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Original article

Asymptomatic *Clostridium difficile* colonization in two Australian tertiary hospitals, 2012–2014: prospective, repeated cross-sectional study

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ABSTRACT

Objectives: To investigate the prevalence and risk factors for asymptomatic toxigenic (TCD) and non-toxigenic *Clostridium difficile* (NTCD) colonization in a broad cross section of the general hospital population over a 3-year period.

Methods: Patients without diarrhoea admitted to two Australian tertiary hospitals were randomly selected through six repeated cross-sectional surveys conducted between 2012 and 2014. Stool specimens were cultured under anaerobic conditions, and *C. difficile* isolates were tested for the presence of toxin genes and ribotyped. Patients were then grouped into noncolonized, TCD colonized or NTCD colonized for identifying risk factors using multinomial logistic regression models.

Results: A total of 1380 asymptomatic patients were enrolled; 76 patients (5.5%) were TCD colonized and 28 (2.0%) were NTCD colonized. There was a decreasing annual trend in TCD colonization, and asymptomatic colonization was more prevalent during the summer than winter months. TCD colonization was associated with gastro-oesophageal reflux disease (relative risk ratio (RRR) = 2.20; 95% confidence interval (CI) 1.17–4.14), higher number of admissions in the previous year (RRR = 1.24; 95% CI 1.10–1.39) and antimicrobial exposure during the current admission (RRR = 2.78; 95% CI 1.23–6.28). NTCD colonization was associated with chronic obstructive pulmonary disease (RRR = 3.88; 95% CI 1.66–9.07) and chronic kidney failure (RRR = 5.78; 95% CI 2.29–14.59). Forty-eight different ribotypes were identified, with 014/020 (n = 23), 018 (n = 10) and 056 (n = 6) being the most commonly isolated.

Conclusions: Risk factors differ between patients with asymptomatic colonization by toxigenic and non-toxigenic strains. Given that morbidity is largely driven by toxigenic strains, this novel finding has important implications for disease control and prevention. **L. Furuya-Kanamori, CMI 2017;23:48.e1–48.e7**

Introduction

Clostridium difficile infection (CDI) is the main cause of healthcare-associated diarrhoea. Toxigenic *C. difficile* (TCD) strains produce toxins A and B, and, increasingly, binary toxin (CDT), which are responsible for the clinical presentation of CDI, ranging from mild diarrhoea to severe life-threatening conditions such as pseudomembranous colitis [1]. It is estimated that up to two-thirds of patients who are exposed to *C. difficile* remain asymptomatic [2]. There is growing evidence that asymptomatic patients colonized

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with TCD can act as a source of *C. difficile* transmission and environmental contamination in hospitals [3,4]. However, not all *C. difficile* strains produce toxins, and it has been proposed that asymptomatic patients colonized with nontoxigenic *C. difficile* (NTCD) strains are protected from colonization by heterologous strains, including toxigenic strains, as a result of niche competition or stimulation of the mucosal immune response in the gastrointestinal tract [5].

Limited evidence indicates that asymptomatic colonized patients may potentially play a role in transmission [6]. The associated host risk factors (e.g. sex, age, comorbidities and medication exposure) and pathogen characteristics (e.g. toxigenic profile and predominant ribotypes) among this group are poorly understood [7]. Few studies have investigated the prevalence of asymptomatic TCD and NTCD colonization in a broad cross section of the general hospital patient population [8], nor have the between-season variability or temporal trends of prevalence been reported.

Therefore, a 3-year study with biannual surveys in adult patients was conducted in two Australian tertiary-care hospitals in different Australian states with the following aims: to estimate the prevalence of asymptomatic *C. difficile* colonization; to compare the prevalence during summer and winter months and over time; to describe the predominant toxin profiles and ribotypes isolated from asymptomatic patients; and to identify host factors associated with TCD and NTCD colonization.

Materials and Methods

Study setting and participants

The study was conducted in two tertiary hospitals in Australia, the Royal Brisbane & Women's Hospital (RBWH), with 929 beds in Brisbane, Queensland, and the Sir Charles Gairdner Hospital (SCGH), with 607 beds in Perth, Western Australia. The patients were prospectively recruited through six repeated cross-sectional surveys conducted between 2012 and 2014. Each year, two surveys were conducted, one starting in late summer (February–March) and the other in late winter (August–September).

On the morning of each survey day, a sampling frame of currently admitted patients in the wards (i.e. medical, surgical, intensive care units) to be surveyed was created in a spreadsheet, with each patient given a unique ID. Patient IDs were drawn at random from the spreadsheet (using a random number generator). If the patient's ID was randomly selected, was 18 years of age or older and did not present diarrhoea (i.e. 3 or more loose or liquid bowel motions per day), the research nurse approached the patient and invited him or her to participate in the study.

