Contamination of Healthcare Workers’ Hands with Clostridium difficile Spores after Caring for Patients with C. difficile Infection

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Contamination of Healthcare Workers’ Hands with *Clostridium difficile* Spores after Caring for Patients with *C. difficile* Infection

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(See the commentary by Pop-Vicas and Baier, on pages 16–17.)

**Objective.** We determined the percentage of healthcare workers’ (HCWs’) hands contaminated with *Clostridium difficile* spores after caring for patients with *C. difficile* infection (CDI) and risk factors associated with contamination.

**Design.** Prospective study.

**Setting.** A French university hospital.

**Methods.** We compared the hand contamination rate among HCWs caring for patients with CDI (exposed group; n = 66) with that among an unexposed group (n = 44). Spores of *C. difficile* were recovered from the hands of HCWs after rubbing their fingers and palms in alcohol shortly after patient care. Associations between hand contamination and HCW category, type (patient or environment), and risk level (high or low risk) of HCW contacts and their respective duration as well as use of gloves were analyzed by bivariate and multivariate analysis.

**Results.** *C. difficile* spores were detected on 24% of HCWs’ hands in the exposed group and on 0% in the unexposed group (P < .001). In the exposed group, logistic regression, which adjusted for high-risk contact (ie, exposure to fecal soiling), contact with the environment, and contact with or without use of gloves, revealed that high-risk contact (adjusted odds ratio [aOR] per 1 contact increment, 2.78; 95% confidence interval [CI], 1.42–5.45; P = .003) and at least 1 contact without use of gloves (aOR, 6.26; 95% CI, 1.27–30.78; P = .02) were independently associated with HCW hand contamination by *C. difficile* spores.

**Conclusions.** Nearly one-quarter of HCWs have hands contaminated with *C. difficile* spores after routine care of patients with CDI. Hand contamination is positively associated with exposure to fecal soiling and lack of glove use.

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*Clostridium difficile*, a gram-positive spore-forming anaerobic bacillus, is a major nosocomial enteropathogen responsible for 15%–25% of antibiotic-associated diarrhea and virtually all cases of pseudomembranous colitis in adults.1,2 The main established risk factors for *C. difficile* infection (CDI) are receipt of antibiotic therapy, age older than 65 years, severity of underlying disease, length of hospital stay,3 and a prior room occupant with CDI.4

Symptomatic patients are known to excrete a large number of organisms in feces, as vegetative organisms or as spores, and bacterial spores are found in abundance in the environment of patients with CDI.5,6,7 *C. difficile* frequently contaminates multiple skin sites, including the groin, chest, abdomen, forearms, and hands of patients with CDI, and can be easily transmitted during contact of those sites.8 Acquisition of spores on gloved hands may occur after contact with commonly touched environmental surfaces (ie, bed rail, bedside table, telephone, and call button) or after contact with commonly examined skin sites.9 Transmission of *C. difficile* strains occurs most commonly via the hands of healthcare workers (HCWs).5,10,11 Several guidelines recommend the use of gloves and washing with soap and water rather than alcohol-based hand rub for mechanical removal of spores from hands in all settings,10–13 whereas the Society for Healthcare Epide-
miology of America (SHEA) guideline advocates hand washing rather than alcohol-based hand rub only during outbreaks or in hyperendemic settings. In prior studies, the proportion of HCWs having hand contamination with both vegetative forms and spores of C. difficile after care of patients with CDI varied between 14% and 59%. Because vegetative forms of C. difficile are killed when exposed to air, whereas their spores are resistant to oxygen, desiccation, and most disinfectants and may persist in the hospital environment for long periods of time, we developed an experimental method for recovery of C. difficile spores by means of alcohol that allowed the destruction of vegetative forms of C. difficile.

The aim of this study was to determine the percentage of HCWs’ hands that are contaminated with C. difficile spores after care of patients with CDI and to analyze risk factors associated with contamination.

