Surveillance snapshot of *Clostridium difficile* infection in hospitals across Queensland detects binary toxin producing ribotype UK 244

Charlotte A Huber, Lisa Hall, Niki F Foster, Mareeka Gray, Michelle Allen, Leisha J Richardson, Jennifer Robson, Renu Vohra, Sanmarie Schlebusch, Narelle George, Graeme R Nimmo, Thomas V Riley, David L Paterson

Abstract

In North America and Europe, the binary toxin positive *Clostridium difficile* strains of the ribotypes 027 and 078 have been associated with death, toxic megacolon and other adverse outcomes. Following an increase in *C. difficile* infections (CDIs) in Queensland, a prevalence study involving 175 hospitals was undertaken in early 2012, identifying 168 cases of CDI over a 2 month period. Patient demographics and clinical characteristics were recorded, and *C. difficile* isolates were ribotyped and tested for the presence of binary toxin genes. Most patients (106/168, 63.1%) were aged over 60 years. Overall, 98 (58.3%) developed symptoms after hospitalisation; 89 cases (53.0%) developed symptoms more than 48 hours after admission. Furthermore, 27 of the 62 (67.7%) patients who developed symptoms in the community had been hospitalised within the last 3 months. Thirteen of the 168 (7.7%) cases identified had severe disease, resulting in admission to the Intensive Care Unit or death within 30 days of the onset of symptoms. The 3 most common ribotypes isolated were UK 002 (22.9%), UK 014 (13.3%) and the binary toxin-positive ribotype UK 244 (8.4%). The only other binary toxin positive ribotype isolated was UK 078 (n = 1). Of concern was the detection of the binary toxin positive ribotype UK 244, which has recently been described in other parts of Australia and New Zealand. No isolates were of the international epidemic clone of ribotype UK 027, although ribotype UK 244 is genetically related to this clone. Further studies are required to track the epidemiology of ribotype UK 244 in Australia and New Zealand. *Commun Dis Intell* 2014;38(4):E279–E284.

Keywords: *Clostridium difficile;* disease surveillance; hospitals

Introduction

Clostridium difficile commonly causes infectious diarrhoea and is the main aetiological agent of pseudomembranous colitis.[1] *C. difficile* strains of the ribotypes UK 027 and UK 078 have been associated with severe adverse outcomes such as toxic megacolon, colonic perforation, peritonitis, and increased mortality rates in North America and Europe.[2] The hypervirulence of these strains has been attributed to the increased production of toxins A and B, as well as the presence of the binary toxin.[3]

Following the first report of local transmission of *C. difficile* ribotype UK 027 in Australia in 2009,[4] a national laboratory survey was performed, but did not detect any strains of potentially hypervirulent ribotypes in Queensland (unpublished data, personal communication, T Riley). Between October 2011 and early 2012, we observed an increase in *C. difficile* infections (CDI) in Queensland Health facilities using passive surveillance of diagnostic laboratory pathology results.[5] Here we provide a more detailed state-wide snapshot of the epidemiology of hospital-identified CDI cases in Queensland in early 2012.

Methods

Study design and definitions

All Queensland public hospitals (n = 170) and 5 private hospitals in Queensland, were invited and agreed to take part in the cross-sectional prevalence study. Between 10 April and 15 June 2012, patients aged over 2 years with diarrhoea at admission or during hospitalisation and testing positive for *C. difficile* were included in the study. The Australian definitions of hospital identified CDI[6] were used to identify cases, and classify recurrence and severe disease. A summary of these definitions are provided in Table 1. Patient samples collected during this time were cultured when available. *C. difficile* isolates were ribotyped and screened for the presence of the binary toxin in order to investigate the prevalence of epidemic strains.

Surveillance data collection and analysis

Basic demographic and clinical data including data on patient location at onset of symptoms, history of recent hospitalisation and antibiotic use were collected by infection control practitioners on

a one page survey. Data were subsequently submitted to the Centre for Healthcare Related Infection Surveillance and Prevention (CHRISP) and entered into a spreadsheet. CHRISP is the statewide unit responsible for overseeing healthcare associated infection surveillance, and prevention and control policy in Queensland public facilities (www.health.qld.gov.au/chrisp). A follow-up survey was conducted 30 days after symptom onset to determine the prevalence of severe CDI using Australian surveillance guidelines (Table 1). De-identified data were imported into IBM SPSS version 20 for further descriptive analysis, including chi-squared tests for differences in proportions.

