MAJOR ARTICLE

Asymptomatic Carriers Are a Potential Source for Transmission of Epidemic and Nonepidemic *Clostridium difficile* Strains among Long-Term Care Facility Residents

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(See the editorial commentary by Muto on pages 999–1000)

Background. Asymptomatic fecal carriage of *Clostridium difficile* is common in patients staying in health care facilities, but the importance of asymptomatic carriers with regard to disease transmission is unclear.

Methods. We prospectively examined the prevalence of asymptomatic carriage of epidemic North American pulsed-field gel electrophoresis type 1 and nonepidemic toxigenic *C. difficile* strains among long-term care patients in the context of an outbreak of *C. difficile*–associated disease and evaluated the frequency of skin and environmental contamination. Molecular typing was performed by pulsed-field gel electrophoresis. Logistic regression was used to assess factors associated with asymptomatic carriage, and a sensitive and specific prediction rule was developed to identify high-risk patients.

Results. Thirty-five (51%) of 68 asymptomatic patients were carriers of toxigenic *C. difficile,* and 13 (37%) of these patients carried epidemic strains. Compared with noncarriers, asymptomatic carriers had higher percentages of skin (61% vs. 19%; $P = .001$) and environmental contamination (59% vs. 24%; $P = .004$). Eighty-seven percent of isolates found in skin samples and 58% of isolates found in environmental samples were identical to concurrent isolates found in stool samples. Spores on the skin of asymptomatic patients were easily transferred to investigators' hands. Previous *C. difficile*–associated disease ($P < .001$) and previous antibiotic use ($P = .017$) were associated with asymptomatic carriage, and the combination of these 2 variables was predictive of asymptomatic carriage (sensitivity, 77%; specificity, 58%; positive predictive value, 66%; negative predictive value, 70%).

Conclusions. Our findings suggest that asymptomatic carriers of epidemic and nonepidemic *C. difficile* strains have the potential to contribute significantly to disease transmission in long-term care facilities. Clinical factors, such as previous *C. difficile*–associated disease and recent antibiotic use, may be predictive of asymptomatic carriage.

Clostridium difficile is the most common cause of health care–associated diarrhea in developed countries [1]. In recent years, large outbreaks of *C. difficile*–associated disease (CDAD) in North America and Europe have been attributed to the emergence of an epidemic strain (North American PFGE type 1 [NAP1]) with unique putative virulence factors and increased resistance to fluoroquinolone antibiotics [2–4]. Infection-control measures have been effective in reducing but not eliminating outbreaks associated with the epidemic strain [2–4]. Control measures typically focus on patients with suspected or documented CDAD [1, 5]. Those individuals are made subject to contact precautions until diarrhea has resolved, and their rooms are cleaned with 10% bleach solution [3, 6, 7].

One factor that could reduce the efficacy of current infection-control strategies for *C. difficile* infection is the failure to address the potential role of asymptomatic carriers in disease transmission. The frequency of asymptomatic carriage of epidemic NAP1 isolates is unknown; however, previous studies have demon-

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strated that approximately two-thirds of patients colonized with *C. difficile* become asymptomatic fecal carriers [5, 8–10]. Two studies found that asymptomatic carriers had lower percentages of environmental contamination than did patients with CDAD (percentages of positive environmental culture results in these studies were 7% vs. 20% [9] and 29% vs. 49% [11]). However, these studies did not examine whether subgroups of asymptomatic carriers may be at increased risk for shedding of organisms (e.g., patients with fecal incontinence) and did not assess contamination of skin, a major potential source of disease transmission. Although some epidemiological studies have suggested that asymptomatic carriers play a relatively minor role in disease transmission [12], Clabots et al. [13] found that most episodes of nosocomial acquisition of CDAD in a study hospital ward were epidemiologically linked to disease transmission from new, asymptomatic patients admitted to the ward. These data suggest that asymptomatic carriers could play an important and underappreciated role in the transmission of *C. difficile* infection.

