Persistence of Skin Contamination and Environmental Shedding of *Clostridium difficile* during and after Treatment of *C. difficile* Infection

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**Background.** Current guidelines for control of *Clostridium difficile* infection (CDI) suggest that contact precautions be discontinued after diarrhea resolves. However, limited information is available regarding the frequency of skin contamination and environmental shedding of *C. difficile* during and after treatment.

**Design.** We conducted a 9-month prospective, observational study involving 52 patients receiving therapy for CDI. Stool samples, skin (chest and abdomen) samples, and samples from environmental sites were cultured for *C. difficile* before, during, and after treatment. Polymerase chain reaction ribotyping was performed to determine the relatedness of stool, skin, and environmental isolates.

**Results.** Fifty-two patients with CDI were studied. *C. difficile* was suppressed to undetectable levels in stool samples from most patients during treatment; however, 1–4 weeks after treatment, 56% of patients who had samples tested were asymptomatic carriers of *C. difficile*. The frequencies of skin contamination and environmental shedding remained high at the time of resolution of diarrhea (60% and 37%, respectively), were lower at the end of treatment (32% and 14%, respectively), and again increased 1–4 weeks after treatment (58% and 50%, respectively). Skin and environmental contamination after treatment was associated with use of antibiotics for non-CDI indications. Ninety-four percent of skin isolates and 82% of environmental isolates were genetically identical to concurrent stool isolates.

**Conclusions.** Skin contamination and environmental shedding of *C. difficile* often persist at the time of resolution of diarrhea, and recurrent shedding is common 1–4 weeks after therapy. These results provide support for the recommendation that contact precautions be continued until hospital discharge if rates of CDI remain high despite implementation of standard infection-control measures.

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*Clostridium difficile* is the most common cause of healthcare-associated diarrhea in developed countries. 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 [NAP1]) with unique putative virulence factors and increased resistance to fluoroquinolone antibiotics. Infection-control measures that have been recommended for control of outbreaks include placement of infected patients under contact precautions, use of 10% bleach for environmental disinfection, and restriction of high-risk antibiotics. Although there is evidence that patients with CDI may continue to shed spores in stool after symptoms resolve, current guidelines suggest that contact precautions may be discontinued after diarrhea resolves. The rationale for discontinuation of contact precautions after resolution of diarrhea is that the risk of transmission is considered to be highest when diarrhea is present. However, minimal information is available with regard to the frequency of skin contamination and environmental shedding of *C. difficile* at different times during and after treatment.

Beginning in 2002, the Cleveland Veterans Affairs Medical Center (Cleveland, OH) experienced a large outbreak of CDI associated with the epidemic strain (authors’ unpublished data). Standard infection-control measures, including placement of infected patients under contact precautions until 2 days after resolution of diarrhea, were effective in reducing the magnitude of the outbreak but did not end it. In a pilot study evaluating skin contamination in patients with CDI, we found that *C. difficile* often persisted on skin after resolution of diarrhea. Therefore, we hypothesized that continued shedding of *C. difficile* after resolution of diarrhea might contribute to ongoing transmission. In the present study, the frequency of skin contamination and environmental shedding of *C. difficile* was examined before, during, and after completion of treatment for CDI. The frequency of skin and envi-
ronmental contamination was also compared between patients infected with epidemic \textit{C. difficile} strains and patients infected with nonepidemic strains and between patients treated with metronidazole and patients treated with vancomycin.

**METHODS**

**Setting and Study Design**

From November 2006 through July 2007, we performed a prospective observational study of 52 patients with CDI at the Cleveland Veterans Affairs Medical Center. The hospital’s institutional review board approved the study protocol. One objective of the study was to compare the timing of resolution of diarrhea and the timing of achievement of undetectable levels in stool samples between patients treated with metronidazole and patients treated with vancomycin; those findings were published elsewhere.9 The diagnosis of CDI was based on presence of diarrhea, defined as 3 or more formed stools in 24 hours for 2 days, and the presence of \textit{C. difficile} toxin in a stool sample (Tox A/B II; Wampole Laboratories).

Information regarding demographic characteristics, coexisting illnesses, fecal incontinence, and medications was obtained through standardized chart review. Presence of diarrhea was assessed by chart review and interviews with patients and nursing staff. For 3 months after diagnosis of CDI, patients were monitored for recurrence of infection.