**METHODS**

This prospective study was performed from June through August 2007 in a 950-bed French university hospital, at a time when the overall incidence of CDI was 1.3 cases per 10,000 bed-days, with 68% of cases classified as hospital acquired. This hospital was not affected by the highly virulent epidemic-related 027/BI/NAP1 strain.

**Observational Study**

Two groups of HCWs were observed for 8 weeks in 1 intensive care unit, 1 surgical ward, and 4 medical wards. The exposed group was comprised of HCWs who took care of a patient with CDI diagnosed during the study period. A CDI case patient was defined as a patient with a diarrheic stool (ie, a stool that took the shape of the sample container) and a toxin test result positive for C. difficile toxin(s) by use of the ImmuNoCard Toxins A&B assay (Meridian Bioscience). Contact precautions were implemented as soon as results were obtained and until 48 hours after the end of diarrhea. Infection control measures consisted of (1) placing patients into a single-bed room with dedicated equipment; (2) donning a disposable gown with full-length sleeves and a pair of gloves on entering the room; (3) hand hygiene with alcohol-based solution (Sterillium; Bode Chemie) before wearing gloves, before an aseptic task and after body fluid exposure, and hand washing with medicated soap and water followed by use of alcohol-based hand rub after glove removal; and (4) daily environmental cleaning with a hypochlorite-based disinfectant according to French guidelines.

The unexposed group comprised HCWs who took care of control (“non-CDI”) patients. A minimum of 2 control patients per case patient were included. A control patient was defined as a patient not having diarrhea or a history of CDI and hospitalized in a ward where no patient with CDI had been identified at the time of the observation. However, these patients were not screened for asymptomatic carriage of C. difficile.

The same trained observer (M.V.) observed all HCWs while in patient rooms. Staff members were informed that they were being observed as part of a study but were unaware of the study objectives; none refused to be observed. Observations were performed daily (except on weekends) while the diarrhea persisted.

The following data were collected on a standardized questionnaire: (1) bacteriological and clinical characteristics of the case patient (date of CDI onset, date of contact precaution implementation, and presence of diarrhea during observation); (2) HCW category (physicians or medical students, nurses or student nurses, nursing assistants, housekeepers, and other, eg, physical therapists, radiology technicians, psychologists, and social workers); (3) room cleaning before observation; (4) level of risk of patient contact (high or low); high-risk contact was defined by the possibility for the HCWs’ hands to be highly contaminated with fecal material [ie, patient washing, diaper change, bed linen change, handling of bedpan, digital rectal exam, colonoscopy, cleansing enema, and care of urogenital or perineal area], and low-risk contact was defined as all other patient care activities not meeting the criteria for high-risk contact; (5) duration of high- or low-risk contact; (6) type of contact (with patient or with environment); (7) use of gloves (contact with gloves or contact without gloves); and (8) compliance with hand hygiene (ie, use of alcohol-based products before donning gloves and between patient care activities).