Since 2002, the acute care and long-term care facilities at the Cleveland Veterans Affairs Medical Center (Cleveland, OH) have experienced a large outbreak of CDAD associated with the epidemic strain (authors' unpublished data). Because infection-control measures, including bleach disinfection, were associated with only a modest reduction in CDAD rates, we suspected that asymptomatic carriers might contribute to ongoing disease transmission. In the present study, the prevalence of asymptomatic carriage of epidemic and nonepidemic *C. difficile* infection was examined among long-term care facility residents. We hypothesized that asymptomatic carriers frequently shed *C. difficile* isolates onto their skin and into the environment and that fecal incontinence is associated with increased shedding. A clinical prediction rule was developed to identify patients at high risk for asymptomatic carriage.

METHODS

Study design. We performed a prospective study of long-term care facility residents in 2 adjacent wards. From July through September 2006, all 73 inpatients in the 2 wards had stool samples or rectal swab specimens and samples from skin sites and environmental sites cultured for *C. difficile* to determine the point-prevalence of carriage. Information regarding demographic characteristics, coexisting illnesses, fecal incontinence, and medication was obtained through standardized chart review. During the subsequent 6 months, the patients with no symptoms of CDAD were monitored for development of CDAD, admission to the acute care facility, and death. The asymptomatic carriers who remained in the long-term care facility had stool cultures performed 1–3 months after the initial culture was performed to assess whether carriage persisted. In addition to 5 patients who had CDAD during the initial survey, 13 patients from the long-term care facility who had CDAD during the 6-month follow-up period had cultures performed as positive controls. Antibiotics were classified as antianaerobic on the basis of the criteria of Donskey et al. [14]. The hospital's institutional review board approved the study protocol.

Skin and environmental cultures. Skin culture specimens (from the groin and chest and/or abdomen) were obtained by swabbing a 5×20 cm area with a premoistened rayon swab. To assess whether spores on skin could be transferred to hands, an investigator donned sterile gloves and contacted the same skin sites of a subset of patients; the gloves were then imprinted directly onto prereduced cycloserine-cefoxitin-fructose agar containing 0.1% taurocholic acid and lysozyme 5 mg/L (CCFA-TAL), and the plates were transferred to an anaerobic chamber (Coy Laboratories). Environmental culture specimens (from the call button, bed rail, bedside table, and telephone) were obtained by donning sterile gloves and applying a sterile premoistened gauze pad (3×3 cm) to a designated area of each surface (5×20 cm for the bed rail and bedside table, and the entire surface area for the call button and telephone receiver). The gauze pads were then placed into a sterile specimen cup.

Microbiologic analysis and molecular typing. Stool samples or rectal swab specimens were transferred into the anaerobic chamber and plated directly onto CCFA-TAL plates. Skin swabs and environmental gauze pads were incubated for 48 h in cycloserine-cefoxitin-fructose broth containing taurocholic acid and lysozyme and then plated onto CCFA-TAL plates and incubated an additional 48 h. 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). All *C. difficile* isolates were tested for in vitro cytotoxin production using *C. difficile* Tox A/B II (Wampole Laboratories), and isolates that did not produce toxin were excluded from the analysis. MICs were determined for moxifloxacin using Etest (AB Biodisk). If *C. difficile* was present in the stool sample, aliquots were incubated 1:1 in 100% ethanol for 30 min, and the density was measured, as described elsewhere [13].

For molecular typing, PFGE was performed using the methods of Alonso et al. [15]. The plugs were run on a 1% agarose gel using the GenePath electrophoresis system (Bio-Rad), and the gel was stained with ethidium bromide. The relatedness of isolates was determined according to the criteria of Tenover et al. [16].

Analyses for binary toxin gene* cdtB *and partial deletions of the* tcdC *gene. Crude DNA was extracted from *C. difficile* isolates using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. PCR was performed on extracted DNA to amplify one of the genes for binary toxin *(cdtB)* using the methods of Terhes et al. [17]. To assess for partial deletions of the *tcdC* gene, PCR was performed using

Table 1. Baseline characteristics and follow-up experience of 68 male long-term care patients with no symptoms of *Clostridium difficile*–associated disease (CDAD), according to stool carriage of *C. difficile* isolates.