Laboratory methods

C. difficile was cultured from stool specimens that tested *C. difficile* toxin positive by either polymerase chain reaction (PCR) or enzyme immunoassay (EIA) at the referring laboratories. At the time of this snapshot a testing algorithm was in place across Queensland to ensure consistency in laboratory methods used. PCR ribotyping was performed as previously described.[7,8] Briefly, genomic DNA was extracted from 24 hour horse blood agar cultures using the UltraClean Microbial DNA Isolation kit (MO BIO Laboratories Carlsbad, CA, United States) then PCR ribotyping was performed, using an ABI 3130 capillary sequencer (Applied Biosystems) with a GeneScan™ 1200 LIZ® Size Standard (Applied Biosystems). The capillary sequencer data were uploaded to the Webribo database (http://webribo.ages.at/). UK ribotypes were determined for a representative of each Webribo ribotype, using the Anaerobe Reference Unit (Cardiff, UK) nomenclature where reference strains were available. This was done by comparing PCR products, concentrated using the Qiagen MinElute PCR purification kit, then separated using the QIAxcel capillary electrophoresis platform (Ambion Inc, Austin, Texas), to the UWA/PathWest reference library as previously described.[10] The library consisted of 50 UK ribotypes including 15 reference strains from the European Centre for Disease Prevention and Control and the most prevalent PCR ribotypes circulating in Australia. If a UK ribotype could not be assigned, local nomenclature (QX type) or webribo (WR type) nomenclature was assigned. Screening for binary toxins was performed as previously described.[10]

Ethics

This study was considered to be a quality assurance audit by the Department.

In Queensland, the use of confidential patient information for audit is legislated under Section 62H of the *Health Services Act 1991*. Approval was obtained from the Chief Health Officer of Queensland Health, and Chief Executive Officers of each private facility.

Ethical approval for this surveillance was not required as: surveillance of CDI in Queensland Health facilities is required in accordance with the Queensland Health Protocol for the Surveillance of Healthcare Associated Infection; private hospitals in Queensland are required to undertake infection control surveillance in accordance with the Infection Control Standard of the *Private Health Facilities Act 1999*.

Results

Patient characteristics

During the audit period, a total of 168 CDI cases were identified. The patients ranged in age from 3 to 100 years, with a median age of 68.5 years. A total of 36 cases were reported from private hospitals. Key descriptive variables such as age and location at onset of symptoms were compared using chi-square tests and did not significantly vary between private and public hospital patients so the data were combined. A summary of patient characteristics is shown in Table 1.

The majority of cases (98/168, 58%) developed symptoms following hospitalisation, however in nine of these patients, symptoms began within 48 hours of admission. Five of the nine had a record of previous hospitalisation in the last month. Of the 62 cases that were community-onset, 27 (43%) had been admitted to a hospital in the previous month and 15 (24%) in the previous 1 to 3 months. Fifteen (9%) of the CDI cases were admitted from a residential or long term care facility (R/LTCF). However, only four of these cases had onset of symptoms within their R/LTCF.

Severe disease

Thirteen (7.7%) CDI cases were classified as severe disease using surveillance definitions. Nine of the 13 had been admitted to hospital in the previous 3 months. No patients with severe CDI had been admitted from an R/LTCF.

The 30-day mortality following diagnosis was 3.6%. Of the 6 patients who died, four had been admitted to hospital within the past month, two had recurrent CDI and two had onset of symptoms in the community. All 6 cases had been prescribed antibiotics in the last month. The mean age of these patients was 75.8 years, (95% CI: 67.1–84.6), which was significantly greater than CDI patients who did not develop severe disease

Table 1: *Clostridium difficile* infections patient characteristics

Survey variable	Number of cases (n=168)	Percentage
Age range		
2–20	10	6.0
21–40	22	13.1
41–60	30	17.9
61–80	59	35.1
80+	47	28.0
Sex		
Male	86	51.2
Female	82	48.8
Patient location at onset of symptoms		
Hospital	98	58.3
Residential/long term care facility	4	2.4
Community	62	36.9
Not reported	4	2.4
Previous hospitalisation		
Within the last month	86	51.2
Within last 1–3 months	33	19.6
More than 3 months ago	16	9.5
No previous hospitalisation	19	11.3
Not reported	14	8.3
Admission from residential/long term care facility		
Within the last month	15	8.9
No previous residential/long term care admission	153	91.1
Is this episode a recurrence (within 8 weeks of onset of previous episode)?		
Yes	20	11.9
No	135	80.4
Not reported	13	7.7
Antibiotic therapy in the last month		
Yes	136	81.0
No	13	7.7
Not reported	19	11.3
Severity of disease		
Intensive care admission for CDI	7	4.2
Colectomy due to CDI	0	0.0
Mortality within 30 days related to infection	6	3.6
No severe disease	91	54.2
Not reported	64	38.1

CDI *Clostridium difficile* infections.