Stool samples, skin samples, and samples from environmental sites were cultured for \textit{C. difficile} before treatment, every 2–3 days during treatment, and each week after completion of treatment while the patients were hospitalized or were residents at the affiliated long-term care facility. Skin samples (from the chest and/or abdomen) were obtained by swabbing a 5 × 20-cm area with a premoistened rayon swab. For collection of environmental samples (from the call button, bed rail, bedside table, and telephone), researchers donned sterile gloves and applied a sterile premoistened gauze pad (3 × 3 cm) to a designated area of each surface (5 × 20 cm for bed rails and bedside tables and the entire surface area of call buttons and telephone receivers). The gauze pads were then placed into a sterile specimen cup. To ensure that the environmental cultures represented ongoing shedding of \textit{C. difficile} in the environment, the environmental sites from which samples were obtained for culture were disinfected with 10% bleach by research staff after each set of samples was obtained, and another set of samples was obtained for follow-up culture to assess whether spores were eradicated. In addition, the hospital cleaning staff performed routine terminal room disinfection with 10% bleach after discharge or transfer of patients with CDI and after discontinuation of contact precautions after resolution of diarrhea.

For a subset of 18 patients who had skin contamination during the period of diarrhea, additional skin samples were obtained for culture to assess the potential for acquisition of \textit{C. difficile} on hands after contacting patients with CDI before and 3 days or more after resolution of diarrhea. An investigator donned sterile gloves and contacted the groin, chest and abdomen, and forearm and hand sites separately with a gloved hand that was then imprinted onto prereduced cycloserine-cefoxitin-fructose broth containing 0.1% taurocholic acid and lysozyme and were then plated onto CCFA-TAL plates and incubated an additional 48 hours.9 Isolates were confirmed to be \textit{C. difficile} on the basis of typical odor and appearance of colonies. All \textit{C. difficile} isolates were tested for in vitro cytotoxin production with use of \textit{C. difficile} Tox A/B II (Wampole Laboratories), and isolates that did not produce toxin were excluded from the analysis.

For a subset of patients, molecular typing was performed to assess the relatedness of isolates from stool, skin, and environmental sites. Crude DNA was extracted from \textit{C. difficile} isolates with use of the QIAamp DNA Mini Kit (Qiagen), according to the manufacturer’s instructions. Polymerase chain reaction (PCR) ribotyping was used to genotype \textit{C. difficile} isolates with use of primers 16S (5′-GTG CGG CTG GAT CAC CTC CT-3′) and 23S (5′-CCC TGC ACC CTT AAT AAC TTG ACC-3′), as described elsewhere.9–10 PCR was performed to amplify 1 of the genes for binary toxin (\textit{cdtB}) with use of the methods of Terhes et al.11 To assess for partial deletions of the \textit{tcdC} gene, PCR was performed using the primers C1 and C2, according to the methods of Spigaglia and Mastrantonio.12 Isolates with partial deletions were identified on the basis of different migration patterns on a 2% agarose gel. For each assay, a known epidemic strain (typed as Bi6 by using restriction enzyme analysis) was used as a positive control, and American Type Culture Collection \textit{C. difficile} 9689 was used as a negative control.

**Statistical Analysis**

Distributions of clinical and demographic characteristics were compared between patients infected with epidemic \textit{C. difficile} strains and patients infected with nonepidemic strains. The unpaired Student \textit{t} test and the Kruskal-Wallis test were used for normally and nonnormally distributed data, respectively. The Pearson \(\chi^2\) test and Fisher exact test were used for categorical data. The proportions of skin and environmental contamination were compared at different times (ie, before treatment, on day 3 of treatment, after resolution of diarrhea, at the end of treatment, and at 2-week intervals after treatment), and the proportions of hand acquisition of \textit{C. difficile}
TABLE Baseline Characteristics and Follow-up Experience of 52 Study Participants with *Clostridium difficile* Infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (range)</td>
<td>68 (50–91)</td>
</tr>
<tr>
<td>Male sex</td>
<td>52 (100)</td>
</tr>
<tr>
<td>Nursing home resident</td>
<td>21 (40.4)</td>
</tr>
<tr>
<td>Clinical condition</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>21 (40.4)</td>
</tr>
<tr>
<td>Cancer</td>
<td>10 (19.2)</td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>13 (25.0)</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>15 (28.8)</td>
</tr>
<tr>
<td>Previous <em>C. difficile</em> infection</td>
<td>8 (15.4)</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>40 (76.9)</td>
</tr>
<tr>
<td>ICU admission in previous month</td>
<td>17 (32.7)</td>
</tr>
<tr>
<td>Recurrence of <em>C. difficile</em> infection</td>
<td>6 (11.5)</td>
</tr>
<tr>
<td>Death, any cause</td>
<td>14 (26.9)</td>
</tr>
</tbody>
</table>