Baseline characteristic	Carrier (n = 35)	Noncarrier (n = 33)	P
Demographic			
Age, mean years ± SD	70 ± 12	71 ± 12	.651
Duration of hospital stay, median no. of days (IQR)	55 (26–160)	44 (18–91)	.193
Clinical condition			
Diabetes mellitus	16 (46)	15 (45)	.983
Cancer	9 (26)	7 (21)	.662
End-stage renal disease	3 (9)	2 (6)	>.99
Previous CDAD	9 (23)	0	.005
Fecal incontinence	15 (43)	10 (30)	.283
Previous exposure			
Acute care facility admission in the previous year	27 (77)	26 (79)	.870
Any antibiotic use in the previous 3 months	25 (71)	14 (42)	.016
Antianaerobic agents	12 (34)	6 (18)	.132
Fluoroquinolones	14 (40)	6 (18)	.048
Cephalosporins	8 (23)	6 (18)	.634
Proton pump inhibitor use	17 (49)	20 (61)	.319
Follow-up experience			
Admission to acute care facility	16 (46)	10 (30)	.191
Development of CDAD	7 (20)	3 (9)	.307
Death	8 (23)	5 (15)	.417

NOTE. Data are no. (%) of patients, unless otherwise indicated. Antianaerobic agents included clindamycin, metronidazole, piperacillin plus tazobactam, ampicillin plus sulbactam, ertapenem, and moxifloxacin. IQR, interquartile range.

the primers C1 and C2, according to the methods of Spigaglia and Mastrantonio [18]. Isolates with partial deletions were identified on the basis of different migration patterns on 2% agarose gel. For both assays, a known epidemic strain (typed as BI6, using restriction enzyme analysis) was used as a positive control, and American Type Culture Collection *C. difficile* 9689 was used as a negative control.

Statistical analysis. Distributions of clinical and demographic characteristics of asymptomatic carriers and noncarriers (i.e., patients with negative stool culture results) were compared. Unpaired Student's t test and Kruskal-Wallis test were used for normally and nonnormally distributed data, respectively. Pearson's χ^2 test and Fisher's exact test were used for categorical data. Logistic regression analysis was used to assess factors associated with asymptomatic carriage of *C. difficile* in stool.

To develop a prediction rule for asymptomatic carriage of *C. difficile*, we used the methods of Furuno et al. [19]. First, sensitivity, specificity, and 95% CIs were calculated to assess the ability of individual and combinations of variables to identify asymptomatic carriers versus noncarriers. The variable with the highest sensitivity was included first, followed by the addition of other variables using the boolean logic terms "and/or" to assess whether they improved the sensitivity without resulting in a decrease in specificity. Goodness-to-fit for logistic models was assessed using the Hosmer-Lemeshow test. The goal for creating a final prediction rule was to maximize sensitivity at the expense of specificity, because the costs associated with false-positive findings are likely to be lower than those associated with false-negative findings in the context of an outbreak (i.e., false-positive culture results would lead to inexpensive measures, including use of bleach for terminal cleaning and routine glove use, whereas not identifying asymptomatic carriers who may transmit *C. difficile* infection may be associated with failure to control an outbreak). Data were analyzed using SPSS, version 10.0 (SPSS), and Stata software, version 9.1 (Stata).

RESULTS

Of the 73 patients in the study wards, all were men and 5 (7%) had CDAD at the time of the point-prevalence survey. The baseline characteristics at the time of the point-prevalence survey and follow-up experience of the 68 long-term care residents with no symptoms of CDAD are shown in table 1. Thirty-five (51%) of the 68 patients were asymptomatic carriers of toxigenic *C. difficile* strains. Twenty of the asymptomatic carriers had stool samples collected, and the remaining 15 had the