**Sampling of HCWs’ Hands**

We developed a method of spore salvage. Twelve milliliters of Schaedler broth (bioMérieux) was inoculated with 2 colonies of a nontoxigenic strain of C. difficile serogroup D (ATCC strain 43597) and incubated anaerobically at 37°C for 5 days. Twelve milliliters of 95% ethanol was added, and the mix was incubated at room temperature for 1 hour. After centrifugation at 3,000g for 20 minutes, the supernatant was discarded, and the pellet containing spores was suspended in 2.2 mL of sterile water. Spore purity was checked by microscopy. Spores were enumerated by plating 100 mL of pure suspension and 10-fold serial dilutions (10⁻¹ to 10⁻⁷) on TCCA (0.1% taurocholate, 250 mg/L cycloserine, and 8 mg/L cefoxitin agar) plates, which were incubated anaerobically at 37°C for 48 hours. One hundred microliters of pure suspension and each dilution were added to 100 mL of 95% ethanol. Filtration through a membrane filter with a pore size of 0.20 μm (Millipore; Billerica) was performed. Filters were placed on TCCA plates. TCCA plates were incubated at 37°C in an anaerobic atmosphere for 48 hours. Colonies of C. difficile were initially identified by their macroscopic appearance and odor, followed by latex agglutination testing (Oxoid C. difficile Test kit; Thermo Scientific). For each sample that
was positive for *C. difficile*, the colonies of *C. difficile* were enumerated (as colony-forming units [CFUs] per plate), and concentration was calculated per 100 μL. The initial and final counts of *C. difficile* spores were compared, and the recovery rate was high (more than 90% of initial count). We validated this method by experimental contamination of hands (data not shown). HCWs’ hands were sampled by the same trained technician (M.V.) at the end of the observation period, just after removing gloves and before performance of hand hygiene with soap and water. Fingers of the dominant hand were rubbed for 1 minute in 80 mL of 95% ethanol, and the palm was rubbed for 30 seconds in square petri dishes (10 cm × 10 cm) with 20 mL of 95% ethanol. Each container was rinsed with 10 mL of 95% ethanol. Alcohol was then filtered through a membrane filter with a pore size of 0.22 μm. Filters were placed on TCCA plates, which were incubated at 37°C in an anaerobic atmosphere for 48 hours. Colonies of *C. difficile* were identified by their macroscopic appearance and odor, followed by latex agglutination testing. *C. difficile* colonies growing on each sample were counted (as CFUs per plate).

### Statistical Analysis

Categorical variables were expressed as numbers and percentages and were compared using the Pearson χ² test or the Fisher exact test, as appropriate. Continuous variables were expressed as medians with interquartile range and were compared using the Mann-Whitney *U* test.

Multivariable logistic regression was used to identify risk factors associated with HCW hand contamination by *C. difficile* spores in the exposed group and estimate their adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Variables with *P* < .05 in the bivariate analyses were selected. Correlation was assessed between high-risk contact and contact with the patient’s environment. If a correlation was detected between 2 variables, only 1 variable was entered in the model. Nonnormally distributed continuous variables were transformed into categorical variables on the basis of the median value. The calibration of the final model was tested using the Hosmer-Lemeshow test. All statistical tests were 2-tailed, and *P* < .05 was considered statistically significant. Analyses were performed with PASW software, version 18 (SPSS).

### Results

During the study observation period, 7 CDI case patients and 16 control patients were included and were cared for by 66 exposed and 44 unexposed HCWs, respectively. All 110 HCWs had their hands sampled. The majority of participating HCWs were nurses and nursing assistants (Table 1).

*C. difficile* spores were recovered from 24% (16/66) samples obtained from hands of exposed HCWs and from none (0/44) of those obtained from unexposed HCWs (*P* < .001). A mean of 2 CFUs (range, 1–6) of *C. difficile* were recovered per positive hand, with the highest numbers found following high-risk care (eg, patient washing and diaper change).

Hand contamination was more common among nursing assistants (42%) than among other HCW categories (19% for nurses and 23% for physicians), consistent with the fact that nursing assistants had more high-risk contacts (47%) than did other HCW categories (15% for nurses and 4% for physicians).

The 66 HCWs in the exposed group had 386 contacts observed with CDI case patients or their environment. In bivariate analysis (Table 2), HCWs with contaminated hands were more likely than their counterparts to have a higher number of contacts overall (*P* = .003) as well as with the patient (*P* = .02) or with his or her environment (*P* = .02). Hand contamination was also associated with a higher number of contacts with a higher duration of high-risk contacts (*P* < .0001). As all CDI patients were under contact precautions, HCWs had to put on gloves to care for patients. Because HCWs with contaminated hands had a higher number of contacts than did HCWs with noncontaminated hands, they also had a higher number of contacts with use of gloves (*P* = .04). However, 7.8% (30/386) contacts occurred with ungloved hands. HCWs with contaminated hands had a higher number of contacts without use of gloves than did HCWs with noncontaminated hands (*P* = .03).