The study 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 onto the study without any additional risk beyond their standard care.

Data collection

Patients were interviewed to obtain demographic data and information on known CDI risk factors (e.g. use of various medications before admission, history of CDI and hospital admissions). Patient medical records were reviewed to determine the date and the reason for the latest admission, recent history of diarrhoea, comorbid conditions, inpatient medication (e.g. antimicrobials, gastric acid suppressants, nonsteroidal anti-inflammatory drugs) and medical procedures (e.g. colonoscopy, surgery) during the admission.

If *C. difficile* was isolated from the patients' stool specimens, the patients were monitored while hospitalized and followed up after discharge on a monthly basis for 3 months. The follow-up interviews were used to determine the patients' clinical outcomes and whether they remained asymptomatic, were readmitted to a hospital, were diagnosed with CDI, developed colitis or died.

Specimen collection and processing

Specimens from the enrolled patients were obtained using a rectal swab from consenting patients. Stool specimens were obtained from patients who were enrolled and able to provide a stool specimen but who did not consent to provide a rectal swab.

Swabs were cultured for *C. difficile* within 30 minutes of collection and stool samples were cultured within 24 hours using our previously described methods [9], except that direct culture was performed on ChromID *C. difficile* agar (bioMérieux, Marcy l'Étoile, France) and plates were examined at 24 and 48 hours for characteristic growth. Broth enrichment in Robertson cooked meat medium containing 5 mg/L of gentamicin, 250 mg/L of cycloserine and 8 mg/L of cefoxitin was performed concurrently and ethanol shocked after 48 to 72 hours for subculture on ChromID agar if direct culture was negative. Putative *C. difficile* colonies were subcultured onto prereduced blood agar plates for identification by characteristic colony morphology and odour, chartreuse fluorescence under UV light and proline aminopeptidase production (Diatabs; Rosco Diagnostica, Taastrup, Denmark) at 48 hours. All agar plate incubations were performed at 35°C under anaerobic conditions.

C. difficile isolates were tested for the presence of toxin genes (*tcdA, tcdB* and *cdtA/cdtB*) and were polymerase chain reaction (PCR) ribotyped following previously described methods [9]. Strains that did not produce banding patterns matching an international ribotype in the reference collection were assigned local nomenclature (QX type).

Statistical analysis

All enrolled patients not experiencing diarrhoea who had *C. difficile* isolated from their stool were considered to have asymptomatic *C. difficile* colonization. If the strain isolated was positive for the presence of *tcdA, tcdB* or *cdtA/cdtB* genes, then the patient was considered asymptomatic TCD colonized; if the isolated strain was negative for all toxin genes, then the patient was considered asymptomatic NTCD colonized. Therefore, for the purpose of the analyses, patients were grouped into three categories according to their status with respect to *C. difficile* colonization at the time of enrolment: noncolonized, TCD colonized and NTCD colonized. The overall and specific survey prevalence of TCD and NTCD colonized patients were calculated.

Pearson's chi-square test and Fisher's exact test were used to compare categorical variables, and the Kruskal-Wallis *H* test was used to compare continuous variables across the three categories of *C. difficile* colonization. Univariate and multivariate multinomial logistic regression models were built with *C. difficile* colonization as the outcome and noncolonized patients as the reference category to identify predictors of TCD and NTCD colonized patients. After adjusting for age and sex of the patients, known risk factors for CDI (i.e. hospital admissions and exposure to antimicrobials), the inclusion of comorbidities and medication exposure during the current admission in the regression model were analysed through a stepwise forward selection with the Akaike information criterion as

the selection criterion. A significance level cutoff of p <0.05 was used for all analyses. All statistical analyses were conducted by Stata SE 14 (StataCorp, College Station, TX, USA).

Results

Prevalence of asymptomatic C. difficile colonization and seasonal variation

During the six surveys throughout the 3 years, 1380 patients were enrolled onto the study (595 and 785 patients from the RBWH and SCGH, respectively) (Supplementary Material 1). The median time between the patients being admitted to hospital and enrolment onto the study was 5 days (interquartile range 2–10 days), and 25% of the patients were enrolled within 48 hours of being admitted. There was no statistically significant difference in time between being admitted and enrolment for both hospitals and across the six surveys (Supplementary Material 2).

