

## ORIGINAL ARTICLE

# Hand Hygiene with Soap and Water Is Superior to Alcohol Rub and Antiseptic Wipes for Removal of *Clostridium difficile*

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**OBJECTIVE.** To evaluate common hand hygiene methods for efficacy in removing *Clostridium difficile*.

**DESIGN.** Randomized crossover comparison among 10 volunteers with hands experimentally contaminated by nontoxicogenic *C. difficile*.

**METHODS.** Interventions included warm water with plain soap, cold water with plain soap, warm water with antibacterial soap, antiseptic hand wipes, alcohol-based handrub, and a control involving no intervention. All interventions were evaluated for mean reduction in colony-forming units (CFUs) under 2 contamination protocols: "whole hand" and "palmar surface." Results were analyzed according to a Bayesian approach, by using hierarchical models adjusted for multiple observations.

**RESULTS.** Under the whole-hand protocol, the greatest adjusted mean reductions were achieved by warm water with plain soap (2.14 log<sub>10</sub> CFU/mL [95% credible interval (CrI), 1.74–2.54 log<sub>10</sub> CFU/mL]), cold water with plain soap (1.88 log<sub>10</sub> CFU/mL [95% CrI, 1.48–2.28 log<sub>10</sub> CFU/mL]), and warm water with antibacterial soap (1.51 log<sub>10</sub> CFU/mL [95% CrI, 1.12–1.91 log<sub>10</sub> CFU/mL]), followed by antiseptic hand wipes (0.57 log<sub>10</sub> CFU/mL [95% CrI, 0.17–0.96 log<sub>10</sub> CFU/mL]). Alcohol-based handrub (0.06 log<sub>10</sub> CFU/mL [95% CrI, –0.34 to 0.45 log<sub>10</sub> CFU/mL]) was equivalent to no intervention. Under the palmar surface protocol, warm water with plain soap, cold water with plain soap, and warm water with antibacterial soap again yielded the greatest mean reductions, followed by antiseptic hand wipes (26.6, 26.6, 26.6, and 21.9 CFUs per plate, respectively), when compared with alcohol-based handrub. Hypothenar (odds ratio, 10.98 [95% CrI, 1.96–37.65]) and thenar (odds ratio, 6.99 [95% CrI, 1.25–23.41]) surfaces were more likely than fingertips to remain heavily contaminated after handwashing.

**CONCLUSIONS.** Handwashing with soap and water showed the greatest efficacy in removing *C. difficile* and should be performed preferentially over the use of alcohol-based handrubs when contact with *C. difficile* is suspected or likely.

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The incidence and severity of *Clostridium difficile* infection (CDI) have increased over the past several years, as documented in numerous reports.<sup>1-6</sup> Prevention of nosocomial transmission is critical to controlling CDI outbreaks, and hand hygiene is an important component of infection prevention and control programs.<sup>7-10</sup> However, the efficacy of different common hand hygiene interventions against *C. difficile* has not been thoroughly investigated. One study, apparently published only in abstract form, revealed that alcohol-based gels were inferior to chlorhexidine soap for removal of *C. difficile* spores.<sup>11</sup> Lacking sufficient experimental evidence, hand hygiene guidelines have been limited to recommendations based on expert opinion, such as those from the Healthcare Infection Control Practices Advisory Committee: "After gloves are removed, hands should be washed with a non-antimicrobial or an antimicrobial soap and water or disinfected with an alcohol-based handrub. During out-

breaks of *C. difficile*-related infections, washing hands with a non-antimicrobial or antimicrobial soap and water after removing gloves is prudent."<sup>12</sup> In this study, 5 commonly used hand hygiene procedures were evaluated for their efficacy in removing *C. difficile* from experimentally contaminated hands of volunteers.