Since high-risk contact was correlated with duration of high-risk contact and contact with the patient was correlated with contact with the patient’s environment (*P* < .001), only the variables depicting high-risk contact and contact with environment were selected for multivariate analysis. Log linearity was verified only for high-risk contact.

Logistic regression, which adjusted for high-risk contact, contact with the environment, contact with use of gloves, and contact without use of gloves, revealed that high-risk contact (aOR per 1 contact increment, 2.78; 95% CI, 1.42–5.45; *P* = .003) and at least 1 contact without use of gloves (aOR, 6.26; 95% CI, 1.27–30.78; *P* = .02) were independently associated with HCW hand contamination by *C. difficile* spores (Table 3).

### Discussion

This study demonstrates that a high proportion of HCWs may carry *C. difficile* spores on their hands after caring for patients with CDI, contrary to HCWs caring for patients without CDI. Contamination of exposed HCWs’ hands was

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**Table 1.** Distribution of Samples by Healthcare Worker Category

<table>
<thead>
<tr>
<th>Category</th>
<th>Exposed group (n = 66)</th>
<th>Unexposed group (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicians or medical students</td>
<td>13 (19.7)</td>
<td>10 (22.7)</td>
</tr>
<tr>
<td>Nurses and student nurses</td>
<td>26 (39.4)</td>
<td>21 (47.7)</td>
</tr>
<tr>
<td>Nursing assistants</td>
<td>19 (28.8)</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>Housekeepers</td>
<td>5 (7.6)</td>
<td>...</td>
</tr>
<tr>
<td>Other</td>
<td>3 (4.5)</td>
<td>...</td>
</tr>
</tbody>
</table>

**Note.** Data are no. (%).
TABLE 2. Bivariate Analysis of Risk Factors Associated with Healthcare Worker Hand Contamination by *Clostridium difficile* Spores after Care of a Patient Having *C. difficile* Infection (Exposed Group)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Contaminated hands (n = 16)</th>
<th>Noncontaminated hands (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthcare worker category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physicians or medical students</td>
<td>3 (18.8)</td>
<td>10 (20.0)</td>
<td>.12</td>
</tr>
<tr>
<td>Nurses and student nurses</td>
<td>5 (31.3)</td>
<td>21 (42.0)</td>
<td></td>
</tr>
<tr>
<td>Nursing assistants</td>
<td>8 (50.0)</td>
<td>11 (22.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>8 (16.0)</td>
<td></td>
</tr>
<tr>
<td>Room disinfection*</td>
<td>5 (31.3)</td>
<td>15 (30.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Total no. of contacts</td>
<td>9 (4–14)</td>
<td>4 (2–6)</td>
<td>.003</td>
</tr>
<tr>
<td>No. of contacts by risk level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>3 (1–5)</td>
<td>0 (0–1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Low risk</td>
<td>4 (3–8)</td>
<td>3 (2–5)</td>
<td>.16</td>
</tr>
<tr>
<td>Contact duration by risk level, minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>12 (3–23)</td>
<td>0 (0–1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Low risk</td>
<td>6 (4–10)</td>
<td>6 (3–8)</td>
<td>.44</td>
</tr>
<tr>
<td>No. of contacts with patient or environment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With patient</td>
<td>3 (1–4)</td>
<td>1 (1–3)</td>
<td>.02</td>
</tr>
<tr>
<td>With environment</td>
<td>5 (2–9)</td>
<td>2 (1–4)</td>
<td>.02</td>
</tr>
<tr>
<td>No. of contacts with and without use of gloves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With gloves</td>
<td>7 (3–13)</td>
<td>4 (2–6)</td>
<td>.04</td>
</tr>
<tr>
<td>Without gloves</td>
<td>0 (0–1)</td>
<td>0 (0–0)</td>
<td>.03</td>
</tr>
<tr>
<td>No. of times alcohol-based hand rub was used or forgotten during care*</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>.7</td>
</tr>
<tr>
<td>Rub used</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td></td>
</tr>
<tr>
<td>Rub forgotten</td>
<td>6 (3–13)</td>
<td>3 (2–5)</td>
<td>.07</td>
</tr>
<tr>
<td>No. of glove withdrawals during care</td>
<td>1 (1–2)</td>
<td>1 (1–1)</td>
<td>.19</td>
</tr>
</tbody>
</table>