C. difficile was isolated from 104 patients (7.5%; 95% confidence interval (CI) 6.2–9.1). A higher prevalence of *C. difficile* colonization was observed at SCGH (9.8%; 95% CI 7.8–12.1) compared to RBWH (4.5%; 95% CI 3.0–6.5). Among the enrolled patients, 76 (5.5%; 95% CI 4.4–6.8) and 28 (2.0%; 95% CI 1.4–2.9) were colonized with TCD and NTCD strains, respectively. A higher prevalence of asymptomatic *C. difficile* colonization was observed during the summer surveys (8.8%; 95% CI 6.9–11.1) compared to the winter surveys (5.9%; 95% CI 4.2–8.1) (Fig. 1). The prevalence of asymptomatic *C. difficile* colonization was highest during the first survey (February–March 2012), when 33 out of 294 patients were colonized (11.2%; 95% CI 7.9–15.4), including 28 (9.5%; 95% CI 6.4–13.5) patients colonized with TCD strains. The lowest prevalence was observed during the fourth survey (August–September 2013); *C. difficile* was isolated from 14 (5.6%; 95% CI 3.1–9.2) patients among the 250 patients enrolled during that survey. The seasonal patterns were similar in both hospitals.

Characterization of C. difficile

Among the 104 *C. difficile* isolates, five toxin profiles were identified, with $A^+B^+CDT^-$ being the most common ($n = 71$, 68.3%). Three isolates (2.9%) were $A^+B^+CDT^+$, one (1.0%) was $A\text{-}B^+CDT^-$, one (1.0%) was $A^-B^-CDT^+$ and the remaining 28 isolates (26.9%) were $A^-B^-CDT^-$. Forty-eight different ribotypes were identified; the most common ribotype was the 014/020 group ($n = 23$, 22.1%), followed by 018 ($n = 10$, 9.6%), 056 ($n = 6$, 5.8%), 010 ($n = 5$, 4.8%) and 103 ($n = 5$, 4.8%). The four binary toxin–positive isolates were PCR ribotypes 063, 127, 251 and QX 220 (Fig. 2, Supplementary Material 3).

Predictors of toxigenic and nontoxigenic C. difficile colonization

The characteristics of patients enrolled onto the study are described in Table 1. There were no differences between non-colonized, TCD colonized and NTCD colonized patients in terms of sex proportion or mean age. Among the comorbidities, cancer prevalence was less common among NTCD colonized patients (7.1% vs. 34.7% (noncolonized) vs. 29.7% (TCD)). Gastro-oesophageal reflux disease and congestive heart failure were more prevalent among TCD colonized patients, while chronic obstructive pulmonary disease and chronic kidney disease were more prevalent among NTCD colonized patients. Five (0.4%), three (4.0%) and two (7.1%) noncolonized, TCD colonized and NTCD colonized patients, respectively, reported having a history of CDI. With regards to healthcare exposure, 64% of TCD and NTCD colonized patients had been admitted to hospital at least once in the previous year compared to 46% of noncolonized patients.

The reasons for the current admission did not significantly differ between the three *C. difficile* colonization categories (Table 2). Exposure to antimicrobials during the admission was common among all the patients; however, it was significantly higher in TCD (83.8%) and NTCD colonized patients (78.6%) compared to non-colonized patients (66.4%; p 0.004). There were no differences in other medication exposure (gastric acid suppressants, aperients, nonsteroidal anti-inflammatory drugs, glucocorticoids, chemotherapy or antidiarrhoeals) or medical procedures (insertion of orogastric tube, gastroscopy, colonoscopy or mechanical ventilation) during the admission across the colonization categories. In terms of surgical procedures, a significantly higher proportion of TCD colonized patients underwent orthopaedic (25.0%) and neurologic (14.5%) surgeries compared to noncolonized patients (12.9% orthopaedic and 5.6% neurologic) and NTCD colonized patients (10.7% orthopaedic and 3.6% neurologic) (p 0.016 and 0.013, respectively).

In the multivariate multinomial logistic regression model, factors associated with an increased relative risk ratio (RRR) of harbouring a TCD strain compared to noncolonized included having gastro-oesophageal reflux disease (RRR 2.20; 95% CI 1.17–4.14), number of hospital admissions in the previous year (RRR 1.24; 95% CI 1.10–1.39), exposure to antimicrobials during the period of

