Epidemiology of *Clostridium difficile* and vancomycin-resistant *Enterococcus* colonization in patients on a spinal cord injury unit

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Background/objective: Patients with spinal cord injury (SCI) are at risk of acquiring colonization with *Clostridium difficile* and vancomycin-resistant *Enterococcus* (VRE) due to prolonged hospitalization and frequent antimicrobial use. We examined the frequency of stool, skin, and environmental contamination with *C. difficile* and VRE in hospitalized patients with SCI.

Methods: We performed a cross-sectional study of 22 hospitalized patients with SCI with no symptoms of *C. difficile* infection. Stool samples, skin, and environmental sites were cultured for *C. difficile* and VRE, and polymerase chain reaction ribotyping was performed for *C. difficile* isolates. Fisher's exact test was used to compare the proportions of skin and environmental contamination among stool carriers and non-carriers. Univariate analysis was used to assess factors associated with asymptomatic carriage of *C. difficile*.

Results: Of 22 asymptomatic patients, 11 (50%) were asymptomatic carriers of toxigenic *C. difficile* and 12 (55%) were carriers of VRE. In comparison with non-carriers, asymptomatic carriers of toxigenic *C. difficile* had higher rates of skin (45 versus 9%) ($P=0.07$) and environmental contamination (55 versus 9%) ($P=0.03$) and longer length of stay (median, 57 versus 6 days; $P=0.04$). A majority of skin and environmental *C. difficile* isolates from individuals were identical to isolates from stool. In comparison with non-carriers, patients with VRE stool colonization had non-significant trends toward more frequent skin (27 versus 9%) and environmental (18 versus 9%) contamination.

Conclusion: Asymptomatic stool carriage of toxigenic *C. difficile* and VRE was common on an acute-care SCI unit. Asymptomatic carriers of toxigenic *C. difficile* had frequent skin and environmental contamination, suggesting the potential to contribute to transmission.

Keywords: *Clostridium difficile*, *Enterococcus*, Vancomycin resistant, Colonization, Environment, Spinal cord injuries, Urinary tract infection

Introduction

Clostridium difficile is the most common cause of healthcare-associated diarrhea in developed countries.¹ In recent years, large outbreaks of *C. difficile* infection (CDI) in North America and Europe have been attributed to the emergence of an epidemic strain (North American pulsed field gel electrophoresis type 1, or NAP1) with unique putative virulence factors and increased resistance to fluoroquinolone antibiotics.²⁻⁴ Multi-faceted infection control measures have been effective in reducing or eliminating some outbreaks

associated with the epidemic strain.³⁻⁵ Control measures typically focus on patients with suspected or documented CDI.^{1,6} Those individuals are placed in contact precautions until their diarrhea resolves and their rooms are disinfected with a 1:10 dilution of household bleach or other sporicidal agents.^{6,7} Because many patients with CDI become asymptomatic carriers of toxigenic *C. difficile* (i.e. continue to shed spores in stool) after their diarrhea has resolved, some experts have recommended that contact precautions be continued until the time of discharge for patients who have recovered from CDI.^{4,7}

Since 2002, the NAP1 strain has become endemic at the Cleveland VA Medical Center.⁸ A particularly high

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rate of infection has been observed on our spinal cord injury (SCI) unit (unpublished data). In our long-term care facility, 51% of 68 asymptomatic patients were found to be asymptomatic carriers of toxigenic *C. difficile* strains, and these carriers frequently had skin and environmental contamination.⁹ Because patients with SCI share similar risk factors with long-term care facility residents (e.g. prolonged length of stay and frequent antibiotic therapy),^{6,9} we examined the prevalence of toxigenic *C. difficile* stool carriage and of skin and environmental contamination in hospitalized patients on our SCI unit. In addition, we examined the prevalence of asymptomatic stool carriage and of skin and environmental contamination with vancomycin-resistant *Enterococcus* (VRE). VRE is another important healthcare-associated pathogen that shares risk factors with and often coexists with *C. difficile*.¹⁰

Methods

From 24 January through 2 February 2007, we performed a point-prevalence culture survey on the 32-bed SCI unit at the Cleveland Veterans Affairs Medical Center to assess the prevalence of asymptomatic carriage of toxigenic *C. difficile* and VRE, and the frequency of associated skin and environmental contamination. Patients with diarrhea of unknown etiology were excluded from the culture survey. Two patients with known CDI on the SCI unit during the study period were included for comparison as positive controls for the assessment of skin and environmental contamination; seven additional patients with CDI cared for on the SCI unit during the 2 months after the initial survey were also included to provide additional data on levels of contamination in CDI patients. The patients' electronic medical record was utilized to obtain the SCI classification, demographic characteristics, coexisting illnesses, fecal incontinence, bowel care regimen, and medications including the use of antibiotics in the preceding 90 days. The Cleveland VA Medical Center's Institutional Review Board approved the study protocol and informed consent was obtained from all subjects.