Note: Data are no. (%) for categorical variables and median (interquartile range) for continuous variables. NS, nonsignificant.

* Before observation.

* Before final sampling of healthcare workers’ hands.

associated with high-risk contact (ie, direct exposure to fecal soiling) and contact without use of gloves.

To our knowledge, this is the first study focusing on carriage of viable *C. difficile* spores on HCWs’ hands. Because of their resistance and persistence, bacterial spores could be the principal form of transmission. Three prior studies have looked for contamination of HCWs’ hands with *C. difficile* after the care of patients with CDI, and these studies reported carriage rates varying between 14% and 59%.

These studies assessed both vegetative forms and spore contamination on hands. However, no previous study has assessed the respective contribution of vegetative versus spore forms to hand contamination. Since vegetative forms are killed in room air, it is possible that only spores were present on hands. The highest *C. difficile* hand carriage rate (59%) was reported by McFarland et al, who obtained extensive cultures at various sites (hand-surface imprints and fingernail impressions on plates and swabs obtained by wiping the interior surface of rings) from the hands of 35 HCWs, immediately after direct contact with a CDI patient. Struelens et al recovered *C. difficile* from the hands of 2 (29%) of 7 HCWs caring for patients with CDI; samples were obtained by imprint of fingertips immediately after direct contact with affected patients. Samore et al reported a 14% hand carriage rate for *C. difficile* among 73 HCWs by taking fingertip-imprint cultures from HCWs while inside an affected patient’s room or within 30 minutes of leaving the room. The time elapsed between care and sampling might explain the lower proportion of hand contamination in this study. The 24% hand contamination rate recorded in our study is consistent with these prior results, taking into account that our study design aimed at recovering only spores. This relatively high rate suggests that recovery of spores from hands after rinsing with alcohol and filtration is an efficient method for detecting only viable spores of *C. difficile*.

In the exposed group, HCWs having contaminated hands had performed a significantly higher number of contacts as well as high-risk contacts than did those with noncontaminated hands. Duration of high-risk contact was positively associated with hand contamination. To our knowledge, this is the first time that duration of contact has been reported as a risk factor for hand contamination. This finding contrasts with those of McFarland et al, who reported that acquisition of *C. difficile* occurred after contacts ranging from direct exposure to fecal soiling to practices generally considered to pose little risk (such as taking the patients’ temperature, physical examination, or feeding patients). While prior studies did not identify HCW category, we also found that the hands of
nursing assistants were more frequently contaminated with \textit{C. difficile} spores, in accordance with their exposure to more high-risk contacts than other HCW categories.

Hand contamination was also associated with number of contacts with the environment of case patients. Numerous studies have documented environmental contamination with \textit{C. difficile} in the rooms of CDI patients.\textsuperscript{3,5,9,12,19,20} Samore et al\textsuperscript{20} reported that the frequency of positive HCW hand culture was strongly correlated with the intensity of environmental contamination. Using real-time polymerase chain reaction to quantify contamination, Mutters et al\textsuperscript{21} reported a significant correlation between \textit{C. difficile} counts on HCWs’ hands and those on the room floor as well as those in the near-patient environment. Furthermore, contaminated gloves could be responsible for environmental contamination. Manian et al\textsuperscript{22} reported that during a CDI outbreak HCWs failed to remove their potentially stool-contaminated gloves prior to touching clean surfaces, which in turn might have contributed to contamination of blood pressure cuffs.