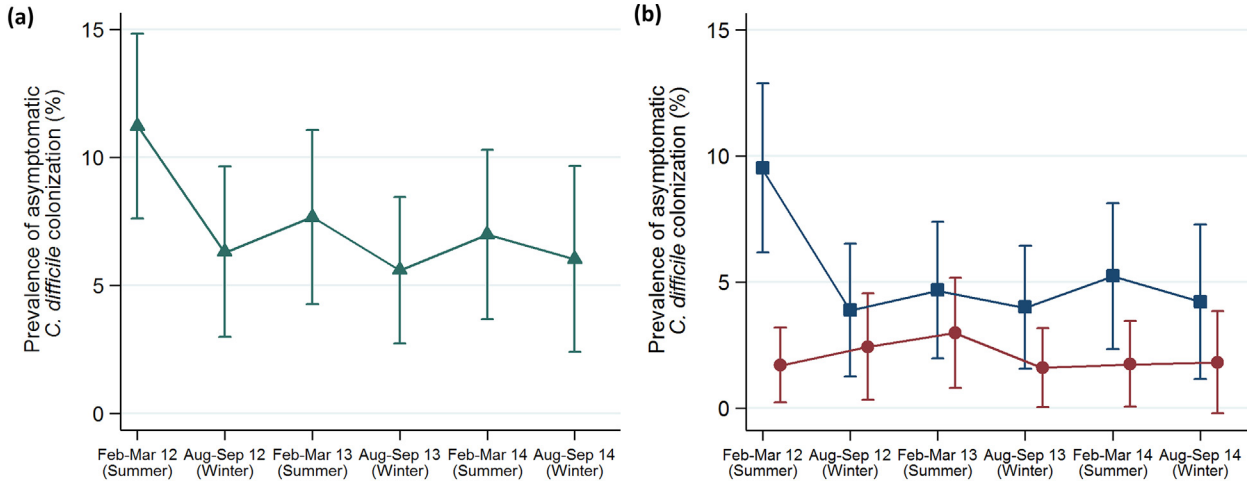

Fig. 1. (a) Seasonal variation of *Clostridium difficile* colonization prevalence and (b) variation by toxigenic profile. Green triangles, blue squares and red circles represent prevalence of overall, toxigenic and nontoxigenic *C. difficile* colonization, respectively. Vertical lines represent 95% confidence interval around prevalence estimates.

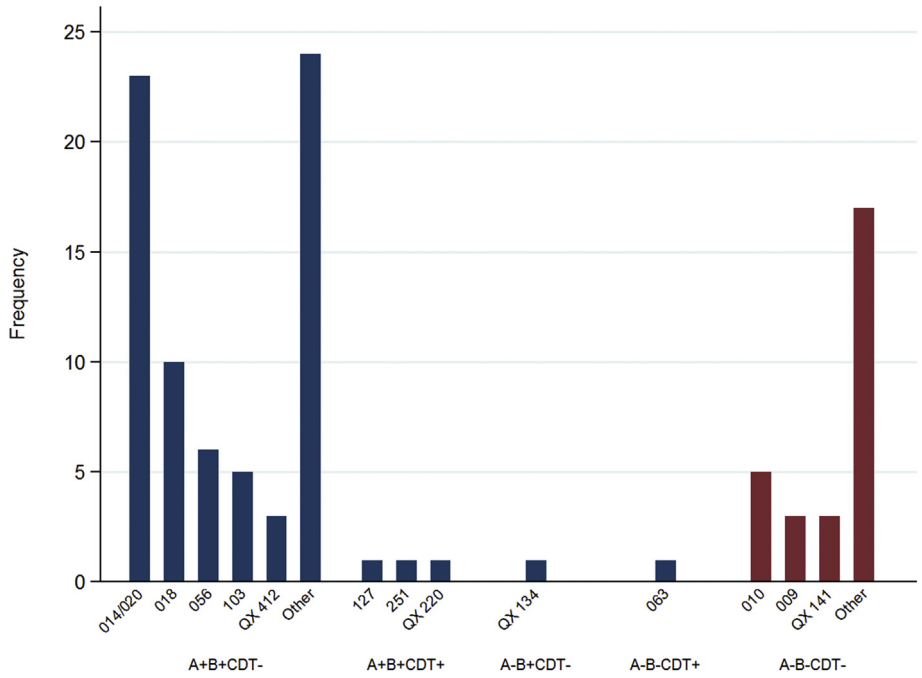

Fig. 2. Distribution of ribotypes among *Clostridium difficile* colonized patients. Blue and red bars represent frequency of toxigenic and nontoxigenic *C. difficile* strains isolated in study, respectively. Ribotypes with toxin profile of $A^{+}B^{+}CDT^{-}$ and $A^{-}B^{-}CDT^{-}$ and frequency of two or fewer were grouped into "other."

admission (RRR 2.78; 95% CI 1.23–6.28) and admission during the summer months (RRR 1.81; 95% CI 1.07–3.06) (Table 3). The regression model also revealed a decreasing annual trend in TCD colonization prevalence (RRR 0.68; 95% CI 0.47–0.97). For harbouring a NTCD strain relative to noncolonized, having chronic obstructive pulmonary disease (RRR 3.88; 95% CI 1.66–9.07) and chronic kidney failure (RRR 5.78; 95% CI 2.29–14.59) were associated with an increased RRR.