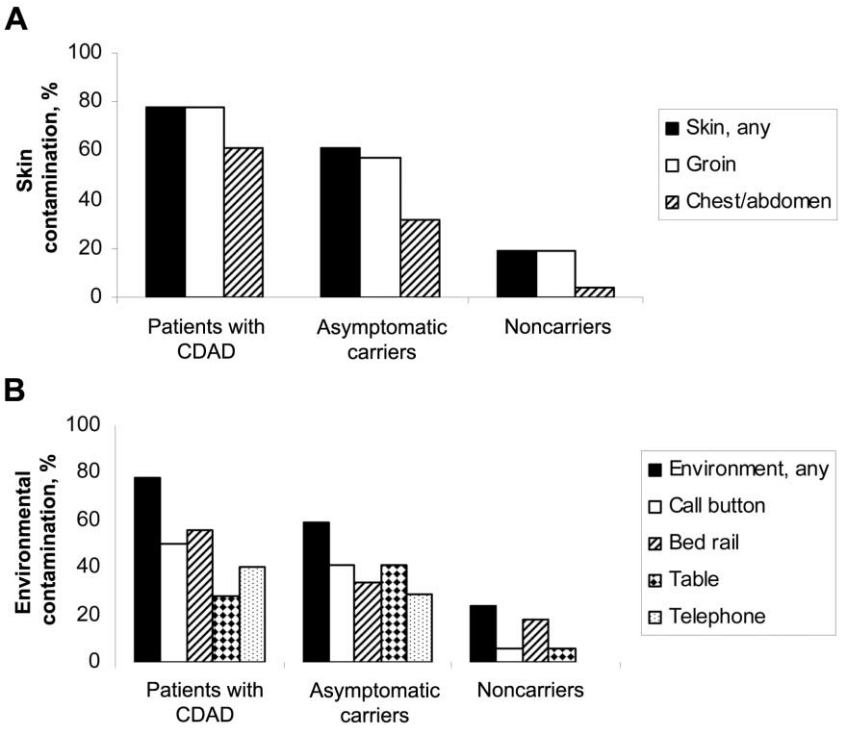

Figure 1. Percentages of *Clostridium difficile* skin *(A)* and environmental *(B)* contamination among study groups. Samples from skin and environmental surfaces were collected for culture concurrently with stool samples from patients with *C. difficile*–associated disease (CDAD; $n = 18$), asymptomatic fecal carriers ($n = 35$), and noncarriers (i.e., patients with negative stool culture results; $n = 33$). Patients with missing skin ($n = 13$) or environmental ($n = 3$) culture samples were excluded.

pathogen detected in rectal swab specimens. The mean densities ($\pm$ SD) of *C. difficile* in stool specimens from the 20 carriers who had stool samples collected and from the 18 positive control subjects with CDAD were 3.6 ± 1.3 $\log_{10}$ colony-forming units (CFU)/g and 5.6 ± 1.4 $\log_{10}$ CFU/g, respectively ($P < .001$). Twelve asymptomatic carriers had follow-up stool cultures performed 1–3 months after the initial culture survey, and 10 (83%) had persistent positive culture results. Asymptomatic carriers were more likely than noncarriers to have had previous CDAD, exposure to any antibiotic in the previous 3 months, and previous exposure to fluoroquinolones. During the 6-month follow-up period, 16 (46%) of the asymptomatic carriers, including 5 of those with persistent positive culture results, were admitted to the acute care facility, and 7 (20%) of these patients developed CDAD. Only 2 of the 7 asymptomatic carriers who developed CDAD had had a previous *C. difficile* infection.

Thirteen (37%) of the 35 asymptomatic carriers had epidemic NAP1 strains in stool samples. All 13 NAP1 isolates had positive PCR reactions for the binary toxin gene *cdtB*, partial deletions of the *tcdC* gene, and were resistant to moxifloxacin (MIC for all isolates, >32 μg/mL). The mean density of stool colonization by the epidemic strain was not significantly different from such density of nonepidemic strains (4.0 $\log_{10}$ CFU/g vs. 3.6 $\log_{10}$ CFU/g; $P = .82$).