Note. Data are no. (%) of participants, unless otherwise indicated. ICU, intensive care unit.

Results

Of 60 patients who received a diagnosis of CDI during the study period, 52 were enrolled in the study; 8 patients were excluded because informed consent was not obtained or no stool samples could be obtained during treatment. The baseline characteristics of the 52 study participants are shown in Table 1. Thirty-four patients had oral metronidazole therapy initiated, and 18 had oral vancomycin therapy initiated; 10 (29%) of the metronidazole-treated patients were switched to vancomycin because of persistent symptoms. Forty-five study participants had molecular typing performed on stool isolates; 31 isolates (69%) were found to be epidemic strains with use of PCR ribotyping and PCR for the binary toxin gene *cdtB* and partial deletions of the *tdC* gene.

Figure 1 shows the proportions of positive stool, skin, and environmental culture results before, during, and after completion of treatment for CDI. The number of participants evaluated at each time point decreased because of discharges or deaths. The mean time to resolution of diarrhea was 4.2 days. *C. difficile* was suppressed to undetectable levels in stool samples from the majority of patients by the time that diarrhea resolved, and only 2 (7%) of 28 patients who had culture performed at the end of treatment still had detectable spores in stool samples. However, 15 (56%) of 27 patients tested 1–4 weeks after treatment had positive stool culture results on 1 or more occasions while asymptomatic; an additional 6 (12%) of the 52 study participants developed symptomatic recurrence of CDI. Skin contamination and environmental shedding remained common at the time of resolution of diarrhea, although the proportions of positive skin and environmental culture results were significantly reduced, compared with these proportions before treatment (*P* < .005). Contamination occurred less frequently at the end of treatment than it

Before and after resolution of diarrhea were compared. The proportions of positive skin and environmental culture results at each time point were also compared between patients infected with epidemic strains and patients infected with non-epidemic strains and between patients treated with metronidazole and patients treated with vancomycin; patients whose therapy was switched from metronidazole to vancomycin were excluded from this comparison. General estimating equations analysis was used to determine the correlation between presence of diarrhea or detectable *C. difficile* in stool and positive skin and environmental culture results; this analysis included data collected from before treatment through the end of treatment (ie, data from 1–6 weeks after treatment were not included).

![Figure 1](image-url)  

Figure 1. Percentage of stool, skin (chest and abdomen), and environmental (bed rail, bedside table, call button, toilet seat) cultures positive for *Clostridium difficile* among 52 patients with *C. difficile* infection. The limit of detection for stool specimens was $\sim 2 \log_{10}$ colony-forming units/g. The numbers of patients who had samples cultured at each time point were 52 before treatment, 48 on day 3 of treatment, 43 after resolution of diarrhea, 28 at the end of treatment, 22 at 1–2 weeks after treatment, 15 at 3–4 weeks after treatment, and 8 at 5–6 weeks after treatment.
did before treatment ($P < .001$); however, *C. difficile* was still detected in skin and environmental samples for 32% and 14% of patients, respectively. In addition, 1–4 weeks after completion of treatment, 15 (58%) of 26 patients who had samples tested had skin contamination on 1 or more occasions, and 13 (50%) of 26 had continued environmental shedding. The frequency of skin and environmental contamination was lower 5–6 weeks after completion of treatment than it was 1–2 or 3–4 weeks after treatment, but the difference was not statistically significant ($P > .099$). However, only 8 patients had samples obtained for culture 5–6 weeks after treatment. Persistent or recurrent skin and environmental contamination after completion of CDI treatment was associated with use of antibiotics for non-CDI indications; skin and environmental contamination after CDI treatment was present in 12 (80%) of 15 patients who had received antibiotic therapy for other indications, compared with only 4 (36%) of 11 patients who did not receive additional antibiotic therapy ($P = .043$).