Over the 3-month follow-up, five colonized patients (4 (5.3%) TCD and 1 (3.6%) NTCD) reported developing CDI, and there were

Table 1
Patient characteristics

Characteristic	Noncolonized ($n = 1276$)	Toxigenic *Clostridium difficile* ($n = 76$)	Nontoxigenic *Clostridium difficile* ($n = 28$)	p[a]
Female sex	600 (47.0%)	40 (52.6%)	13 (46.4%)	0.633
Age, years, mean (SD)	61.8 (17.4)	64.1 (16.1)	64.3 (20.96)	0.414
Medical condition				
Cancer	441 (34.7%)	22 (29.7%)	2 (7.1%)	0.003
Diabetes mellitus	297 (23.4%)	18 (24.3%)	8 (28.6%)	0.806
Neurologic disorder	283 (22.3%)	23 (31.1%)	8 (28.6%)	0.165
GORD	256 (20.1%)	24 (32.4%)	7 (25.0%)	0.035
COPD	218 (17.2%)	17 (23.0%)	11 (38.3%)	0.005
Chronic kidney disease	107 (8.4%)	14 (18.9%)	9 (32.1%)	<0.001
Congestive heart failure	98 (7.7%)	12 (16.2%)	3 (10.7%)	0.029
Liver disease	90 (7.1%)	4 (5.4%)	2 (7.1%)	0.896
Inflammatory bowel disease	53 (4.2%)	3 (4.1%)	2 (7.1%)	0.578
Pregnancy	24 (1.9%)	0 (0.0%)	1 (3.6%)	0.384
Solid organ transplant	21 (1.7%)	1 (1.4%)	2 (7.1%)	0.118
HIV	5 (0.4%)	0 (0.0%)	0 (0.0%)	1.000
Smoking status				
Current	171 (13.4%)	7 (9.3%)	1 (3.6%)	0.218
Ever	754 (59.2%)	43 (58.1%)	13 (46.4%)	0.391
History of CDI (ever)	5 (0.4%)	3 (4.0%)	2 (7.1%)	<0.001
History of CDI in the last year	1 (0.1%)	0 (0.0%)	2 (7.1%)	0.002
Healthcare exposure 12 months before admission				
Admitted to hospital	584 (46.4%)	47 (64.4%)	18 (64.3%)	0.002
No. of admissions, median (IQR)	0 (0–2)	1 (0–3)	2 (0–3)	<0.001
LOS in the last admission, median (IQR)	4 (1–9)	6 (3–9)	8 (3–17)	0.997
Medication exposure 30 days before admission				
Antimicrobials	770 (63.4%)	51 (69.9%)	17 (60.7%)	0.506
Gastric acid suppressants	550 (44.5%)	29 (40.3%)	11 (39.3%)	0.685
Aperients	479 (43.4%)	29 (51.8%)	12 (46.2%)	0.453

CDI, *Clostridium difficile* infection; COPD, chronic obstructive pulmonary disease; GORD, gastro-oesophageal reflux disease; IQR, interquartile range; LOS, length of stay.
[a] p for comparison across noncolonized, toxigenic *C. difficile* and nontoxigenic *C. difficile*.

