A comparison of *Clostridium difficile* ribotypes circulating in Australian hospitals and communities

Running title: *C. difficile* ribotypes in Australia

Luis Furuya-Kanamori[1#], Thomas V. Riley[2,3], David L. Paterson[4], Niki F. Foster[2,3], Charlotte A. Huber[4], Stacey Hong[2], Tiffany Harris-Brown[4], Jenny Robson[5], Archie C.A. Clements[1]

[1] Research School of Population Health, The Australian National University, Canberra, ACT, Australia

[2] Microbiology & Immunology, School of Pathology & Laboratory Medicine, The University of Western Australia, Nedlands, WA, Australia

[3] Department of Microbiology, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands, WA, Australia

[4] UQ Centre for Clinical Research, The University of Queensland, Herston, QLD, Australia

[5] Sullivan Nicolaides Pathology, Taringa, QLD, Australia

Correspondence during review process to:

Luis Furuya-Kanamori (Luis.Furuya-Kanamori@anu.edu.au)

Post-publication correspondence to:

Archie C.A. Clements (director.rsph@anu.edu.au)

Abstract

Clostridium difficile infection (CDI) is becoming less exclusively a healthcare-associated (HA) infection. Community-associated (CA)-CDI has increased over the past decades. It has been postulated that asymptomatic toxigenic *C. difficile* (TCD)-colonised patients may play a role in the transfer of *C. difficile* between the hospital setting and the community. Thus, to investigate the relatedness of *C. difficile* across the hospital and community settings, we compared host and pathogen characteristics among symptomatic and asymptomatic patients in these two settings over a 3-year period. Two studies were simultaneously conducted, the first study enrolled symptomatic CDI patients from two tertiary hospitals and the community in two Australian states; while the second study enrolled asymptomatic TCD-colonised patients from the same tertiary hospitals. A total of 324 patients (96 HA-CDI, 152 CA-CDI and 76 TCD-colonised) were enrolled. The predominant *C. difficile* ribotypes isolated in the hospital setting corresponded with those isolated in the community, 79% of the *C. difficile* isolates from the hospitals had a matching ribotype isolated from the community, suggesting that transmission between these two settings is occurring. Toxigenic *C. difficile* strains causing symptomatic infection were similar to those causing asymptomatic infection and patients exposed to antimicrobials prior to admission were more likely to develop symptomatic infection (OR 2.94; 95%CI 1.20-7.14). Our findings suggest that development of CDI symptoms in a setting without establishment of hospital epidemics with binary toxin producing *C. difficile* strains may be driven mainly by host susceptibility and exposure to antimicrobials, rather than by *C. difficile* strain characteristics.

Introduction

Over the past three decades, the epidemiology of *Clostridium difficile* infection (CDI) has markedly changed, and several countries have reported a significant increase in incidence and severity of the disease as well as numerous hospital outbreaks. The changes have been partly attributed to the emergence of specific *C. difficile* strains (PCR ribotypes 001, 027, 078) with increased toxin production and in some cases resistance to later generations of fluoroquinolones (1-3). CDI was previously considered exclusively a healthcare-associated (HA) infection affecting elderly patients with multiple comorbidities and a recent history of antimicrobial exposure. However, patients in the community are now also considered at risk of CDI and *C. difficile* strains that are known to be highly pathogenic are now frequently isolated among community-associated (CA)-CDI cases (1). Severe cases of CA-CDI were reported among populations that were considered at low risk of CDI including pregnant women and healthy young adults without antimicrobial exposure nor contact with healthcare facilities (4, 5).

Symptoms of CDI can range from mild diarrhoea to life-threatening conditions such as pseudomembranous colitis and are precipitated by the capacity of some *C. difficile* strains to produce toxins A and B and binary toxin (CDT). Similar to other infectious diseases, not all patients colonised with toxigenic *C. difficile* (TCD) strains become symptomatic. Loo et al. found that *C. difficile* ribotype 027 was the predominant strain isolated from symptomatic patients with HA-CDI; whereas, asymptomatic patients were more likely colonised with other strains (6). However, it is unclear which host and pathogen features determine whether a colonised patient with *C. difficile* will remain asymptomatic or develop mild or severe forms of the disease in a non-027 endemic setting. Although, cases of *C. difficile* ribotypes 027 have been reported in Australia (7, 8); *C. difficile* 027 has not yet established and the most common ribotypes circulating are 014/020, 056, and 002 (9, 10).

Journal of Clinical Microbiology

It has also been proposed that asymptomatic TCD-colonised patients act as a source of environmental contamination and may result in emergence of new CDI cases particularly in a hospital setting (11, 12). Furthermore, epidemiological studies and a mathematical modelling study have demonstrated that CA-CDI importation into the hospital may play a role in maintaining HA-CDI transmission (13-15).

Despite the growing evidence that HA-CDI, CA-CDI and asymptomatic TCD-colonisation are interrelated and all three play a significant role in *C. difficile* epidemiology, no reported study has previously evaluated these three components of *C. difficile* at the same time. Therefore, the current study aimed to determine whether these three components are in fact interrelated by comparing the predominant *C. difficile* ribotypes and symptomatic and asymptomatic patients' characteristics in the healthcare setting and in the community, over a 3-year period.