## METHODS

### Test Organism

A nontoxicogenic strain of *C. difficile* was used for the study (ATCC 700049, American Type Culture Collection). The organism was incubated anaerobically in brain-heart infusion broth at 37°C for 48 hours to produce a seed culture, which was used to inoculate flasks containing 500 mL of brain-heart infusion broth. The flasks were incubated anaerobically at 37°C for 48 hours and portioned into 20-mL aliquots (hence-

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forth referred to as “the inoculum”). The inoculum was stored at  $-80^{\circ}\text{C}$  until use. Quantitation of the inoculum was assessed by serial dilution and incubation on cycloserine-cefoxitin fructose agar plates supplemented with 5% sheep’s blood and 0.1% taurocholate (CCFA-T),<sup>13</sup> and the percentage of spore forms in the inoculum was measured with the use of an alcohol shock method followed by serial dilution onto CCFA-T.<sup>14</sup>

### Subjects

Ten hospital laboratory workers volunteered for this study, and informed consent was obtained prior to participation in the study. No volunteers had any dermatologic diseases affecting their hands at any point during the study. No limitations were set on fingernail length or on the presence of rings or other hand jewelry, nor were any data collected on these variables. No specific training in handwashing techniques was provided to the volunteers at any time during the study. Ethical approval was obtained from the Research Ethics Board of the McGill University Health Centre.

### Hand Hygiene Interventions

A crossover format was used so that all volunteers would be exposed to all interventions once for each contamination protocol during the observation period of June–July 2007. The order of interventions for each volunteer was assigned by means of computer-generated random number lists. The hand hygiene interventions studied were (1) warm ( $30^{\circ}\text{C}$ ) water with plain soap (Hygenipak Instafoam, Deb Canada), (2) cold ( $15^{\circ}\text{C}$ ) water with plain soap (Hygenipak Instafoam, Deb Canada), (3) warm ( $30^{\circ}\text{C}$ ) water with 2% chlorhexidine antibacterial soap (Hygenipak 2% CHG foaming skin cleanser, Deb Canada), (4) antiseptic hand wipes with 40% vol/vol ethanol and 0.5% parachlorometaxylenol (Sani-dex antimicrobial hand wipes, PDI), (5) alcohol-based handrub with 70% vol/vol isopropanol (Manorapid, Antiseptica, Pulheim-Brauwiler), and (6) a no-wash negative control. Each intervention using soap and water was timed for 10 seconds of lathering, followed by brief rinses with water and drying with paper towels. Use of antiseptic hand wipes and alcohol rubs was each timed for 15 seconds. Hands were allowed to air dry for 1 minute after use of antiseptic handwipe and alcohol-based handrub. Participants washed their hands with plain soap and water every time they entered or exited the laboratory. A minimum of 24 hours between interventions for each volunteer was mandated to reduce carryover of residual handwashing agents or bacteria.

### Whole-Hand Protocol

The 20 mL of inoculum was poured into a clean, tight-fitting nitrile glove. Each hand was sequentially placed into the glove, and the inoculum was gently dispersed throughout the glove for 30 seconds. The hands were allowed to air dry for 1–2 minutes, and then one of the hand hygiene interventions was

performed. Subsequently, each hand was placed into a new glove containing 20 mL of sterile brain-heart infusion broth, which was gently dispersed for 30 seconds. A 1-mL sample of the broth was removed via pipette for quantification by serial 10-fold dilution on CCFA-T and incubated anaerobically at  $37^{\circ}\text{C}$  for 48 hours. If a plate yielded 1–100 colony-forming units (CFUs), the colony counts were recorded.

### Surface Contamination Protocol

A ceramic tile measuring  $20 \times 25$  cm was painted with 1 mL of the inoculum and dried for at least 30 minutes at room temperature. Both palms were placed on the contaminated surface for 5 seconds, and then one of the hand hygiene interventions was performed. Subsequently, hand impressions were made onto 6 separate CCFA-T plates for 5 seconds each: 1 plate each for the fingertips, hypothenar surface, and thenar surface of each hand. If a plate yielded 0–50 colonies, the colony counts were recorded; plates with more than 50 colonies per plate or confluent growth were evaluated as “too numerous to count.”

### Statistical Analysis

Previous studies of hand hygiene have shown that effective agents can reduce bacterial loads by approximately  $2.0 \log_{10}$ , with a standard deviation of  $0.4 \log_{10}$ .<sup>7</sup> A difference of at least  $1 \log_{10}$  between interventions was considered to be an important difference. With 10 paired assessments for each product, a power of more than 99% to detect a  $1.0 \log_{10}$  difference was calculated. Hierarchical models, adjusted for the effect of multiple observations on each subject, were used to compare the different interventions. For the whole-hand data, the model was fit by using the base-10 logarithm of the *C. difficile* colony count (CFUs per milliliter). For the surface data, a 2-part model was used that estimated (1) the proportion of observations with scores of “too numerous to count” for each intervention and (2) the median colony count among all plates with countable colonies (0–50 colonies per plate). Both models adjusted for the effect of right versus left hand. The surface model also adjusted for the hand area (hypothenar, thenar, or fingertips) tested. Both models were estimated using a Bayesian approach. A noninformative prior distribution was used for both models, and the posterior median and 95% credible interval (CrI) for the parameters of interest were extracted. The R statistical software package (version 2.3.1; R Foundation for Statistical Computing) was used for descriptive statistics and graphs. The models were fit with WinBUGS (version 1.4.1; MRC Biostatistics Unit).