Table 2
Medication exposure and procedures during admission

Characteristic	Noncolonized ($n = 1276$)	Toxigenic *Clostridium difficile* ($n = 76$)	Nontoxigenic *Clostridium difficile* ($n = 28$)	p[a]
Reason for admission				
New medical/surgical problem	460 (36.8%)	35 (47.3%)	11 (39.3%)	0.175
Exacerbation of chronic condition	392 (31.4%)	19 (25.7%)	6 (21.4%)	
Infection	208 (16.7%)	12 (16.2%)	10 (35.7%)	
Elective surgery	171 (13.7%)	8 (10.8%)	1 (3.6%)	
Obstetric condition	18 (1.4%)	0 (0.0%)	0 (0.0%)	
Current length of stay, days, median (IQR)[b]	5 (2–10)	7 (4–17)	4 (2–8)	0.974
Medication exposure				
Any antimicrobial	836 (66.4%)	62 (83.8%)	22 (78.6%)	0.004
Cephalosporins	416 (32.6%)	34 (44.7%)	9 (32.1%)	0.092
Penicillins and β-lactamase inhibitors	377 (29.6%)	21 (27.6%)	13 (46.4%)	0.141
Penicillins	186 (14.6%)	12 (15.8%)	3 (10.7%)	0.866
Vancomycin	117 (9.2%)	12 (15.8%)	4 (14.3%)	0.095
Metronidazole	106 (8.3%)	16 (21.1%)	5 (17.9%)	<0.001
Macrolides	95 (7.5%)	3 (4.0%)	4 (14.3%)	0.174
Trimethoprim/sulfamethoxazole	75 (5.9%)	6 (7.9%)	3 (10.7%)	0.287
Ciprofloxacin	75 (5.9%)	5 (6.6%)	2 (7.1%)	0.770
Aminoglycosides	55 (4.3%)	5 (6.6%)	2 (7.1%)	0.340
Carbapenems	44 (3.5%)	6 (7.9%)	1 (3.6%)	0.114
Fluoroquinolones[c]	32 (2.5%)	3 (4.0%)	1 (3.6%)	0.448
Clindamycin	29 (2.3%)	4 (5.3%)	0 (0.0%)	0.233
Tetracyclines	22 (1.7%)	0 (0.0%)	2 (7.1%)	0.093
Other antimicrobials	33 (2.6%)	2 (2.6%)	0 (0.0%)	1.000
Gastric acid suppressants	686 (54.4%)	50 (67.6%)	16 (57.1%)	0.086
Proton pump inhibitors	643 (51.0%)	46 (62.2%)	16 (57.1%)	0.150
H2 blocker	75 (5.9%)	6 (7.9%)	1 (3.6%)	0.672
Aperients	590 (46.8%)	45 (60.8%)	15 (53.6%)	0.202
NSAIDs	382 (30.4%)	19 (26.0%)	12 (42.9%)	0.593
Glucocorticoids	331 (26.3%)	23 (31.1%)	7 (25.0%)	0.654
Chemotherapy	85 (6.8%)	2 (2.7%)	1 (3.6%)	0.406
Antidiarrhoeal	29 (2.3%)	3 (4.1%)	3 (10.7%)	0.080
Medical procedures				
Insertion of orogastric tubes	124 (9.8%)	8 (10.8%)	2 (7.1%)	0.885
Gastroscopy	81 (6.4%)	4 (5.4%)	2 (7.1%)	0.886
Colonoscopy	40 (3.2%)	1 (1.4%)	0 (0.0%)	0.780
Mechanical ventilation[d]	86 (6.8%)	10 (13.5%)	1 (3.6%)	0.158
Surgical procedures				
Orthopaedic	165 (12.9%)	19 (25.0%)	3 (10.7%)	0.016
Abdominal	137 (10.7%)	6 (7.9%)	1 (3.6%)	0.480
Cardiologic/thoracic	120 (9.4%)	4 (5.3%)	2 (7.1%)	0.499
Neurologic	72 (5.6%)	11 (14.5%)	1 (3.6%)	0.013
Oncologic	36 (2.8%)	0 (0.0%)	0 (0.0%)	0.381
Other surgical procedures	121 (9.5%)	5 (6.6%)	1 (3.6%)	0.571

IQR, interquartile range; NSAID, nonsteroidal anti-inflammatory drug.
[a] p for comparison across noncolonized, toxigenic *C. difficile* and nontoxigenic *C. difficile*.
[b] Time between admission and patient enrolment.
[c] Ciprofloxacin not included.
[d] Excludes mechanical ventilation during surgical procedures.

five deaths (4 (5.3%) TCD and 1 (3.6%) NTCD) not related to CDI. Given the small number of events, no statistical analysis was possible to compare the clinical outcomes of TCD and NTCD strains.

Discussion

The current study identified an asymptomatic *C. difficile* colonization prevalence of 7.5% across all hospital care wards, which was significantly lower than estimates recently reported in the United Kingdom (11%) [10] and the United States (21%) [11]. Likewise, the TCD colonization prevalence (5.5%) was lower compared to the pooled prevalence reported in a meta-analysis by Zacharioudakis *et al.* [8] (8.1% (95% CI 5.7–11.1) worldwide and 10.0% (95% CI 7.1–13.4) in North America). The prevalence of NTCD colonized patients in our study (2.0%) was significantly lower than that reported by Alasmari *et al.* (5.8%) [11], yet the ratios between nontoxigenic and toxigenic strains were similar in both studies (1:2.7). Inpatient hospital transfer has been identified as an important vehicle of *C. difficile* (symptomatic and asymptomatic) spread [12,13]. Given that hospital transfers in Australia mainly occur within a circumscribed health service area (http://www0.health.nsw.gov.au/policies/pd/2011/pdf/PD2011_031.pdf), the spread of any infectious disease may be limited and may contribute to the observed low prevalence of *C. difficile* colonization in our study. Australia's low population density might also contribute to less intense transmission in the community [14].