Journal of Clinical Microbiology

Methods

Study setting

Two studies were simultaneously conducted over a three-year period (2012-2014) in two Australian States. The first study examined symptomatic patients with HA and CA-CDI; whereas, the second study examined asymptomatic *C. difficile*-colonised patients in a healthcare setting.

The first study enrolled patients in two tertiary hospitals, The Royal Brisbane and Women's Hospital (RBWH), with 929 beds in Brisbane, Queensland, and The Sir Charles Gairdner Hospital (SCGH), with 607 beds in Perth, Western Australia. Patients in the community who submitted specimens through their general practitioner (GP) to coordinating laboratories (Sullivan Nicolaides Pathology in Brisbane, Queensland and PathWest Laboratory Medicine, Clinipath Laboratories and Western Diagnostic Pathology in Perth, Western Australia) were also enrolled. This study used a census design in which all the stool specimens submitted during the study period to the hospitals and the laboratories by patients 18 year of age or older and experiencing diarrhoea were screened for *C. difficile*. If the specimen was positive for *C. difficile* toxin A or B genes, the patient was invited to participate in the study. HA-CDI was defined as healthcare facility-onset, healthcare facility-associated CDI, constituting onset of diarrhoea 48 hours or more after admission to a hospital, and community-onset, healthcare facility-associated disease constituting onset of symptoms in a patient who had been discharged from a healthcare facility within the previous 4 weeks. CA-CDI was defined as community-onset CDI in a patient who had not been admitted to a healthcare facility in the previous 12 weeks, or healthcare facility-onset CDI within 48 hours or less of admission to the hospital (16).

The second study has been previously described elsewhere (17). In brief, six cross-sectional surveys (two per year) were conducted at the RBWH and SCGH. Randomly

Journal of Clinical Microbiology

selected hospitalised patients aged 18 years or older, without diarrhoea, were approached and invited to participate in the study. Patients not experiencing diarrhoea and who had a toxigenic *C. difficile* strain (positive for the presence of *tcdA*, *tcdB* and/or *cdtA/cdtB* genes) isolated from their stool were considered to have asymptomatic TCD-colonisation and were included in the current analysis.

The studies received the approval of the RBWH (HREC/11/QRBW/223), the Sir Charles Gairdner Group (2011-088), The University of Queensland (2011000898), and The University of Western Australia (RA/4/1/5186) Human Research Ethics Committees. All the participants (or a legal proxy) provided written informed consent for their inclusion in the study. In Western Australia, a Waiver of Consent was granted when a person was unable to provide consent but the person could be enrolled in the study without any additional risk beyond their standard care.

Data collection

A questionnaire was administered to all patients from both studies regarding the patient's age, sex, occupation, previous hospital admissions and use of medications in the previous 30 days and their co-habitants' ages. For hospitalised patients at the RBWH and SCGH, medical records were accessed to obtain additional information and to determine the date and the reason for the current admission, comorbidities, as well as the in-patient medication and procedures prior to specimen collection. Each participant was followed up on a monthly basis for 3 months by examination of the patients' records and a short interview for hospital patients, and by a telephone interview for discharged or CA-CDI cases. The follow-up interviews were used to determine clinical outcomes of the patients (whether they developed symptoms, had a recurrence of CDI, underwent a colectomy, were admitted to ICU or died).

Stool specimen collection and processing

As previously described (17), direct stool specimen culture was performed on ChromID™ *C. difficile* agar (bioMerieux). Broth enrichment in Robertson's cooked meat medium, followed by ethanol shock and subculture on ChromID at 48-72 h was performed if direct culture was negative. Putative *C. difficile* colonies were subcultured onto pre-reduced blood agar plates under anaerobic conditions. *C. difficile* isolates were tested for the presence of toxin genes and were polymerase chain reaction (PCR) ribotyped as previously described (18). Strains that did not produce banding patterns matching an international ribotype in the reference collection were assigned a local nomenclature (QX type).

Statistical analysis

The frequency of *C. difficile* ribotypes was tabulated by year and *C. difficile* category (HA-, CA-CDI and asymptomatic TCD-colonisation) to identify the predominant ribotypes circulating in each category and to examine the changes in ribotype profile over the study period. The Simpson's index of diversity was calculated for each category to compare the diversity of ribotypes isolated across the three categories.

Pearson's chi-squared test and Fisher's exact test were used to compare categorical variables; whereas, the Wilcox-Mann-Whitney U test and Kruskal-Wallis H test were used to compare continuous variables between participant groups. Multivariate logistic regression models were built to identify predictors of symptomatic disease. After adjusting for age and sex of the patients, known risk factors for CDI (i.e. prior hospital admissions and exposure to antimicrobials and gastric-acid suppressive agents); the inclusion of comorbidities in the regression model was done through a stepwise forward selection with the AIC as the selection criterion. A significance level cut off of $p = 0.05$ was used for all analyses. All

statistical analyses were conducted using Stata® SE, version 14 (Stata Corporation; College Station, TX).

Journal of Clinical Microbiology

158 **Results**

159 Over the three-year study period, 324 patients (96 HA-, 152 CA-CDI and 76
160 asymptomatic TCD-colonisation) were enrolled. One hundred and sixty-five patients (50.9%)
161 were enrolled in Queensland, while 159 (49.1%) were enrolled in Western Australia.