## RESULTS

### Inoculum of Test Organism

The concentration of *C. difficile* test organism used was  $1.4 \times 10^5$  CFU/mL, consisting of 62% spores. Preliminary testing, including turbidimetry, indicated that this concen-

TABLE 1. Mean *Clostridium difficile* Colony Counts after Different Hand Hygiene Interventions According to the Whole-Hand Protocol

Intervention	Mean count (95% CI), log <sub>10</sub> CFU/mL
Warm water and plain soap	1.99 (1.80–2.09)
Cold water and plain soap	1.90 (1.58–2.22)
Warm water and antibacterial soap	2.31 (2.04–2.58)
Antiseptic hand wipe	3.25 (3.04–3.45)
Alcohol-based handrub	3.74 (3.40–4.07)
No intervention	3.82 (3.54–4.10)

NOTE. CI, confidence interval; CFU, colony-forming unit.

tration was routinely achievable (data not shown). This concentration was selected to ensure that *C. difficile* colonies remain detectable following hand hygiene interventions, thus allowing a precise measurement of effect size. Furthermore, this concentration is biologically and clinically relevant, because in a previous study, 1 research group has measured concentrations of *C. difficile* directly from stool specimens of patients both before and during treatment for CDI that were within 1.5 log<sub>10</sub> of this value.<sup>15</sup>

#### Whole-Hand Protocol

A total of 120 observations (10 participants, 2 hands each, 5 interventions, and 1 negative control) were compared in a pairwise fashion. The results are summarized as mean log<sub>10</sub> colony counts after intervention (Table 1) and comparison of log<sub>10</sub> colony count reductions between pairs of interventions (Table 2). The largest reductions were observed for plain soap with the use of either warm or cold water versus either no hand hygiene or alcohol-based handrub. Antibacterial soap had the next largest statistically significant effect, fol-

lowed by antiseptic hand wipes. Finally, the effect of the alcohol-based handrub was not significantly different from the effect of not washing.

#### Palmar Surface Protocol

A total of 318 observations (for interventions: 10 participants, 2 hands each, 3 surfaces per hand, 5 interventions; for negative control: 3 participants, 2 hands each, 3 surfaces per hand) were taken; note that the negative control group included only 3 participants, as colony counts were uniformly too numerous to count. For each intervention, the probability of observing plates evaluated as heavily contaminated (too numerous to count) is displayed in Table 3. The likelihood of heavy contamination was high for alcohol-based handrub although significantly lower than for no hand hygiene intervention. Thus, alcohol-based handrub did produce a significant reduction in contamination although of a lesser magnitude than did the other hand hygiene interventions. The comparison of colony count reductions between pairs of interventions is shown in Table 4. The largest reductions in colony counts were observed after any of the interventions involving plain or antibacterial soap versus alcohol-based handrub; the next largest reductions were after antiseptic handwipe versus alcohol-based handrub, with soap-based interventions demonstrating a small advantage over antiseptic hand wipes.

Comparing the 3 different anatomical areas of the hand, odds ratios for a score of “too numerous to count” for observations from hypothenar versus fingertips (median odds ratio, 7.98 [95% CrI, 1.96–37.65]) and thenar versus fingertips (median odds ratio, 5.14 [95% CrI, 1.25–23.41]) both suggested that fingertips were less likely than other hand areas

TABLE 2. Pairwise Comparison of Mean Reduction in *Clostridium difficile* Colony Count (Intervention 1 vs Intervention 2) for Hand Hygiene Interventions Used According to the Whole-Hand Protocol