(62.1 years, 95% CI: 57.1–67.0). Due to the presence of significant co-morbidities it was difficult to ascertain whether the primary cause of death in these patients was CDI.

Ribotyping data

C. difficile isolates were obtained from 83 (81.4%) of 102 specimens submitted for culture. The prevalence of ribotypes detected is shown in Table 2. The most commonly isolated ribotype was UK 002 (22.9%), and UK 014 was the 2nd most prevalent (13.3%). Ribotypes UK 014 and UK 020 are often combined due to difficulty distinguishing between their ribotyping patterns. The UK 014/020 group constituted 19.3% of isolates.

There were no isolates of ribotype UK 027, and only 1 isolate was ribotype UK 078. This case (a 46-year-old male) had onset of symptoms more than 48 hours after admission to hospital, and did not develop severe disease.

Ribotype UK244 was the 3rd most common ribotype circulating with 7 cases detected. Further detail on the cases infected with ribotype UK 244 is presented in Table 3.

Table 2: Prevalence of ribotypes detected during the study n = 83

Ribotype	N	% of isolates
UK 002	19	22.9
UK 014	11	13.3
UK 244	7	8.4
QX 014 WR AI-37	5	6.0
UK 056	5	6.0
UK 043	4	4.8
UK 020	3	3.6
QX 025	2	2.4
QX 150 WR 632	2	2.4
UK 014/020	2	2.4
UK 017	2	2.4
UK 049	2	2.4
UK 054	2	2.4
UK 070	2	2.4
Unique*	15	18.1

* Includes UK 005, UK 015/191, UK 018, UK 070, UK 076, UK 078, UK 087, UK 126, QX 026 WR 404, QX 029 WR 409, QX 033 WR AI-83, QX 072 WR 629, QX 095 WR AI-34, QX 266 WR 637 and QX 291 WR 641.

Discussion

The results of this snapshot give insight into the burden of disease as well as the prevalence of hypervirulent *C. difficile* strains in Queensland. In Queensland all public hospitals (n = 170) are serviced by Pathology Queensland or Mater Pathology laboratories, with consistent laboratory protocols, and data stored in a centralised database. As a result we were able to identify all public hospital CDI cases during this time. In addition, with the support of five of the largest private facilities in the state, and their pathology providers, this study provided a detailed state-wide picture of the prevalence of cases, demographic and clinical risk factors and circulating ribotypes.

The study emphasised the importance of timely surveillance by infection control practitioners (ICPs). We were able to gather very useful information with the support of ICPs, to confirm that CDI is not just a hospital issue in Queensland, with 37% (62/168) of cases developing symptoms in the community.

We believe previous admission to hospital may still play a role in cases with onset of symptoms in the community and residential care facilities. The majority of cases in this study developed

Table 3: Characteristics of patients infected with *Clostridium difficile* ribotype 244

Age (years)	Sex	Hospital type	Onset of symptoms	Resident	Severity of disease
54	F	Private	Community	No	No severe disease
85	F	Public	Community	No	ICU admission
42	F	Public	Community	No	No severe disease
25	M	Public	Hospital	No	No severe disease
82	F	Public	Hospital	No	Died
88	F	Public	Hospital	Yes	Unknown
52	M	Public	Hospital	No	No severe disease

ICU Intensive care unit.

symptoms only after hospitalisation (98/168 58%). Of the 9 patients who had their onset within 48 hours of hospital admission, five had had a previous hospital admission in the last month. Of the 62 cases who developed symptoms in the community, 27 (44%) had been admitted to a hospital in the previous month and 15 (24%) in the previous 1 to 3 months. Frequent movement of patients between hospital and the community may contribute to the challenge of determining the true place of exposure, making traditional classifications of healthcare versus community associated infection misleading.[11]

Thirteen of the 168 (7.7%) CDI cases were classified as severe. The 30-day mortality was 3.6%. However, due to the advanced age of the 6 patients who died, as well as the presence of co-morbidities, it was difficult to ascertain whether the primary cause of death in these patients was CDI.