162

163 *Characteristics of C. difficile isolates*

164 Five different toxin profiles were identified amongst the toxigenic *C. difficile* strains
165 isolated (Table 1). The proportion of toxin profiles did not significantly differ between *C.*
166 *difficile* categories (p-value = 0.816). The most common toxin profile was A+B+CDT-
167 (n=293, 83.2%). A-B+CDT+ *C. difficile* were only isolated among symptomatic patients
168 (n=3), while A-B-CDT+ was only isolated from one asymptomatic patient. Non-toxigenic *C.*
169 *difficile* strains were isolated from ten symptomatic patients (7 HA-CDI, 3 CA-CDI), most
170 likely due to co-infection with TCD strains that were not isolated.

171 The Simpson's indices of diversity were 0.89, 0.89 and 0.88 for HA-CDI, CA-CDI
172 and asymptomatically TCD-colonisation, respectively. Although, a high diversity of
173 ribotypes (over 90) were identified during the study period, four *C. difficile* ribotypes (i.e.
174 014/020, 056, 002 and 018) accounted for over 50% of the isolates. *C. difficile* ribotype
175 014/020 (n=97, 29.9%) was the predominant ribotype throughout the 3-year study period
176 among symptomatic (both HA-CDI and CA-CDI) and asymptomatic patients (Figure 1 and
177 supplementary material). *C. difficile* 056 (n=31, 9.6%) was the second most common
178 ribotype isolated followed by 002 (n=21, 6.5%) which was predominantly found in CA-CDI
179 and 018 (n=18, 4.9%) which was mainly found among asymptomatic TCD-colonised
180 patients. Amongst all study patients, particularly virulent ribotypes *C. difficile* 244, 078, 251
181 and 027 were isolated from only four, two, one and one CDI patients, respectively.

182 The predominant *C. difficile* ribotypes isolated from symptomatic HA-CDI patients
183 were concordant with the ribotypes identified among asymptomatic TCD-colonised patients;

Journal of Clinical Microbiology

over 70% of the isolates from symptomatic patients had a matching ribotype isolated from an asymptomatic patient. Likewise, 79% of the *C. difficile* isolates from the hospitals had a matching ribotype isolated from the community.

Patients' pre-admission characteristics

The pre-admission characteristics of patients constituting to the three *C. difficile* categories (HA-CDI, CA-CDI and TCD-colonisation) are presented in Table 2. The proportion of females significantly differed between the three groups, with a higher proportion having CA-CDI (73.7%) than HA-CDI (52.1%) or asymptomatic TCD-colonisation (52.6%, p-value < 0.001). Across the three groups, there was no statistical difference in healthcare exposure in the previous year. With regards to medication exposure in the month prior to enrolment, antimicrobials (p-value = 0.031) and gastric acid suppressant (p-value < 0.001) were more often prescribed to patients that developed HA-CDI compared to the other two groups, while laxatives (p-value < 0.001) were more often prescribed to patients that were asymptomatically colonised. Household exposure to toddlers, elderly people, domestic animals or livestock did not significantly differ between groups. Ten percent of the symptomatic patients (HA- [10.4%] and CA-CDI [10.0%]) reported having a prior episode of CDI in the past 12 months compared to none of the asymptomatic TCD-colonised patients (p-value < 0.001).

Characteristics during admission and prior to specimen collection

With regards to the reason for admission, and the procedures, comorbidities and medication exposure during admission (Table 3). More patients with HA-CDI (11.5%) underwent a colonoscopy compared with asymptomatic TCD-colonised patients (1.4%, p-value = 0.006); however, more asymptomatic TCD-colonised patients required mechanical

ventilation (p-value = 0.006) and underwent orthopaedic (p-value < 0.001) and neurological (p-value < 0.001) interventions than HA-CDI patients. Patients with HA-CDI presented a significantly lower proportion of COPD (p-value = 0.026) and neurological disorder (p-value = 0.042) compared to asymptomatic TCD-colonised patients. Conversely, patients with HA-CDI had a higher proportion of IBD (16.7%) compared to asymptomatic colonised patients (4.1%, p-value = 0.008). In terms of medication exposure during the hospital admission, HA-CDI (74.0%) and TCD-colonised patients (77.6%, p-value = 0.578) were equally exposed to antimicrobials. However, penicillins and β-lactamase inhibitors (p-value =0.010) were more often prescribed to patients who went on to develop HA-CDI than asymptomatic TCD-colonised patients. HA-CDI patients were more likely to have had chemotherapy (p-value = 0.019) and antidiarrhoeal medication (p-value = 0.019) than asymptomatic TCD-colonised patients, while the latter group of patients were more commonly exposed to laxatives (p-value = 0.029).

Predictors of symptomatic and severe forms of the disease

The multivariate logistic regression model (Table 4) revealed that patients exposed to antimicrobials within 30 days prior to hospitalisation were at higher risk of developing symptoms (OR 2.94; 95%CI 1.20-7.14); whereas, patients with COPD were at lower risk of developing symptoms of the infection (OR 0.31; 95%CI 0.12-0.83).

During the follow-up period, four TCD-colonised patients developed symptomatic CDI. Fifty-three and six patients with HA-CDI and CA-CDI, respectively, had recurrent CDI. Nine deaths were recorded including three participants with HA-CDI, two with CA-CDI and four asymptomatically colonised with TCD. Three patients, all with HA-CDI, were admitted to ICU. No colectomies were recorded.