Notably, findings from this study conducted in two Australian cities located in a temperate climate zone suggest that asymptomatic *C. difficile* colonization has decreased from 2012 to 2014. In addition, it was noted that asymptomatic *C. difficile* colonization and symptomatic CDI displayed a synchronous seasonal trend, with higher prevalence during summer compared to winter months [15–19]. Understanding asymptomatic *C. difficile* seasonality is important because well-timed preventive and control measures targeting patients at high risk of asymptomatic colonization can be put in place to reduce transmission and emergence of new CDI cases.

Forty-eight different ribotypes were identified among the 104 asymptomatically colonized patients. Similar to the findings of

Table 3
Multinomial logistic regression models for predictors of toxigenic and nontoxigenic *Clostridium difficile* colonization

Characteristic	Toxigenic *C. difficile*		Nontoxigenic *C. difficile*	
	Univariate model, RRR (95% CI)	Multivariate model, RRR (95% CI)	Univariate model, RRR (95% CI)	Multivariate model, RRR (95% CI)
Female	1.25 (0.79–1.99)	1.33 (0.76–2.33)	0.98 (0.46–2.07)	0.82 (0.37–1.82)
Age (per decade)	1.08 (0.94–1.24)	1.07 (0.90–1.28)	1.09 (0.87–1.34)	0.99 (0.78–1.25)
Medical conditions				
Diabetes mellitus	1.06 (0.61–1.83)	1.26 (0.67–2.36)	1.31 (0.57–3.02)	0.96 (0.39–2.36)
Neurologic disorder	1.57 (0.95–2.62)	1.73 (0.94–3.17)	1.40 (0.61–3.20)	1.19 (0.49–2.87)
GORD	1.90 (1.15–3.16)	2.20 (1.17–4.14)	1.32 (0.56–3.14)	1.43 (0.54–3.73)
COPD	1.44 (0.82–2.52)	0.87 (0.42–1.80)	3.13 (1.44–6.77)	3.88 (1.66–9.07)
Chronic kidney disease	2.54 (1.37–4.69)	1.77 (0.83–3.75)	5.15 (2.28–11.67)	5.78 (2.29–14.59)
No. of admissions in year	1.25 (1.13–1.38)	1.24 (1.10–1.39)	1.24 (1.06–1.44)	1.14 (0.96–1.36)
Antimicrobial exposure 30 days before admission	1.34 (0.80–2.24)	0.95 (0.50–1.81)	0.90 (0.42–1.93)	0.60 (0.25–1.46)
Length of stay during current admission	0.99 (0.98–1.01)	1.00 (0.97–1.02)	0.99 (0.98–1.01)	1.00 (0.99–1.02)
Medications during admission				
Antimicrobials	2.62 (1.40–4.92)	2.78 (1.23–6.28)	1.86 (0.75–4.62)	2.40 (0.88–6.61)
Proton pump inhibitors	1.58 (0.97–2.55)	0.92 (0.50–1.72)	1.28 (0.60–2.73)	0.76 (0.31–1.82)
H2 blocker	1.39 (0.59–3.32)	1.14 (0.33–3.93)	0.58 (0.08–4.37)	0.70 (0.09–5.63)
Glucocorticoids	1.26 (0.76–2.10)	1.48 (0.82–2.66)	0.93 (0.39–2.22)	0.76 (0.30–1.93)
Year	0.78 (0.58–1.05)	0.68 (0.47–0.97)	0.94 (0.59–1.50)	0.84 (0.52–1.37)
Season—summer	1.73 (1.06–2.82)	1.81 (1.07–3.06)	1.13 (0.53–2.41)	1.25 (0.57–2.76)

CI, confidence interval; COPD, chronic obstructive pulmonary disease; GORD, gastro-oesophageal reflux disease; RRR, relative risk ratio.

Alasmari *et al.* [11] in the United States, our study found that the 014/020 group was the most common ribotype among asymptomatically colonized patients. However, none of the other ribotypes reported by Alasmari *et al.* (012, 053, 077 and 027) was identified among the colonized patients in Australia. The diversity of ribotypes identified in our study corresponds with surveillance studies among symptomatic CDI cases in hospitals in Queensland [20] and Western Australia [9]. Furthermore, the predominant ribotypes among symptomatic patients (014/020 group and 056) in the surveillance studies matches our findings in asymptomatic patients. These findings suggest that patients colonized with *C. difficile* may act as a source of transmission in the hospital for new CDI cases [3,4,21].