Collection of rectal, skin, and environmental cultures

Rectal cultures were obtained by introducing a sterile swab into the rectum. A previous study has demonstrated that rectal swabs detected 100% of *C. difficile* carriers with positive stool cultures.¹¹ Skin cultures for *C. difficile* (groin and chest/abdomen) were obtained by donning sterile gloves and rubbing a standardized 5 × 20 cm² area with a pre-moistened sterile gauze pad

(3 × 3 cm²). The gauze pad was then placed into a sterile specimen cup. Skin cultures for VRE were obtained by rubbing a sterile swab on a standardized 5 × 20 cm² area. For each patient the *C. difficile* specimen was obtained prior to the VRE specimen and different areas were cultured for each pathogen. We have previously demonstrated that gauze pads are more sensitive than swabs for the recovery of *C. difficile* from environmental surfaces,⁹ whereas gauze pads and swabs yield similar rates of recovery of VRE from skin or the environment (authors' unpublished data).

Environmental cultures for *C. difficile* (bed rail and hand grip of trapeze) were obtained by donning sterile gloves and rubbing a sterile pre-moistened gauze pad (3 × 3 cm) on a designated area of each surface (5 × 20 cm for bed rail and the entire inferior surface of the hand grip). Environmental cultures for VRE were obtained by rubbing a sterile pre-moistened swab on a separate 5 × 20 cm area of the bed rail and the entire inferior surface of the hand grip. All cultures were obtained by one of the investigators (DMD) using standardized methods for each subject.

C. difficile microbiologic analysis and molecular typing

Culture specimens were incubated for 48 hours in pre-reduced cycloserine–cefoxitin–fructose (CCF) broth containing 0.1% taurocholic acid and lysozyme 5 mg/l, and then plated onto CCF agar plates containing taurocholic acid and lysozyme and incubated for an additional 48 hours.^{8,9} Isolates were confirmed to be *C. difficile* on the basis of typical odor and appearance of colonies and by a positive reaction using *C. difficile* latex agglutination (Microgen Bioproducts, Camberly, UK). All *C. difficile* isolates were tested for *in vitro* cytotoxin production using *C. difficile* Tox A/B II (Wampole Laboratories, Princeton, NJ, USA), and isolates that did not produce toxin were excluded from the analysis.

For a subset of patients, molecular typing was performed to compare isolates from stool, skin, and environmental sites and to determine the prevalence of epidemic NAP1 strains. Crude DNA was extracted from *C. difficile* isolates using QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) ribotyping was used to genotype *C. difficile* isolates as previously described.¹² PCR was performed to amplify one of the genes for binary toxin (*cdtB*) using the methods of Terhes *et al.*¹³ To assess for partial deletions of the *tcdC* gene, PCR was performed according to the methods of Spigaglia and Mastrantonio.¹⁴

Isolates with partial deletions were identified based on different migration patterns on a 2% agarose gel. For each assay, a known epidemic strain (typed as BI6 using restriction enzyme analysis) was used as a positive control and American Type Culture Collection (ATCC, Manassas, VA, USA) *C. difficile* 9689 was used as a negative control.

VRE microbiologic analysis

Swab tips were incubated in Enterococcosel™ broth (Becton Dickinson, Sparks, MD, USA) containing vancomycin 20 µg/ml for 48 hours, followed by plating onto Enterococcosel agar™ (Becton Dickinson) containing vancomycin 20 µg/ml for an additional 48 hours. Identification was performed in accordance with Clinical Laboratory Standards Institute guidelines.¹⁵

Data analysis

Distributions of clinical and demographic characteristics of asymptomatic carriers and non-carriers (i.e. patients with negative stool cultures) were compared. Unpaired *t*-test was used for normally distributed data. Fisher's exact test was used for categorical data. Data were analyzed with the use of SPSS statistical software version 10.0 (SPSS Inc., Chicago, IL, USA) and STATA 9.1 (StataCorp, College Station, TX, USA).

Results

Of 30 patients admitted to the SCI unit during the study, 2 had CDI and the other 28 had no diarrhea or other changes in bowel habits. Of the 28 subjects with no diarrhea, 22 (78.6%) patients were enrolled and 6 patients

were excluded because they were discharged prior to enrollment or were unavailable for cultures due to appointments or procedures. Of the 22 subjects, 11 (50%) had asymptomatic carriage of toxigenic *C. difficile* in stool and 11 did not. Table 1 shows a comparison of the characteristics of the subjects with and without asymptomatic stool carriage of toxigenic *C. difficile*. The average length of stay prior to the culture being obtained was significantly longer for the asymptomatic carriers of toxigenic *C. difficile* versus the non-carriers (median, 57 versus 6 days; $P = 0.04$). There were non-significant trends toward more frequent indwelling devices ($P = 0.08$), recent intensive care unit admission, and more frequent antibiotic use within the past 90 days for the asymptomatic carriers of toxigenic *C. difficile* versus non-carriers.