Discussion

Previous studies that examined the relationship between *C. difficile* strains and the development of symptoms were conducted during an outbreak (19) or in settings where binary toxin producing *C. difficile* strains were predominant (6); this is the first epidemiological study of *C. difficile* conducted simultaneously in a healthcare and a community setting that examined symptomatic and asymptomatic patients in a setting without establishment of hospital epidemics with binary toxin producing *C. difficile* strains. There was no difference in ribotype diversity across HA-CDI, CA-CDI and asymptomatically TCD-colonised patients reflecting similar pathogen population structures. Furthermore, the most prevalent *C. difficile* ribotypes were similar across HA-CDI, CA-CDI and asymptomatically TCD-colonised patients suggesting that transmission of *C. difficile* is occurring between the hospitals and the communities, and asymptomatic TCD-colonised as well as symptomatic patients may be acting as a vehicle of transmission between these two settings.

The finding also suggests that *C. difficile* ribotypes may not be determinants of the development of symptomatic infection, but rather development of symptoms may be mainly driven by host factors such as immune state and disruption of the gut microbiome by exposure to antimicrobials or underlying conditions affecting the gastrointestinal tract (20-22). Our findings differ from a previous study in which a binary toxin *C. difficile* strain (i.e. ribotype 027) was more likely to cause symptomatic disease compared to other strains (6). This difference could be explained by the very low prevalence of *C. difficile* ribotype 027 and other highly virulent binary toxin producing strains in Australia and therefore our findings may be expected in other settings without hospital epidemics with binary toxin producing *C. difficile* strains.

Journal of Clinical Microbiology

Several meta-analyses have described risk factors for HA-CDI (23) and CA-CDI (24); however, female sex is not a well-documented risk factor for CA-CDI and few studies have described this association (25-29). In our study we found that nearly three-quarters of the CA-CDI cases occurred in women; whereas, HA-CDI and asymptomatic cases were equally distributed between the sexes. This observation may be mostly related to behavioural risk factors among women that occur in the community rather than physiological differences between sexes. Among the behavioural factors occurring in the community that may put females at risk of CDI are higher rates of antimicrobial prescriptions (30, 31), vegetable consumption (32) and contact with children (33).

Whilst there is no conclusive evidence that contaminated food leads to CDI in humans, studies have found that retail vegetables are contaminated with *C. difficile* strains similar to those affecting humans (34, 35). Likewise, *C. difficile* ribotypes frequently isolated in the current study, such as 014/020 and 056, were common ribotypes found in piglets and veal calves, respectively, in Australia (36, 37). Therefore, the possibility of food being a vehicle of *C. difficile* transmission cannot be ruled out. Although, our study did not find an association between CDI category and contact with toddlers (33), this association needs to be assessed in the context of gender as an effect modifier. Due to the small number of participants that reported living with toddlers, this analysis was not possible.

Another interesting finding was that 10% of symptomatic patients in both settings (hospital and community) reported having CDI in the previous year compared to 0% among the asymptomatic TCD-colonised patients. While this may be explained by recall bias, given the greater awareness of the disease among the symptomatic patients, this finding may also reflect differences in immune system capacity, with previous infection not offering protection against further infection in these individuals. Those with some degree of immunosuppression might develop symptoms and those with a fully functioning immune system might not

Journal of Clinical Microbiology

develop symptoms irrespective of the toxigenicity of the *C. difficile* strains to which the patient had been previously exposed. This hypothesis warrants further investigation that would require measurement and comparison of serum antibody, pro-inflammatory cytokines and chemokines levels of non-colonised, asymptomatic *C. difficile* colonised and symptomatic CDI patients. However, indirect evidence from the current study supports our hypothesis given that patients with some degree of immunosuppression (patients on chemotherapy) were more likely to develop symptoms.

This study supports reports elsewhere that inflammatory bowel disease is a risk factor for developing CDI (38); however, a finding that requires further investigation is that patients with COPD were less likely to develop symptoms. Wojciechowski and colleagues reported a reduced risk of CDI for patients with a COPD diagnosis and when systemic corticosteroids were used during antimicrobial treatment (39). This was corroborated by the present study whereby COPD was statistically associated with reduced risk of CDI. Wojciechowski and colleagues argued that corticosteroids attenuate the host immune response to *C. difficile* toxins, thus reducing toxin-induced cytokine release that is associated with systemic symptoms of CDI. Further studies are required to confirm the mechanism behind the association.

There are some limitations to this study. Although, a large sample size (n = 342) of patients was enrolled, the small number of significant health outcomes (i.e. deaths, ICU admission) recorded during the follow-up period precluded statistical analyses to elucidate whether HA-CDI was associated with more severe outcomes than CA-CDI. In addition, more discriminatory strain typing methods (e.g. multilocus variable-number tandem repeat analysis and whole-genome sequencing) are required to conclusively determine specific transmission events between community and hospital CDI cases as well the role of asymptomatic colonised patients.

In summary, similar *C. difficile* ribotypes were circulating in the community and hospitals in this study of two Australian states suggesting carryover of strains between settings. Furthermore, asymptomatic and symptomatic patients were colonised with similar *C. difficile* ribotypes suggesting that, in a setting without establishment of hospital epidemics with binary toxin producing *C. difficile* strains, the development of symptoms may be primarily driven by host characteristics rather than *C. difficile* toxigenicity or ribotype. Future epidemiological studies in settings without hospital epidemics with binary toxin producing *C. difficile* strains are needed to confirm our findings and determine the role of patient-, antibiotic- and *C. difficile* strain-related factors in the development of symptoms.