Only 49% of cases had an isolate ribotyped. We believe this is still representative of circulating ribotypes, as there did not appear to be any particular hospitals or laboratories more likely to have their samples successfully typed. Unfortunately, we were not able to ribotype strains from all cases of severe disease. Clinicians should be reminded to notify ICPs of suspected cases of severe disease in a timely fashion, as they are best placed to liaise with laboratories to ensure typing occurs.

Of the 102 specimens obtained for culture, 83 were successfully cultured and ribotyped. Of those ribotyped the most frequently isolated ribotype was UK 002 (19/83, 22.9%), which has also been most commonly detected in other regions of the world, such as Southern Scotland,[12] and Hong Kong where it has been associated with increased sporulation.[13] Ribotype UK 014 was the 2nd most prevalent ribotype (13.3%). Ribotypes UK 014 and UK 020 are often combined due to difficulty distinguishing between their ribotyping patterns. The UK 014/020 group constituted 19.3% of isolates. This was concordant with a European study reporting a combined prevalence of 16%.[14]

There were no isolates of ribotype UK 027, which is known to have caused severe disease in North America and Europe.[2] Only 1 isolate was ribotype UK 078, a community-associated strain with increased potential to cause severe disease.[15] However, this case (a 46-year-old male) had onset of symptoms more than 48 hours after admission to hospital, and did not develop severe disease. The link between the presence of hypervirulent strain type and severity of disease remains difficult to elucidate, although evidence for the importance of strain type as an independent risk factor is increasing.[3] In the United States of America, the CDI epidemic saw the emergence of 'hypervirulent' strains in an older and sicker population, which made it challenging to determine whether the rise in severity was due to the pathogen, or due to other host factors such as age and co-morbidities.[16]

There has been Australia-wide interest in the prevalence of ribotype 244 – a recently described strain that appears to be closely related to 027, and which is possibly of similarly high virulence.[17] In Australia, ribotype 244 was first detected by The University of Western Australia in specimens from a large Sydney hospital (personal communication, T Riley, 22 April 2013). Ribotype 244 has since been detected in several parts of Australia, and in New Zealand where it was associated with severe disease and community onset of infection.[18,19]

The risk of ribotype UK 244 in the Queensland population is unknown. Although in other Australian jurisdictions ribotype 244 has previously been reported to be a community ribotype associated with a high probability of severe disease, this was not uniformly the case in this Queensland study. Lim et al have recently published a case-control study of 14 ribotype 244 cases, matched by hospital locality and date of diagnosis to 24 controls; demonstrating a clinically, but not statistically significant increase in severity and 30 day mortality.[19]

Further larger studies, incorporating a more geographically representative range of hospitals, may be required to determine the risk associated with this ribotype in Australia. In addition, regardless of ongoing efforts to monitor hospital infections,[5] it would be useful for a continuous national passive surveillance program to be considered, with periodic ribotyping. This type of surveillance, such as that undertaken for nationally notifiable conditions, would assist decision makers to determine the level of risk to the community, and plan public health interventions in a more timely fashion.

Acknowledgements

This study was a collaborative effort between: Infection Control Practitioners within Queensland Health and the following private hospitals, Mater Health Services (Private), Greenslopes Private Hospital, St Andrew's Hospital, Wesley Hospital, Holy Spirit Northside; Pathology Queensland including the Microbiology Discipline Working Party and the Central Sequencing Service; Sullivan and Nicolaides Pathology, Queensland Medical Laboratory and Mater Pathology; the Queensland Health Centre for Healthcare

Related Infection Surveillance and Prevention and the University of Queensland Centre for Clinical Research.

Author details

Dr Charlotte A Huber[1]
Dr Lisa Hall[2,3]
Dr Niki F Foster [4]
Ms Mareeka Gray[2]
Ms Michelle Allen[2,3]
Ms Leisha J Richardson[1]
Dr Jennifer Robson[5]
Dr Renu Vohra[6]
Dr Sanmarie Schlebusch[7,8]
Ms Narelle George[9]
Prof Graeme R Nimmo[9]
Prof Thomas V Riley[4,10]
Prof. David L Paterson[1,2,9]

1. University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, Brisbane, Queensland
2. Centre for Healthcare Related Infection Surveillance and Prevention, Queensland Health, Brisbane, Queensland
3. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland
4. School of Pathology and Laboratory Medicine, University of Western Australia, Crawley, Western Australia
5. Sullivan Nicolaides Pathology, Brisbane, Queensland
6. Queensland Medical Laboratories, Brisbane, Queensland
7. Mater Pathology, Brisbane, Queensland
8. University of Queensland, Brisbane, Queensland
9. Central Microbiology Laboratory, Pathology Queensland, Brisbane, Queensland
10. PathWest Laboratory Medicine WA, Nedlands, Western Australia