Comparing patients with CDI, asymptomatic carriers of toxigenic *C. difficile*, and non-carriers, the percentages of positive cultures for any skin site were 89, 45, and 9, respectively, and for any environmental site, it was 78, 55, and 9, respectively (Fig. 1). In comparison with non-carriers, asymptomatic carriers of toxigenic *C. difficile* had a significantly higher percentage of environmental ($P = 0.03$) contamination and a trend toward a higher percentage of skin ($P = 0.07$) contamination. In comparison with CDI patients, asymptomatic carriers of toxigenic *C. difficile* had trends toward lower percentages of skin ($P = 0.07$) and environmental ($P = 0.24$) contamination. Of the 11 patients with negative stool cultures, one had both skin and environmental contamination with *C. difficile*.

Table 1 Baseline characteristics of 22 male SCI patients with no symptoms of CDI, according to stool carriage of *C. difficile*

Characteristic (n (%)) unless otherwise specified)	Asymptomatic <i>C. difficile</i> carrier (n = 11)	Non-carrier (n = 11)	P value, (unless specified as relative risk (RR))
Age (median, IQR)	54 (43–72)	58 (49–61)	NS
Length of stay (median days, IQR)	57 (9–243)	6 (1–23)	0.04
Level of SCI			
Cervical	3 (27)	5 (45)	NS
Thoracic	6 (55)	6 (55)	NS
Lumbar or undetermined	2 (18)*	0 (0)	NS
VRE colonization	7 (64)	5 (45)	1.4 (0.64–3.07)
Transfer from outside hospital	6 (55)	3 (27)	RR = 2 (0.66–6.04)
Long-term care facility resident	3 (27)	1 (9)	RR = 3 (0.37–24.6)
Infection during admission	10 (91)	6 (55)	RR = 1.7 (0.94–2.95)
Antibiotics within past 90 days	9 (82)	5 (45)	RR = 1.8 (0.89–3.64)
Prior <i>C. difficile</i> infection	2 (18)	2 (18)	RR = 1 (0.17–5.4)
Indwelling device†	7 (64)	2 (18)	RR = 3.5 (0.92–13.2)
Bowel care regimen including rectal stimulation	5 (45)	4 (36)	RR = 1.25 (0.45–3.45)
Acid-suppressive medication (proton pump inhibitor or H ₂ blocker)	3 (27)	4 (36)	RR = 0.75 (0.14–3.24)
Intensive care admission past 90 days	3 (27)	1 (9)	RR = 3 (0.13–3.24)

IQR = interquartile range; NS = not statistically significant and indicates that the *P* value was ≥ 0.05 .

*One subject had lumbar SCI and the other had multiple sclerosis.

†Indwelling devices included chronic indwelling urinary catheters, tracheostomies, and percutaneous endoscopic gastostomy tubes.

Figure 1 *C. difficile* skin (A) and environmental (B) contamination among study groups. Cultures of skin and environmental surfaces were collected concurrently with stool cultures from patients with *C. difficile* infection (9 patients), asymptomatic fecal carriers (11 patients), and non-carriers (i.e. negative stool cultures) (11 patients).

Of the 11 asymptomatic carriers of toxigenic *C. difficile*, 1 (9%) was found to be a carrier of the NAP1 strain and the other 10 had non-epidemic strains. All 12 of the skin isolates from 6 asymptomatic carriers of toxigenic *C. difficile* were identical to concurrent stool isolates by PCR-ribotyping. Of 11 environmental isolates from 5 asymptomatic carriers of toxigenic *C. difficile* that were subjected to typing, 9 (82%) were identical to concurrent stool isolates.

Of the 22 subjects, 12 (55%) were found to have unsuspected colonization of their stool with VRE. Of the 11 asymptomatic carriers of toxigenic *C. difficile*, 7 (64%) were also carriers of VRE. As shown in Fig. 2, the proportions of positive skin and environmental contamination were higher for the patients with stool colonization versus those with negative stool cultures, but the differences were not statistically significant.