For patients with CDAD, asymptomatic carriers, and noncarriers, the percentages of positive culture results were 78%, 61%, and 19%, respectively, for samples from any skin site and 78%, 59%, and 24%, respectively, for samples from any environmental site (figure 1). Asymptomatic carriers had significantly higher rates of skin and environmental contamination ($P \leq .004$) than did noncarriers; but they had lower rates than did patients with CDAD ($P \leq .007$). Two or more environmental sites yielded positive culture results for an equal proportion of asymptomatic carriers (14 [44%] of 32) and patients with CDAD (8 [44%] of 18; $P = .962$). Carriers of epidemic and nonepidemic strains had similar percentages of skin (67% vs. 55%; $P = .782$) and environmental (55% vs. 62%; $P = .522$) contamination. Compared with continent asymptomatic carriers, incontinent asymptomatic carriers did not have significantly higher percentages of skin (77% vs. 47%; $P = .735$) or environmental (69% vs. 52%; $P = .089$) contamination.

PFGE demonstrated that 13 (87%) of 15 isolates in skin samples tested were identical to concurrent isolates in stool samples, whereas only 11 (58%) of 19 isolates in environmental samples tested were identical to isolates in stool samples; a

representative PFGE gel is shown in figure 2. Hand imprint cultures from a subset of patients confirmed that *C. difficile* spores could easily be acquired on sterile gloves; *C. difficile* was acquired on hands after contact with the skin of 8 (57%) of 14 patients who had positive skin culture results.

C. difficile was cultured from the skin of 5 (19%) of 27 patients with negative stool culture results who also had skin cultures performed. None of the 5 subjects with positive skin but negative stool culture results had previously had CDAD. None of the subjects developed CDAD during the follow-up period.

In univariate logistic regression analysis (table 2), antibiotic use in the previous 3 months ($P = .017$) and history of previous CDAD ($P < .001$) were associated with asymptomatic carriage. Two prediction rules for asymptomatic carriage were developed on the basis of the logistic regression analysis (table 3). Rule 1, including antibiotic use in the previous 3 months or previous CDAD, has a sensitivity of 77%, specificity of 66%, positive predictive value of 66%, and negative predictive value of 70% and correctly classifies 68% of the individuals' goodness-to-fit tests. Rule 2, including antibiotic use in the previous 3 months or previous CDAD or fecal incontinence, has increased sensitivity (83%), but the modest decrease in specificity (42%) resulted in fewer people being correctly classified.