Corresponding author: Professor David Paterson, University of Queensland Centre for Clinical Research, Level 8, Building 71/918, Royal Brisbane and Women's Hospital, HERSTON QLD 4029. Telephone: +61 7 3346 6074. Email: david.antibiotics@gmail.com

References

1. Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. *N Engl J Med* 1994;330(4):257–262.
2. Clements AC, Magalhaes RJ, Tatem AJ, Paterson DL, Riley TV. *Clostridium difficile* PCR ribotype 027: assessing the risks of further worldwide spread. *Lancet infect Dis* 2010;10(6):395–404.
3. Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, et al. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* Infection. *Clin Infect Dis* 2013.
4. Richards M, Knox J, Elliott B, Mackin K, Lyras D, Waring LJ, et al. Severe infection with *Clostridium difficile* PCR ribotype 027 acquired in Melbourne, Australia. *Med J Aust* 2011;194(7):369–371.
5. Slimings C, Armstrong P, Beckingham WD, Bull AL, Hall L, Kennedy KJ, et al. Increasing incidence of *Clostridium difficile* infection, Australia, 2011–2012. *Med J Aust* 2014;200(5):272–276.
6. Australian definitions of hospital identified CDI [online] Available from: http://www.safetyandquality.gov.au/our-work/healthcare-associated-infection/national-hai-surveillance-initiative/
7. Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kernbichler S, Fiedler A, et al. Characterization of *Clostridium difficile* isolates using capillary gel electrophoresis-based PCR ribotyping. *J Med Microbiol* 2008;57(Pt 11):1377–1382.
8. Stubbs SL, Brazier JS, O'Neill GL, Duerden BI. PCR targeted to the 16S–23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. *J Clin Microbiol* 1999;37(2):461–463.
9. Carson KC, Boseiwaqa LV, Thean SK, Foster NF, Riley TV. Isolation of *Clostridium difficile* from faecal specimens—a comparison of chromID *C. difficile* agar and cycloserine-cefoxitin-fructose agar. *J Med Microbiol* 2013;62(Pt 9):1423–1427.
10. Stubbs S, Rupnik M, Gibert M, Brazier J, Duerden B, Popoff M. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. *FEMS Microbiol Lett* 2000;186(2):307–312.
11. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, et al. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. *N Engl J Med* 2013;369(13):1195–1205.
12. Taori SK, Hall V, Poxton IR. Changes in antibiotic susceptibility and ribotypes in *Clostridium difficile* isolates from southern Scotland, 1979–2004. *J Med Microbiol* 2010;59(Pt 3):338–344.
13. Cheng VC, Yam WC, Lam OT, Tsang JL, Tse EY, Siu GK, et al. *Clostridium difficile* isolates with increased sporulation: emergence of PCR ribotype 002 in Hong Kong. *Eur J Clin Microbiol Infect Dis* 2011;30(11):1371–1381.
14. Bauer MP, Notermans DW, van BBH, Brazier JS, Wilcox MH, Rupnik M, et al. *Clostridium difficile* infection in Europe: a hospital-based survey. *Lancet* 2011;377(9759):63–73.
15. Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, et al. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. *Clin Infect Dis* 2013;56(11):1589–1600.
16. Aronoff DM. Editorial commentary: Host-pathogen interactions in *Clostridium difficile* infection: it takes two to tango. *Clin Infect Dis* 2014;58(10):1401–1403.
17. Valiente E, Dawson LF, Cairns MD, Stabler RA, Wren BW. Emergence of new PCR ribotypes from the hypervirulent *Clostridium difficile* 027 lineage. *J Med Microbiol* 2012;61(Pt 1):49–56.
18. De AMN, Heffernan H, Dervan A, Bakker S, Freeman JT, Bhally H, et al. Severe *Clostridium difficile* infection in New Zealand associated with an emerging strain, PCR-ribotype 244. *N Z Med J* 2013;126(1380):9–14.
19. Lim SK, Stuart RL, Mackin KE, Carter GP, Kotsanas D, Francis MJ, et al. Emergence of a ribotype 244 strain of *Clostridium difficile* associated with severe disease and related to the epidemic ribotype 027 strain. *Clin Infect Dis* 2014;58(12):1723–1730.