Our study corroborates data reporting that recent hospital admission increases the risk of TCD [2,4,8,22–24]. For each admission to a hospital in the previous 12 months, we found that the risk of TCD colonization increased by 24%. Gastro-oesophageal reflux disease was also associated with TCD; conversely, exposure to proton pump inhibitors (PPIs) during the admission was not a significant factor. Interestingly, medication exposure as a risk factor for TCD colonization remains uncertain. Our findings align with those reported by Kong *et al.* [23], who found no association between PPIs and TCD colonization; however, other studies have identified exposure to PPIs as a risk factor for asymptomatic *C. difficile* colonization [24,25]. Likewise, exposure to antimicrobials during the admission was associated with an increased risk of TCD, while previous studies found that TCD was instead associated with immunosuppressant use [23,24].

With regards to colonization by NTCD, a positive association was observed with chronic obstructive pulmonary disease (but not with smoking history). Chronic renal disease has been previously reported as a risk factor for TCD colonization [22,24], yet from our study findings, chronic renal disease was only associated with NTCD colonization. There is evidence that suggests that colonization with NTCD is protective against infection with TCD strains; hence, it is important to identify this group of patients and prevent the disruption of their "naturally" protected gut microbiome against TCD strains through the use of antimicrobials. Faecal microbiota transplantation has proven to be a highly effective therapeutic alternative for recurrent CDI; thus, future studies need to investigate the potential additional benefits of NTCD colonized donors compared to noncolonized donors.

Screening all inpatients without symptoms of diarrhoea for *C. difficile* will not be a cost-effective disease control measure; thus, by understanding the risk factors, resources could be allocated to those patients who are at high risk of being colonized by a TCD strain. Now that Longtin *et al.* [26] have reported that infection control measures (i.e. isolation precautions and environmental control) targeting asymptomatic TCD colonized patients significantly reduces the incidence of healthcare-associated CDI, identification of risk factors becomes crucial for screening patients at high risk of TCD colonization and allocating resources to reduce CDI transmission in the hospitals.

A striking finding of this study was that TCD and NTCD colonized patients did not share risk factors. This finding may suggest that colonization by TCD and NTCD strains are two different conditions. TCD colonization is likely more closely related to symptomatic CDI than NTCD colonization, given the fact that TCD colonized patients and CDI patients (and not NTCD colonized patients) share exposure to antimicrobials as their main risk factor.

We acknowledge that the study is limited by a number of factors. First, given the small number of events (new CDI cases and deaths) recorded during the follow-up period, it was not possible to elucidate patient and strain characteristics associated with clinical outcomes. Second, the majority of the specimens were collected using rectal swabs (84.3%). The positivity rate with rectal swabs was lower (6.79%) than with stool samples (11.52%), which could have influenced the low prevalence of asymptomatic *C. difficile* colonization identified in this study. However, collection of stool specimens was less convenient and less appealing to patients and would have negatively affected recruitment. Of further note is the fact that rectal swabs were guaranteed to be collected, as they were taken at the time of recruitment. Finally, the study was not designed to capture when a patient was exposed to *C. difficile;* thus, our study population may contain patients that acquired *C. difficile* in the community or in the hospital. Future studies need to investigate if community- and healthcare-associated asymptomatic colonized patients have different epidemiologic profiles as has been reported for symptomatic community- and healthcare-associated CDI cases.

One major strength of the current study was the number of enrolled patients. This is the first study with a sufficient sample size to determine independent (adjusted) risk factors separately for asymptomatic TCD and NTCD colonization. Additionally, given the long study period, ours is the first study to report seasonal differences in asymptomatic carriage over multiple years. Finally, it examined not only factors associated with asymptomatic colonization before hospital admission but also included factors to which patients were exposed during the admission, such as medical procedures (e.g. insertion of nasogastric tubes), surgical procedures and a detailed record of medication exposure, as well as after hospital discharge.

In conclusion, our study found a lower prevalence of asymptomatic TCD and NTCD colonized patients compared to previous studies elsewhere. It also found that risk factors for TCD and NTCD colonization were distinct from each other and that the prevalence of asymptomatic carriage was seasonal, indicating that carriage in the population is dynamic. Additional research is required to elucidate if current international guideline recommendations of not routinely screening and not providing treatment to asymptomatic colonized patients are still the best approach.

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Transparency Declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.08.030.

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