The frequencies of skin and environmental contamination at each time point did not differ significantly between patients infected with epidemic strains and patients infected with non-epidemic strains ($P > .155$ for each comparison) or between patients treated with vancomycin and patients treated with metronidazole ($P > .178$ for each comparison), excluding the 10 patients who were switched from metronidazole to vancomycin. Of 17 patients whose isolates were subjected to PCR ribotyping, 16 (94%) had skin isolates and 14 (82%) had environmental isolates that were identical to concurrent stool isolates.

Figure 2 shows the mean density of *C. difficile* in stool samples at each time point. Patients with negative culture results were assigned a value equal to the limit of detection (ie, 2 logs). The mean densities of *C. difficile* in stool 1–2, 3–4, and 5–6 weeks after treatment were lower than the densities before treatment and during diarrhea ($P < .001$). However, the mean densities at 2–4 weeks after treatment were not statistically different from those in stool samples obtained before treatment ($P = .178$).

Figure 3 shows the mean number of cultures positive for *C. difficile* in environmental samples at each time point. The mean number of cultures positive for *C. difficile* in skin and environmental samples was lower than the mean number of cultures positive for *C. difficile* in stool samples ($P < .001$).
weeks after treatment were significantly lower than the mean density before treatment ($P < .001$) and were higher than the mean densities at the time of resolution of diarrhea and at end of treatment ($P < .032$). The mean density of *C. difficile* in stool samples was significantly lower 5–6 weeks after treatment, compared with 1–2 and 3–4 weeks after treatment ($P < .005$).

Because an increased number of positive environmental sites in patient rooms has been associated with an increased likelihood of contamination of healthcare workers’ hands, the mean number of positive environmental sites was also examined at each time point (Figure 3). The mean number of positive environmental sites was significantly higher before treatment than at any time during treatment and 1–6 weeks after treatment ($P < .001$). However, the mean numbers of positive environmental sites 1–2 and 3–4 weeks after treatment were not significantly different from the numbers on day 3 of treatment ($P > .646$) or at the time of resolution of diarrhea ($P > .510$). The mean number of positive environmental sites 5–6 weeks after treatment was not significantly different from the number at the time of resolution of diarrhea ($P = .724$).

Figure 4 shows the comparison of acquisition of *C. difficile* on investigators’ gloved hands after contact with skin sites of 18 patients before and after resolution of diarrhea. Samples were obtained 3–22 days after resolution of diarrhea. For each skin site, spores were acquired on hands more frequently during the period of diarrhea than after resolution of diarrhea (Figure 4A; however, the difference was only statistically significant for the groin site. The mean number of colonies acquired on hands was significantly greater after contact with the chest and/or abdomen or arm and/or hand, but not after contact with the groin, of patients with diarrhea (Figure 4B).

By general estimating equations analysis including data from before treatment through the end of treatment, skin contamination with *C. difficile* was positively correlated with presence of diarrhea (hazard ratio, 3.70; 95% confidence interval [CI], 1.88–7.26) and detectable levels of *C. difficile* in stool samples (hazard ratio, 3.80; 95% CI, 1.91–7.58). Shedding on environmental surfaces before and during treatment was positively correlated with presence of diarrhea (hazard ratio, 3.87; 95% CI, 1.97–7.63), detectable levels of *C. difficile* in stool samples (hazard ratio, 5.07; 95% CI, 2.48–10.36), and presence of *C. difficile* on skin (hazard ratio, 9.97; 95% CI, 4.53–21.95).

**DISCUSSION**

In this prospective study, we found that treatment of CDI was associated with reductions in skin and environmental contamination. However, 60% of patients with CDI had skin contamination, and 37% continued to shed spores in the environment at the time that diarrhea resolved. The frequencies of skin contamination and environmental shedding were lower at the end of treatment (32% and 14%, respectively) than during treatment; however, more than one-half of patients tested 1–4 weeks after treatment had asymptomatic stool carriage of *C. difficile*, and most had concurrent skin and environmental contamination. There were no statistically significant differences in the frequencies of skin and environmental contamination between patients infected with epidemic strains and patients infected with nonepidemic strains or between patients treated with metronidazole and patients treated with vancomycin. After contact with skin of patients with CDI, acquisition of *C. difficile* on investigators’ hands was reduced after resolution of diarrhea; however, hand contamination still occurred approximately 50% of the time after contact with patients’ chest and abdomen or arm and hand. These results support the hypothesis that continued shedding of *C. difficile* after resolution of diarrhea could contribute significantly to transmission.