Journal of Clinical Microbiology

Funding information

This research was funded by a National Health and Medical Research Council grant (#1006243). LFK is funded by an Endeavour Postgraduate Scholarship (#3781_2014), an Australian National University Higher Degree Scholarship, and a Fondo para la Innovación, Ciencia y Tecnología Scholarship (#095-FINCyT-BDE-2014). ACAC is funded by an Australian National Health and Medical Research Council Senior Research Fellowship (#1058878). The study sponsors had no further role in the study design, data collection, analyses, interpretation of results, writing of the article or the decision to submit it for publication.

Declaration of interests

The authors have no competing interest.

Acknowledgements

We would like to thank all patients, physicians, nurses, laboratories and hospitals who took part in the study. We will also like to thank Tanya Scheller, Christine Duncan, Suzanne Ditchburn, Welma Van Schalkwyk, Noellene Foster, Sarah MacArthur, Jessica Macfarlane who assisted with the enrolment of the patients and data extraction.

334 **References**

335 1. **Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW,**
336 **Bergwerff AA, Dekker FW, Kuijper EJ**. 2008. Emergence of Clostridium difficile
337 infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin
338 Infect Dis. **47**:1162-1170.

339 2. **Borgmann S, Kist M, Jakobiak T, Reil M, Scholz E, von Eichel-Streiber C,**
340 **Gruber H, Brazier JS, Schulte B**. 2008. Increased number of Clostridium difficile
341 infections and prevalence of Clostridium difficile PCR ribotype 001 in southern Germany.
342 Euro Surveill. **13**:19057.

343 3. **Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault**
344 **AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson**
345 **TJ, Horn R, Rene P, Monczak Y, Dascal A**. 2005. A predominantly clonal multi-
346 institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and
347 mortality. N Engl J Med. **353**:2442-2449.

348 4. **Centers for Disease Control and Prevention**. 2005. Severe Clostridium difficile–
349 associated disease in populations previously at low risk four states. Morb Mortal Wkly Rep.
350 **54**:1201-1205.

351 5. **Centers for Disease Control and Prevention**. 2008. Surveillance for community-
352 associated Clostridium difficile--Connecticut, 2006. Morb Mortal Wkly Rep. **57**:340-343.

353 6. **Loo VG, Bourgault A-M, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye**
354 **B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Béliveau C, Oughton**
355 **M, Brukner I, Dascal A**. 2011. Host and Pathogen Factors for Clostridium difficile
356 Infection and Colonization. N Engl J Med. **365**:1693-1703.

357 7. **Riley TV, Thean S, Hool G, Golledge CL**. 2009. First Australian isolation of
358 epidemic Clostridium difficile PCR ribotype 027. Med J Aust. **190**:706-708.

Journal of Clinical Microbiology

8. **Richards M, Knox J, Elliott B, Mackin K, Lyras D, Waring LJ, Riley TV**. 2011. Severe infection with Clostridium difficile PCR ribotype 027 acquired in Melbourne, Australia. Med J Aust. **194**:369-371.

9. **Huber CA, Hall L, Foster NF, Gray M, Allen M, Richardson LJ, Robson J, Vohra R, Schlebusch S, George N, Nimmo GR, Riley TV, Paterson DL**. 2014. Surveillance snapshot of Clostridium difficile infection in hospitals across Queensland detects binary toxin producing ribotype UK 244. Commun Dis Intell Q Rep. **38**:E279-284.

10. **Foster NF, Collins DA, Ditchburn SL, Duncan CN, van Schalkwyk JW, Golledge CL, Keed AB, Riley TV**. 2014. Epidemiology of Clostridium difficile infection in two tertiary-care hospitals in Perth, Western Australia: a cross-sectional study. New Microbes New Infect. **2**:64-71.

11. **Lanzas C, Dubberke ER, Lu Z, Reske KA, Grohn YT**. 2011. Epidemiological model for Clostridium difficile transmission in healthcare settings. Infect Control Hosp Epidemiol. **32**:553-561.

12. **Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN**. 1992. Acquisition of Clostridium difficile by hospitalized patients: evidence for colonized new admissions as a source of infection. J Infect Dis. **166**:561-567.

13. **Walker AS, Eyre DW, Wyllie DH, Dingle KE, Harding RM, O'Connor L, Griffiths D, Vaughan A, Finney J, Wilcox MH, Crook DW, Peto TE**. 2012. Characterisation of Clostridium difficile hospital ward-based transmission using extensive epidemiological data and molecular typing. PLoS Med. **9**:e1001172.

14. **Yakob L, Riley T, Paterson D, Clements A**. 2013. Clostridium difficile exposure as an insidious source of infection in healthcare settings: an epidemiological model. BMC Infect Dis. **13**:376.

15. **Curry SR, Muto CA, Schlackman JL, Pasculle AW, Shutt KA, Marsh JW, Harrison LH**. 2013. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. Clin Infect Dis. **57**:1094-1102.

16. **Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH**. 2010. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect Control Hosp Epidemiol. **31**:431-455.

17. **Furuya-Kanamori L, Clements AC, Foster NF, Huber CA, Hong S, Harris-Brown T, Yakob L, Paterson D, Riley TV**. 2016. Asymptomatic Clostridium difficile colonisation in two Australian tertiary hospitals, 2012–2014: A prospective, repeated cross-sectional study. Clin Microbiol Infect. [In press].