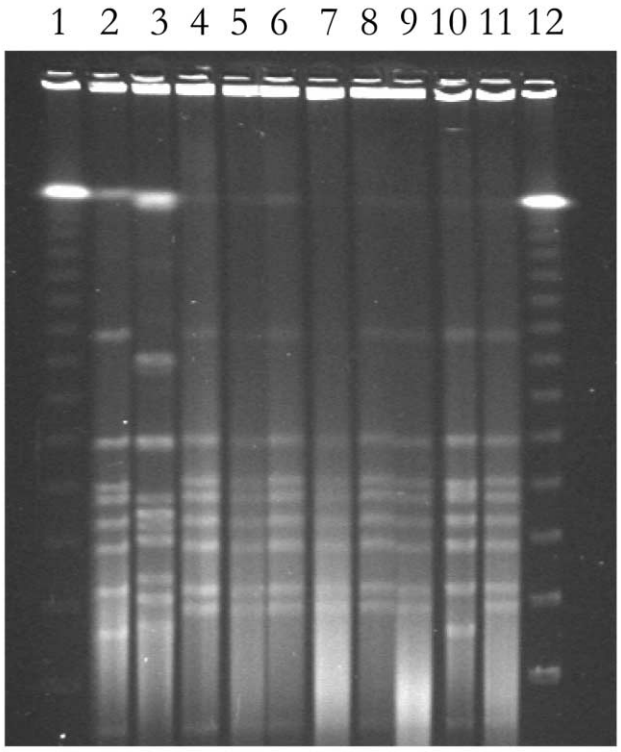

Figure 2. PFGE results demonstrating contamination of skin and environmental surfaces with epidemic NAP1 *Clostridium difficile* isolates that are identical to concurrent stool isolates from an asymptomatic carrier. The patient was an 84-year-old man with lung cancer and fecal incontinence, with 4.6 $\log_{10}$ colony-forming units per g of *C. difficile* in stool. *Lanes 1* and *12,* Lambda ladder. *Lane 2,* Epidemic control strain (BI6 by restriction enzyme analysis). *Lane 3,* Nonepidemic control strain (American Type Culture Collection 9689). *Lanes 4* and *5,* Isolates found in stool samples collected 1 month apart. *Lanes 6* and *7,* Skin isolates found in samples from the groin and chest and/or abdomen. *Lanes 8–11,* Environmental isolates found in samples from the call button, bed rail, and bedside table. The isolate found in the sample from the bed rail was not identical to the isolate found in the stool sample from the patient who stayed in the room but was an epidemic strain that was identical to the control strain in our institution *(lane 2).*

Table 2. Univariate logistic regression analysis of characteristics associated with carriage of *Clostridium difficile* in stool for 68 long-term care patients with no symptoms of *C. difficile*–associated disease (CDAD).

	Carriage of *C. difficile* in stool	
Characteristic	OR (95% CI)	*P*
Fecal incontinence	1.73 (0.63–4.67)	.285
Any antibiotic use in the previous 3 months	3.39 (1.24–9.23)	.017
Antianaerobic agents	2.35 (0.76–7.24)	.138
Fluoroquinolones	3.00 (0.99–9.13)	.053
Cephalosporins	1.33 (0.41–4.36)	.634
Proton pump inhibitor use	0.61 (0.23–1.61)	.321
Previous CDAD[a]	20.71 (2.41–8)	<.001

[a] The OR was not calculable by logistic regression, because previous CDAD predicts current carriage perfectly; the OR was estimated by 2×2 analysis after adding 0.5 to each cell; the lower bound of 95% CI was estimated by Cornfield approximation.

DISCUSSION

In the context of a *C. difficile* infection outbreak, we found that 52% of long-term care facility residents who had no symptoms of CDAD were asymptomatic fecal carriers of toxin-producing *C. difficile* strains, more than one-third of which were epidemic NAP1 strains. Asymptomatic carriers outnumbered patients with CDAD in the study wards 7 to 1. The frequency of contamination of skin and environmental surfaces among asymptomatic carriers was nearly as high as that among patients with CDAD, and spores on the skin of asymptomatic patients were easily acquired on investigators' hands. These findings suggest that asymptomatic carriers have the potential to contribute significantly to the transmission of epidemic and nonepidemic *C. difficile* infection in long-term care facilities.

Previous studies have demonstrated that asymptomatic carriage of *C. difficile* may be common in outbreak settings, with percentages of carriage as high as 30% reported in long-term care facilities [20–23]. The very high prevalence of asymptomatic carriage in our study population may be attributable

Table 3. Sensitivity and specificity of factors predicting carriage of *Clostridium difficile* in stool among 68 long-term care patients with no symptoms of *C. difficile*–associated disease (CDAD).

Characteristic	Carriage of *C. difficile* in stool			
	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
Fecal incontinence	43	70	60	53
Any antibiotic use in the previous 3 months	71	57	64	65
Antianaerobic agents	34	83	67	54
Fluoroquinolones	40	82	70	56
Cephalosporins	23	81	57	50
Proton pump inhibitor use	50	39	46	42
Previous CDAD	23	100	100	55
Previous CDAD or antibiotic use	77	58	66	70
Previous CDAD or antibiotic use or fecal incontinence	83	42	60	70
Previous CDAD or antibiotic use or fecal incontinence or proton pump inhibitor use	89	24	55	67

NOTE. The prevalence of carriage of *C. difficile* in stool was 52%.

to the presence of multiple risk factors in many patients (e.g., long duration of hospital stay and frequent antibiotic therapy) in the context of a large outbreak. In addition, supplementation of selective media with both taurocholic acid and lysozyme may have increased the sensitivity for detection of low levels of spores, compared with such sensitivity found in previous studies that included supplementation with taurocholic acid alone [24].