Discussion

In a setting in which the NAP1 strain has become endemic, we found that 50% of subjects on the acute-care SCI unit at the Cleveland VA Medical Center were asymptomatic carriers of toxigenic *C. difficile*. These patients frequently had contamination of skin and environmental sites, and molecular typing demonstrated that most skin and environmental isolates were

Figure 2 VRE skin (A) and environmental (B) contamination among study groups. Cultures of skin and environmental surfaces were collected concurrently with stool cultures from asymptomatic fecal VRE carriers (12 patients) and non-carriers (i.e. negative stool cultures) (10 patients).

identical to concurrent stool isolates. Unsuspected stool colonization with VRE was also common among the study patients, with frequent skin and environmental contamination. It is likely that the requirement for assistance with bowel care may contribute to frequent skin and environmental contamination among carriers of healthcare-associated pathogens on SCI units. Because asymptomatic carriers of toxigenic *C. difficile* outnumbered patients with CDI by greater than five-fold, our findings suggest that these individuals could contribute significantly to transmission on SCI units.

There are limited data on the epidemiology of *C. difficile* in SCI units. There are a few case reports of CDI in SCI units,¹⁶⁻¹⁸ but we are not aware of previous studies that examined rates of asymptomatic carriage in hospitalized patients with SCI. Marciniak *et al.*¹⁸ did investigate the prevalence of toxigenic *C. difficile* carriage upon admission to a rehabilitation unit that included spinal cord injury patients and found a rate of 16%. Our rate was significantly higher, possibly due to the fact that patients were cultured at various points of hospitalization, with many of the carriers having been hospitalized for weeks to months. It is also possible that the high prevalence of asymptomatic carriage of toxigenic

C. difficile could be in part due to ineffective or insufficient environmental cleaning on the ward. However, it is notable that the point-prevalence study was conducted several months after implementation of a facility-wide cleaning intervention that included the use of sodium hypochlorite (i.e. a 1:10 dilution of household bleach) for routine disinfection of all hospital rooms.¹⁹ The cleaning intervention resulted in significant reductions in *C. difficile* and VRE contamination in isolation rooms,¹⁹ and with a reduction in the incidence of CDI from 15 to 10 cases per 1000 patient discharges (authors' unpublished data). We also cannot exclude the possibility that poor compliance with hand washing or glove use contributed to the high prevalence of asymptomatic carriage of toxigenic *C. difficile*. Based on Infection Control Department surveillance data, the rate of compliance with hand hygiene recommendations on the ward during the year of the study was ~70%. However, monitoring was only performed during daytime shifts with no evening or weekend monitoring, and no monitoring of glove use was performed.

In our previous study in long-term care, we found that recent antibiotic use and previous CDI were significant risk factors for asymptomatic carriage of toxigenic *C. difficile*.⁸ In the present study, we found that 82% of asymptomatic carriers of toxigenic *C. difficile* had received recent antibiotic therapy, but only 18% had a previous history of CDI. There was a significant association between increased length of stay and asymptomatic carriage of toxigenic *C. difficile*. Previous studies have demonstrated that increased length of stay is a risk for CDI.⁶ We also found that presence of indwelling devices was much more common in asymptomatic carriers of toxigenic *C. difficile* versus non-carriers (64 versus 18%), but this difference was not statistically significant ($P = 0.08$). Others have recently associated the presence of indwelling devices with more frequent colonization with healthcare-associated antibiotic-resistant pathogens, including VRE and methicillin-resistant *Staphylococcus aureus*.^{20,21} The association between devices and colonization by healthcare-associated pathogens may in part be due to confounding factors such as frequent antibiotic use and increased exposure to healthcare workers.^{20,21}

Our study had several limitations. Our population included only male veterans, and therefore additional studies are needed in other settings. The short study period and small number of subjects has the potential to overestimate the actual prevalence of asymptomatic carriage of toxigenic *C. difficile* in this population, as we may not have been able to study a representative sample of the population. The small number of subjects

also limits our ability to analyze the various potential risk factors for asymptomatic carriage of toxigenic *C. difficile* in this population, and may account for the lack of a significant association between prior antibiotic use and *C. difficile* carriage. Although the association between length of stay and asymptomatic carriage is plausible and consistent with previous studies,⁶ this finding should also be interpreted with caution given the small sample size. Another limitation is the use of broth enrichment cultures, which does not allow the quantification of organisms on skin or in the environment. Therefore, our findings do not allow a comparison of the burden of spores associated with CDI versus asymptomatic carriers of toxigenic *C. difficile*. Finally, although our findings suggest that asymptomatic carriers may contribute to the transmission of *C. difficile*, molecular typing of strains that are acquired by patients will be necessary to confirm that strains from asymptomatic carriers are being transmitted.

Conclusion

We found that asymptomatic carriage of toxigenic *C. difficile* and VRE was common among with SCI, and carriers frequently had skin and environmental contamination. Further studies are indicated to determine whether these findings can be generalized to other SCI units.

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Conflict of interest statement

Dr Donskey has received research support from ViroPharma, Merck, Optimer, Elan, and Ortho-McNeil. All other authors: no conflicts.

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