Several studies have demonstrated that the appropriate use of gloves can prevent transmission of \textit{C. difficile} and reduce the incidence of CDI.\textsuperscript{2,3,5,23} When caring for patients with CDI, HCWs should don gloves immediately upon entering the patient room according to our hospital protocol. Several explanations may account for the contamination of HCWs’ hands despite the wearing of gloves. First, HCWs may have contaminated their hands during the withdrawal of gloves. Although glove removal technique was not evaluated, there was no association between hand contamination and glove removal in our study. Second, gloves may not provide absolute protection against hand contamination because barrier protection could be compromised during use.\textsuperscript{24}

An important observation in our study was that 7 (44%) of 16 HCWs with contaminated hands and 9 (18%) of 50 HCWs without contaminated hands had at least 1 contact without gloves. Furthermore, HCWs with contaminated hands performed a higher number of contacts without gloves than did HCWs with noncontaminated hands. Glove use is the only CDI prevention recommendation with the highest strength of recommendation and quality of evidence rating.

European and CDC guidelines recommend hand hygiene with soap and water after the removal of gloves.\textsuperscript{13,15} The current SHEA guidelines for prevention of CDI during acute care\textsuperscript{16} recommend hand washing only in outbreak or hyperendemic settings and hand hygiene with soap and water or alcohol in routine or endemic settings. Although soap and water is superior to removing \textit{C. difficile} spores from hands of volunteers compared with alcohol-based hand rub products, the rationale for this recommendation\textsuperscript{16,25} is based on a lack of studies in acute care settings demonstrating an increase in CDI with alcohol-based hand rub products or a decrease in CDI with soap and water as well as the lower overall compliance with hand hygiene when using soap and water. Our real-life study identifies contact with ungloved hands as a risk factor for HCW hand contamination and suggests that routine hand washing with soap and water after care of CDI patients should be recommended in all settings.

Our study has some limitations. First, as few patients developed CDI during the study period, the same HCW could be observed on several days and, as a result, improve their infection control practices and adherence to glove use. Thus, we may have underestimated the frequency of hand contamination. Second, samples were not obtained from HCWs’ hands before entry into the room. However, Mutters et al\textsuperscript{21} found no difference in \textit{C. difficile} counts on the hands of HCWs working in either a \textit{C. difficile}–positive ward or a \textit{C. difficile}–negative ward. We thus included in our study a group of HCWs unexposed to patients with CDI as a control group expected to have no hand contamination with \textit{C. difficile} but without matching on high-risk care. Third, the exact number of spores needed to result in infection is not known, so the exact risk of transmission of CDI by a HCW having hands contaminated with spores is unknown. Finally, we evaluated only the proportion of HCWs with \textit{C. difficile} spores on their hands after routine care of patients with CDI and risk factors of contamination; further studies are needed to determine how long spores can remain viable on HCWs’ hands.

### Acknowledgments

We are grateful to the staff of the wards who participated in the study and Dr. Andrew Stewartson for his assistance.

**Potential conflicts of interest.** All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

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### References


2. Bartlett JG. Narrative review: the new epidemic of \textit{Clostridium difficile}.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariate model</th>
<th>aOR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of high-risk contacts</td>
<td>2.78 (1.42–5.45)</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>More than 2 contacts with environment</td>
<td>0.33 (0.03–3.48)</td>
<td>.35</td>
<td></td>
</tr>
<tr>
<td>More than 4 contacts with gloves</td>
<td>0.59 (0.05–6.17)</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>At least 1 contact without gloves</td>
<td>6.26 (1.27–30.78)</td>
<td>.02</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** The Hosmer-Lemeshow $\chi^2$ test was 0.52 ($P = .99$), indicating good calibration of the final model. aOR, adjusted odds ratio; CI, confidence interval.