18. **Carson KC, Boseiwaqa LV, Thean SK, Foster NF, Riley TV**. 2013. Isolation of Clostridium difficile from faecal specimens-a comparison of chromID C. difficile agar and cycloserine-cefoxitin-fructose agar. J Med Microbiol. **62**:1423-1427.

19. **Johnson S, Clabots CR, Linn FV, Olson MM, Peterson LR, Gerding DN**. 1990. Nosocomial Clostridium difficile colonisation and disease. Lancet. **336**:97-100.

20. **Walk ST, Micic D, Jain R, Lo ES, Trivedi I, Liu EW, Almassalha LM, Ewing SA, Ring C, Galecki AT, Rogers MA, Washer L, Newton DW, Malani PN, Young VB, Aronoff DM**. 2012. Clostridium difficile ribotype does not predict severe infection. Clin Infect Dis. **55**:1661-1668.

21. **Walker AS, Eyre DW, Crook DW, Wilcox MH, Peto TEA**. 2013. Regarding "Clostridium difficile ribotype does not predict severe infection". Clin Infect Dis. **56**:1845-1846.

22. **Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, Oakley S, O'Connor L, Finney J, Vaughan A, Crook DW, Wilcox MH, Peto TE**. 2013. Relationship between bacterial strain type, host biomarkers, and mortality in Clostridium difficile infection. Clin Infect Dis. **56**:1589-1600.

23. **Slimings C, Riley TV**. 2014. Antibiotics and hospital-acquired Clostridium difficile infection: update of systematic review and meta-analysis. J Antimicrob Chemother. **69**:881-891.

24. **Furuya-Kanamori L, Stone JC, Clark J, McKenzie SJ, Yakob L, Paterson DL, Riley TV, Doi SA, Clements AC**. 2015. Comorbidities, Exposure to Medications, and the Risk of Community-Acquired Clostridium difficile Infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol. **36**:132-141.

25. **Dial S, Delaney JC, Barkun AN, Suissa S**. 2005. USe of gastric acid–suppressive agents and the risk of community-acquired Clostridium difficile–associated disease. JAMA. **294**:2989-2995.

26. **Itskowitz MS, Lebovitz PJ**. 2003. Non-antibiotic associated pseudomembranous colitis: a case report and review of the literature. Adv Stud Med. **3**:571-574.

27. **Chen Y, Glass K, Liu B, Riley T, Korda R, Kirk M**. 2016. A population-based longitudinal study of Clostridium difficile infection-related hospitalization in mid-age and older Australians. Epidemiol Infect. [In press].

28. **Aronsson B, Mollby R, Nord CE**. 1982. Clostridium difficile and antibiotic associated diarrhoea in Sweden. Scand J Infect Dis Suppl. **35**:53-58.

29. **Chitnis AS, Holzbauer SM, Belflower RM, Winston LG, Bamberg WM, Lyons C, Farley MM, Dumyati GK, Wilson LE, Beldavs ZG, Dunn JR, Gould LH, MacCannell DR, Gerding DN, McDonald LC, Lessa FC**. 2013. Epidemiology of

community-associated Clostridium difficile infection, 2009 through 2011. JAMA Intern Med. **173**:1359-1367.

30. **Barlam TF, Morgan JR, Wetzler LM, Christiansen CL, Drainoni ML**. 2015. Antibiotics for respiratory tract infections: a comparison of prescribing in an outpatient setting. Infect Control Hosp Epidemiol. **36**:153-159.

31. **Sun C, Jew S, Dasta SL**. 2006. Osteopathic physicians in the United States: antibiotic prescribing practices for patients with nonspecific upper respiratory tract infections. J Am Osteopath Assoc. **106**:450-455.

32. **Milligan RA, Burke V, Beilin LJ, Dunbar DL, Spencer MJ, Balde E, Gracey MP**. 1998. Influence of gender and socio-economic status on dietary patterns and nutrient intakes in 18-year-old Australians. Aust N Z J Public Health. **22**:485-493.

33. **Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN**. 2008. A case-control study of community-associated Clostridium difficile infection. J Antimicrob Chemother. **62**:388-396.

34. **Metcalf DS, Costa MC, Dew WM, Weese JS**. 2010. Clostridium difficile in vegetables, Canada. Lett Appl Microbiol. **51**:600-602.

35. **Marwah MB, Derek JB, John PB, Alistair DS**. 2009. Clostridium difficile in Ready-to-Eat Salads, Scotland. Emerg Infect Dis. **15**:817.

36. **Knight DR, Thean S, Putsathit P, Fenwick S, Riley TV**. 2013. Cross-Sectional Study Reveals High Prevalence of Clostridium difficile Non-PCR Ribotype 078 Strains in Australian Veal Calves at Slaughter. Appl Environ Microbiol. **79**:2630-2635.

37. **Knight DR, Squire MM, Riley TV**. 2015. Nationwide surveillance study of Clostridium difficile in Australian neonatal pigs shows high prevalence and heterogeneity of PCR ribotypes. Appl Environ Microbiol. **81**:119-123.

