriage of multiple *C. difficile* strains are not common (30, 31). A possible source of nontoxigenic *C. difficile* is the environment, and RT010 and RT039 have been found in lawns and compost in WA (26).

This study has some limitations. As reported in our earlier publication, there was a lack of information about the clinical significance of isolates (8). Diagnostic testing remains controversial in Australia and, in recent years, many laboratories that moved to nucleic acid amplification tests (NAATs) for the detection of *C. difficile* toxin genes have changed to a 2-step algorithm due to lack of specificity of NAATs (32). Thus, while many CDI isolates in this study were derived from PCR-positive stool specimens, the Australian Commission on Safety and Quality in Health Care describes NAATs as suitable for surveillance purposes (4). Last, as mentioned earlier, there may be some classification inconsistencies for HI and CA isolates due to the organizational structure of the health care systems in Australia. However, this is an ongoing surveillance study for *C. difficile* with the aim of monitoring emerging strains (8), and consistency in methodology remains critical for the purpose of conducting surveillance (33).

To summarize, a heterogeneous *C. difficile* strain population was identified in Australia between 2013 and 2018. *C. difficile* RT014/020 remained the most prevalent type found in humans with CDI. The detection of identical RTs commonly isolated from humans, animals, and the environment supports a zoonotic paradigm for CDI and the need for a One Health approach to CDI management. Future work using high-resolution whole-genome-based typing will determine the true extent of genetic relatedness of identical *C. difficile* RTs as well as possible bidirectional transmission of *C. difficile* between health care and community settings. Continued surveillance of CDI in Australia remains important for the detection of emerging strain lineages and for developing improved diagnostic tools and therapeutic options.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.05 MB.

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