Interventions compared		Mean log reduction (95% CI), log <sub>10</sub> CFU/mL
Intervention 1	Intervention 2	
Warm water and plain soap	No hand hygiene	2.14 (1.74–2.54)
Warm water and plain soap	Alcohol-based handrub	2.08 (1.69–2.47)
Cold water and plain soap	No hand hygiene	1.88 (1.48–2.28)
Cold water and plain soap	Alcohol-based handrub	1.82 (1.43–2.22)
Warm water and plain soap	Antiseptic hand wipe	1.57 (1.18–1.96)
Warm water and antibacterial soap	No hand hygiene	1.51 (1.12–1.91)
Warm water and antibacterial soap	Alcohol-based handrub	1.46 (1.06–1.85)
Cold water and plain soap	Antiseptic hand wipe	1.31 (0.92–1.71)
Warm water and antibacterial soap	Antiseptic hand wipe	0.94 (0.55–1.34)
Warm water and plain soap	Warm water and antibacterial soap	0.63 (0.23–1.02)
Antiseptic hand wipe	No hand hygiene	0.57 (0.17–0.96)
Antiseptic hand wipe	Alcohol-based handrub	0.51 (0.12–0.91)
Cold water and plain soap	Warm water and antibacterial soap	0.37 (–0.03 to 0.76)
Warm water and plain soap	Cold water and plain soap	0.26 (–0.14 to 0.66)
Alcohol-based handrub	No hand hygiene	0.06 (–0.34 to 0.45)

NOTE. Adjusted for multiple observations on each individual and the effect of left versus right hand. CI, confidence interval; CFU, colony-forming unit.

TABLE 3. Probability of Heavy Contamination (Colonies Too Numerous to Count) Following Different Hand Hygiene Interventions Used According to the Palmar Surface Protocol

Intervention	Median probability (95% CI)
Warm water and plain soap	0 ( $4.0 \times 10^{-11}$ to 0.017)
Cold water and plain soap	0 ( $4.7 \times 10^{-11}$ to 0.018)
Warm water and antibacterial soap	0 ( $3.8 \times 10^{-11}$ to 0.018)
Antiseptic hand wipe	0.05 (0.012 to 0.12)
Alcohol-based handrub	0.43 (0.31 to 0.55)
No hand hygiene	1

NOTE. Adjusted for multiple observations on each individual.

to remain heavily contaminated with *C. difficile* following a hand hygiene intervention.

## DISCUSSION

Spore-forming organisms such as *C. difficile* pose unique problems for infection control in healthcare facilities. Any hand hygiene intervention involves some combination of both chemical and physical actions, but none of the antiseptic agents routinely used in hand hygiene is reliably sporicidal.<sup>12</sup> Physical removal of spores from the hands of healthcare workers therefore seems to be important in limiting the spread of these organisms within an institution. Recent work suggesting that asymptomatic carriers may be an important source for transmission of epidemic and non-epidemic *C. difficile* further complicates this problem.<sup>16</sup> The role of alcohol-based hand-rub solutions in the spread of CDI remains controversial. Some clinicians have hypothesized that alcohol-based hand-rub solutions, because they lack sporicidal activity, may have contributed to increased CDI incidence. While some data exist to support this hypothesis,<sup>17</sup> several other studies have revealed no change or decreased CDI rates when alcohol-based handrub use has increased.<sup>18-20</sup> Healthcare Infection Control Practices Advisory Committee guidelines reflect this

uncertainty by stating that the use of soap and water after glove removal is prudent in the setting of a *C. difficile* outbreak, although this is not emphasized in their final list of recommendations.<sup>12</sup> Our study provides experimental evidence to support the use of soap and water for hand hygiene if contamination by *C. difficile* is known or probable.

Our data that demonstrate in vitro superiority of soap-based hand hygiene to an alcohol-based handrub for removal of *C. difficile* are consistent with prior observations.<sup>11</sup> The reason that antibacterial soap seems slightly inferior to plain soap according to the whole-hand protocol but not according to the palmar surface protocol is uncertain. This may be due to a higher concentration of organic matter present in the whole-hand protocol, which interferes with the activity of chlorhexidine.<sup>21</sup> Antiseptic hand wipes probably remove *C. difficile* spores by mechanical means, and, although demonstrated to be inferior to soap and water, they may be an alternative in situations where handwashing is impossible, such as for immobilized patients. The partial effectiveness demonstrated by alcohol-based handrub in the palmar surface protocol may have been due to displacement of organisms onto dorsal areas of the hand, which were not measured under this protocol. However, partial removal of *C. difficile* from areas of the hand frequently used during routine clinical care may still be preferable to no removal.