Journal of Clinical Microbiology

455 38. **Rodemann JF, Dubberke ER, Reske KA, Seo da H, Stone CD**. 2007. Incidence of
456 Clostridium difficile infection in inflammatory bowel disease. Clin Gastroenterol Hepatol.
457 **5**:339-344.
458 39. **Wojciechowski AL, Parameswaran GI, Mattappallil A, Mergenhagen KA**. 2014.
459 Corticosteroid use is associated with a reduced incidence of Clostridium difficile-associated
460 diarrhea: a retrospective cohort study. Anaerobe. **30**:27-29.
461

462 **Table 1.** Frequency distribution of *C. difficile* toxin profiles by source.

Toxin profile	Symptomatic HA-CDI (n= 96)*	CA-CDI (n=152)*	Asymptomatic TCDc (n=76)
A+B+CDT+	4 (4.2%)	7 (4.6%)	3 (4.0%)
A+B+CDT-	83 (86.5%)	139 (91.4%)	71 (93.4%)
A-B+CDT+	1 (1.0%)	2 (1.3%)	0 (0.0%)
A-B+CDT-	1 (1.0%)	1 (0.7%)	1 (1.3%)
A-B-CDT+	0 (0.0%)	0 (0.0%)	1 (1.3%)

HA healthcare-associated; CA community-associated; CDI *C. difficile* infection; TCDc toxigenic *C. difficile* colonisation

*Non-toxigenic (A-B-CDT-) *C. difficile* was isolated from seven HA-CDI and three CA-CDI patients.

463

464 **Table 2.** Patients' characteristics and healthcare, medication and environmental exposure prior to enrolment.

	Symptomatic HA-CDI (n= 96)	CA-CDI (n=152)	Asymptomatic TCDc (n=76)	p-value[1]	p-value[2]	p-value[3]
Female sex	50 (52.1%)	112 (73.7%)	40 (52.6%)	<0.001	0.943	<0.001
Age in years, median (IQR)	61.7 (49.2-75.0)	66.4 (49.1-75.4)	66.2 (54.8-76.8)	0.765	0.317	0.607
Healthcare exposure 12 months prior to enrolment						
Admitted to a hospital	62 (69.7%)	105 (69.1%)	47 (64.4%)	0.924	0.476	0.729
Number of admissions, median (SD)	2.1 (2.2)	1.5 (1.6)	2.0 (2.6)	0.128	0.328	0.323
LOS in the last admission, median (IQR)	7 (4-16)	6 (3-10)	6 (3-9)	0.191	0.140	0.215
Medication exposure 30 days prior to enrolment						
Antimicrobials	83 (86.5%)	117 (77.0%)	51 (69.9%)	0.066	0.008	0.031
Gastric acid suppressants	52 (54.7%)	34 (22.4%)	29 (40.3%)	<0.001	0.64	<0.001
Laxatives	28 (29.2%)	17 (14.2%)	29 (51.8%)	0.007	0.005	<0.001
Household exposure prior to enrolment						
People <2 years	3 (3.1%)	6 (4.0%)	4 (5.3%)	1.000	0.365	0.817
People >65 years	24 (25.3%)	52 (34.2%)	22 (29.3%)	0.138	0.553	0.322
Cats	21 (21.9%)	23 (15.1%)	12 (15.8%)	0.176	0.314	0.363
Dogs	30 (31.3%)	63 (41.5%)	28 (36.8%)	0.106	0.441	0.269
Livestock	8 (8.3%)	15 (9.9%)	7 (9.2%)	0.685	0.840	0.921
Smoking status						
Current	8 (8.3%)	10 (6.6%)	7 (9.3%)	0.604	0.819	0.740
Ever	52 (54.2%)	61 (40.1%)	43 (58.1%)	0.031	0.608	0.016
History of CDI in the last year	10 (10.4%)	15 (10.0%)	0 (0.0%)	0.916	0.003	<0.001

HA healthcare-associated; CA community-associated; CDI *C. difficile* infection; TCDc toxigenic *C. difficile* colonisation; NTCD non-toxigenic *C. difficile* colonisation; IQR interquartile range; SD standard deviation; LOS length of stay.

p-value[1] for HA- and CA-CDI; p-value[2] for HA-CDI and TCD; p-value[3] for HA-, CA-CDI, and TCDc comparison.

Table 3. Reason for admission, procedures, comorbidities and medication exposure during admission but prior to specimen collection among patients with healthcare-associated *C. difficile* infection and asymptomatic toxigenic *C. difficile* colonisation.