Although numerous studies have evaluated environmental *C. difficile* contamination, we are not aware of published data regarding the frequency or distribution of skin contamination among patients with CDAD or asymptomatic carriers. Both the groin and chest and/or abdomen of colonized or infected patients were frequently contaminated with *C. difficile*, and 87% of isolates found in skin samples were identical to concurrent isolates found in stool samples on the basis of PFGE typing. In contrast, 42% of isolates found in environmental samples were not related to concurrent isolates found in stool samples. Because routine cleaning of rooms in the study wards did not include the use of a sporicidal disinfectant, it is possible that many *C. difficile* isolates from the environment were from prior occupants of the rooms. Alternatively, the discordance isolates in stool samples and environmental samples could have been a result of carriage of multiple strains of *C. difficile* by some patients. In this study, we did not assess whether individual patients carried >1 strain type.

Some observations from our study were unexpected. First, approximately one-fifth of patients with negative stool culture results had skin and environmental culture results positive for *C. difficile*. We hypothesize that the presence of *C. difficile* on skin may have been attributable to prior stool carriage or to levels of stool colonization that were below the limit of detection. The positive environmental culture results, on the other hand, may have been a result of shedding from previous patients in the rooms. Second, 7 (20%) of the asymptomatic carriers developed CDAD, 5 of whom were primary symptomless carriers (i.e., patients with no history of previous CDAD). Previous studies suggest that primary asymptomatic carriage of toxigenic or nontoxigenic strains confers a low risk (~1%) for subsequent CDAD, even in the presence of antibiotic therapy [25]. One potential explanation for the frequent development of CDAD in patients with primary asymptomatic carriage in our study could be related to differences between epidemic and nonepidemic strains; 3 of the 5 patients with primary asymptomatic carriage who developed CDAD were carriers of the epidemic strain. Finally, we hypothesized that fecal incontinence would be associated with increased skin and environmental contamination, but the proportion of contamination was similar among continent and incontinent patients.

Our findings have several important implications for infection control of outbreaks of *C. difficile* infection. First, because environmental surfaces in asymptomatic carriers' rooms may frequently be contaminated with spores, use of sporicidal disinfectants may be indicated in outbreak settings. Given the high rate of asymptomatic carriage in our long-term care facility, we use a 10% bleach solution for terminal cleaning of all rooms after patients are discharged or transferred from the ward. Second, because alcohol-based hand hygiene products do not kill spores, use of gloves may be indicated when caring for patients at high risk for asymptomatic carriage in the context of an outbreak of *C. difficile* infection. Third, current guidelines recommend discontinuation of contact precautions for patients with CDAD after diarrhea resolves [5]. Our findings suggest that it may be

reasonable to extend the duration of contact precautions until the time when the patient is discharged from the hospital, because nearly one-fourth of asymptomatic carriers had had a previous episode of CDAD. A previous study found that as many as one-half of patients with CDAD continued to excrete spores in stool after resolution of diarrhea [26].

Because active surveillance for asymptomatic carriage of *C. difficile* may not be feasible in many health care facilities, a sensitive clinical prediction rule would be useful to allow identification of patients at high risk for carriage. We found that a prediction rule based on history of previous CDAD and antibiotic use in the previous 3 months had 77% sensitivity to detect asymptomatic carriage. Addition of fecal incontinence as a third variable in the prediction rule increased sensitivity to 83%, with only a modest reduction in specificity. Further prospective studies are needed to validate the prediction rule.

Our study has some limitations. The study population included only long-term care residents and entirely consisted of male patients; therefore, additional studies are needed in other contexts. Although our findings strongly suggest that asymptomatic carriers may contribute to transmission of *C. difficile* infection, molecular typing of strains acquired by patients is necessary to confirm that strains from asymptomatic carriers are being transmitted. We have shown that 3 of the NAP1 PFGE subtypes isolated from asymptomatic carriers are identical to isolates causing CDAD in our institution (authors' unpublished data). As noted previously, Clabots et al. [8] performed molecular typing and found that most episodes of nosocomial acquisition of CDAD in a study ward were epidemiologically linked to transmission from new, asymptomatic patients admitted to the ward.

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