As noted previously, current guidelines do not recommend continuation of contact precautions beyond the period of resolution of diarrhea as a routine infection-control mea-
sure. However, a recent guideline recommended that contact precautions be continued until hospital discharge as a special approach if CDI is not effectively controlled by the use of basic measures. Our findings provide support for this recommendation. There was a reduction in the frequency and density of *C. difficile* in stool samples and in the frequency of skin and environmental contamination 5–6 weeks after treatment, suggesting that the first month after completion of treatment may be the time of the highest risk for continued transmission. However, additional studies are needed to confirm that shedding decreases with time, because only 8 study patients had culture performed 5–6 weeks after CDI treatment. Continued use of antibiotics for non-CDI indications was associated with skin and environmental contamination after CDI treatment. Therefore, it may be reasonable to target patients with CDI who continue to receive antibiotics for non-CDI indications for extension of contact precautions. In a previous study involving long-term care facility residents, we found that recent antibiotic therapy is also a risk factor for asymptomatic carriage of *C. difficile*.

In a survey of 23 hospitals in northeastern Ohio in 2005, we found that 10 (43%) had policies requiring that patients with CDI remain under contact precautions until discharge (authors’ unpublished data). The most common reasons cited for continuing contact precautions until hospital discharge were inadequate control of outbreaks with use of standard infection-control measures and concern that patients who develop recurrent CDI might spread *C. difficile* during the period before contact precautions are renewed. In that regard, it should be noted that 6 (12%) of the 52 patients in our study developed recurrent CDI within 2 months after treatment.

We are not aware of published studies that have examined the benefit of extending the duration of isolation of patients with CDI in comparison with standard infection-control measures. Muto et al included continuation of contact precautions until hospital discharge as a component of the comprehensive bundle of measures that were effective in controlling an outbreak associated with NAP1 strains. However, others have reported control of outbreaks of NAP1 infection without extending the duration of contact precautions.

Our study has several limitations. First, the study population consisted predominantly of male patients and older patients, and the sample size was small. Therefore, additional studies are needed in other settings and with larger numbers of participants. Second, the study was performed in the context of an outbreak associated with epidemic NAP1 isolates. However, there were no differences in the proportions of skin contamination and environmental shedding between patients infected with epidemic strains and patients infected with non-epidemic strains. Third, patients were followed up only while they were hospitalized or admitted to the long-term care facility. It is possible that patients with CDI who are discharged to home have lower rates of skin contamination and environmental shedding than do patients discharged to a long-term care facility; however, our goal was to assess contamination in patients in the hospital or long-term care facility. Fourth, hand acquisition was assessed after contact with skin by investigators wearing sterile gloves. Additional studies are needed to assess hand acquisition by healthcare workers performing patient care activities. Fifth, we did not perform typing to assess whether strains shed by patients after treatment were the same as the original infecting isolates. Sixth, we did not correlate the timing or type of bathing performed by patients with their skin culture results. Because decontamination of patients’ skin with chlorhexidine gluconate has been an effective infection-control measure for vancomycin-resistant enterococci, further research is indicated to evaluate strategies to reduce the burden of *C. difficile* spores on skin. Finally, only small numbers of spores were acquired on hands. However, we demonstrated that spores acquired on hands could be transferred to an agar plate. Duckro et al found that even small numbers of vancomycin-resistant enterococci on surfaces could easily be transferred to clean sites on hands of healthcare workers.

In summary, we found that skin contamination and environmental shedding of *C. difficile* often persisted after resolution of diarrhea, and recurrent shedding was common 1–6 weeks after therapy. These results lend support for the recommendation that contact precautions be continued until hospital discharge if rates of CDI remain high despite implementation of standard infection-control measures. Studies are needed to assess whether extending the duration of contact precautions for patients with CDI will be helpful as a strategy to reduce transmission.

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