	Symptomatic HA-CDI (n= 96)	Asymptomatic TCDc (n=76)	p-value
Reason for admission			
New medical/surgical problem	25 (28.1%)	35 (47.3%)	0.022
Exacerbation of chronic condition	25 (28.1%)	19 (25.7%)	
Infection	31 (34.8%)	12 (16.2%)	
Elective surgery	8 (9.0%)	8 (10.8%)	
Medical procedures			
Insertion of orogastric tubes	8 (8.3%)	8 (10.8%)	0.680
Gastroscopy	13 (13.5%)	4 (5.4%)	0.049
Colonoscopy	11 (11.5%)	1 (1.4%)	0.006
Mechanical ventilation‡	2 (2.1%)	10 (13.5%)	0.006
Surgical procedures			
Orthopaedic	7 (7.3%)	19 (25.0%)	<0.001
Abdominal	12 (12.5%)	6 (7.9%)	0.327
Cardiological/Thoracic	2 (2.1%)	4 (5.3%)	0.238
Neurological	0 (0.0%)	11 (14.5%)	<0.001
Oncological	5 (5.2%)	0 (0.0%)	0.052
Other surgical procedures	2 (2.1%)	5 (6.6%)	0.138
Medical conditions			
Cancer	42 (43.8%)	22 (29.7%)	0.061
Diabetes mellitus	21 (21.9%)	18 (24.3%)	0.706
Neurological disorder	17 (17.7%)	23 (31.1%)	0.042
Gastro oesophageal reflux disease	26 (27.1%)	24 (32.4%)	0.448
Chronic obstructive pulmonary disease	10 (10.4%)	17 (23.0%)	0.026
Chronic kidney disease	22 (22.9%)	14 (18.9%)	0.527
Congestive heart failure	11 (11.5%)	12 (16.2%)	0.369
Liver disease	10 (10.4%)	4 (5.4%)	0.274
Inflammatory bowel disease	16 (16.7%)	3 (4.1%)	0.008
Diverticulosis	9 (9.4%)	2 (2.7%)	0.072
Solid organ transplant	7 (7.3%)	1 (1.4%)	0.069
Medication exposure			
Any antimicrobial¶	71 (74.0%)	59 (77.6%)	0.578
Penicillins and β-lactamase inhibitors	45 (46.9%)	21 (27.6%)	0.010
Cephalosporins	29 (30.2%)	34 (44.7%)	0.050
Penicillins	11 (11.5%)	12 (15.8%)	0.407
Trimethoprim/sulfamethoxazole	11 (11.5%)	6 (7.9%)	0.437
Carbapenems	11 (11.5%)	6 (7.9%)	0.437
Ciprofloxacin	9 (9.4%)	5 (6.6%)	0.354
Aminoglycosides	8 (8.3%)	5 (6.6%)	0.448
Fluoroquinolones†	1 (1.0%)	3 (4.0%)	0.228
Clindamycin	1 (1.0%)	4 (5.3%)	0.120
Tetracyclines	1 (1.0%)	0 (0.0%)	0.442
Macrolides	0 (0.0%)	3 (4.0%)	0.084
Metronidazole	17 (17.7%)	7 (9.2%)	0.110
Vancomycin	7 (7.3%)	6 (7.9%)	0.882

Journal of Clinical Microbiology

470 **Table 3.** Reason for admission, procedures, comorbidities and medication exposure during
471 admission but prior to specimen collection among patients with healthcare-associated *C.*
472 *difficile* infection and asymptomatic toxigenic *C. difficile* colonisation (continued).
473

	Symptomatic HA-CDI (n= 96)	Asymptomatic TCDc (n=76)	p-value
Gastric acid-suppressive agents	59 (61.5%)	41 (54.0%)	0.321
Proton pump inhibitors	57 (59.4%)	37 (48.7%)	0.162
H2 blocker	4 (4.2%)	5 (6.6%)	0.480
Laxatives	28 (29.2%)	34 (45.3%)	0.029
Nonsteroidal anti-inflammatory drugs	18 (18.8%)	13 (17.1%)	0.780
Glucocorticoids	35 (36.5%)	18 (23.7%)	0.072
Chemotherapy	12 (12.5%)	2 (2.6%)	0.019
Antidiarrheal	12 (12.5%)	2 (2.6%)	0.019

HA-CDI healthcare-associated *C. difficile* infection; TCDc toxigenic *C. difficile* colonisation.

‡ Excludes mechanical ventilation during surgical procedures.
¶ Excludes metronidazole and vancomycin.
† Ciprofloxacin not included.

474

475 **Table 4.** Logistic regression for predictors of symptomatic healthcare-associated *C. difficile*
476 infection compared to asymptomatic toxigenic *C. difficile* colonisation.
477

	Univariate model OR (95% CI)	Multivariate model OR (95% CI)
Female	0.98 (0.53-1.79)	0.92 (0.45-1.85)
Age (per decade)	0.91 (0.76-1.09)	0.96 (0.78-1.19)
Admitted to a hospital in the past 12 months	1.27 (0.66-2.44)	1.05 (0.48-2.27)
Medication exposure 30 days prior to admission		
Antimicrobials	**2.78 (1.28-5.88)**	**2.94 (1.20-7.14)**
Gastric acid-suppressive agents	1.79 (0.96-3.33)	1.67 (0.76-3.57)
Medical conditions		
Cancer	1.85 (0.97-3.45)	1.15 (0.52-2.50)
Diabetes mellitus	0.87 (0.43-1.79)	0.72 (0.30-1.69)
Neurological disorder	**0.48 (0.23-0.98)**	0.50 (0.21-1.15)
Gastro oesophageal reflux disease	0.78 (0.40-1.49)	0.74 (0.33-1.64)
Chronic obstructive pulmonary disease	**0.39 (0.17-0.91)**	**0.31 (0.12-0.83)**
Chronic kidney disease	1.27 (0.60-2.70)	1.16 (0.47-2.86)
Congestive heart failure	0.67 (0.28-1.61)	1.03 (0.35-3.03)

OR odds ratio; CI confidence interval.
478

Figure 1 legend. Distribution of ribotypes by years among symptomatic healthcare- (HA) and community-associated (CA) *C. difficile* infection (CDI) and asymptomatic toxigenic *C. difficile* colonized (TCDc) patients. Ribotypes with a frequency of less than 3 isolates in a year were grouped into "Other".