We chose to simulate real-life conditions by including hand hygiene interventions, methods, and durations that more closely reflect actual practice<sup>22-25</sup> than do guideline recommendations.<sup>12</sup> Handwashing with cold water was evaluated because it is frequently used by healthcare workers, as a result of time constraints, inadequate facilities, or concern that warm water contributes to skin irritation.<sup>26</sup> Antiseptic hand wipes were evaluated because they are used in some institutions for patients who cannot ambulate or be brought to sinks for routine handwashing.

This study has several limitations. This in vitro study was

TABLE 4. Pairwise Comparison of Mean Reduction in *Clostridium difficile* Colony Count (Intervention 1 vs Intervention 2) for Hand Hygiene Interventions Used According to the Palmar Surface Protocol

Interventions compared		Mean log reduction (95% CI), log <sub>10</sub> CFU per plate
Intervention 1	Intervention 2	
Warm water and antiseptic soap	Alcohol-based handrub	26.64 (24.47-28.81)
Warm water and plain soap	Alcohol-based handrub	26.63 (24.48-28.77)
Cold water and plain soap	Alcohol-based handrub	26.56 (24.42-28.71)
Antiseptic hand wipe	Alcohol-based handrub	21.92 (19.73-24.09)
Warm water and antibacterial soap	Antiseptic hand wipe	4.72 (2.59-6.59)
Warm water and plain soap	Antiseptic hand wipe	4.71 (2.94-6.50)
Cold water and plain soap	Antiseptic hand wipe	4.64 (2.85-6.45)
Warm water and plain soap	Cold water and plain soap	0.07 (-1.71-1.84)
Warm water and antibacterial soap	Warm water and plain soap	0.01 (-1.77-1.83)
Warm water and antibacterial soap	Cold water and plain soap	0.08 (-1.74-1.90)

NOTE. Adjusted for multiple observations on each individual and the effect of left vs right hand. CI, confidence interval; CFU, colony-forming unit.

designed to assess hand carriage of *C. difficile*, but results may not extrapolate to clinically important outcomes such as nosocomial CDI rates. All of the handwashing interventions studied were performed for less time than recommended by the manufacturers of the products used, so their comparative efficacy when used according to the manufacturer's instructions remains uncertain. Although specific instructions on hand hygiene technique were not provided to the healthcare workers participating in this study, education is provided to all employees, and signage illustrating proper technique is posted at all handwashing sinks in our institution. More diligent adherence to recommended hand hygiene technique might further reduce hand contamination, but overall compliance with handwashing practices by healthcare workers is regrettably low<sup>27</sup> and, in any case, would be unlikely to alter the relative efficacy of the interventions. Finally, our study protocols relied on direct contamination of bare hands; the effect of gloving hands prior to contamination was not evaluated, although it would have increased the applicability of our results to include healthcare workers following current contact precaution guidelines.<sup>28</sup>

The use of 2 different contamination protocols in this study provides complementary information. The whole-hand protocol is based on a well-studied method for comparing relative efficacies of different handwashing agents, commonly referred to as the glove juice method.<sup>7</sup> This method allows for the precise calculation of residual contamination, usually quantified as "log reductions" in the infection control literature. The palmar surface protocol could detect only a limited range of contamination densities but more closely simulated the type of hand contamination that occurs within healthcare facilities, and it examined the areas of the hand most likely involved in nosocomial transmission. Higher colony counts from hypothenar and thenar surfaces than from fingertips after hand hygiene interventions may be due to less vigorous washing of palmar surfaces or to better adhesion of *C. difficile* to these surfaces, although an effect from a smaller total surface area for fingertips than other hand regions cannot be excluded, because colony counts were not adjusted for surface area. The overall similarity of the intervention effects between the 2 study protocols supports the validity of our findings.

Alcohol-based handrubs have been documented to be at least as effective as soap and water in a variety of healthcare settings and for several different microorganisms.<sup>29,30</sup> In addition, the convenience of these handrubs may result in higher user adherence rates than for soap and water,<sup>31</sup> while the relative ease and lower cost of installing dispensers instead of sinks makes them attractive for institutions. As the current study demonstrates, however, *C. difficile* is a particular organism for which alcohol-based handrubs seem to be inferior to soap and water. These data could be incorporated into future hand hygiene guidelines supporting the use of soap and water after known or probable contamination with *C. difficile*. The challenge facing healthcare facilities is how best to reduce the transmission of *C. difficile* without compro-

promising the important benefits of using alcohol-based rubs in preventing the spread of other nosocomial pathogens.

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