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Major Article

Efficacy of the World Health Organization–recommended handwashing technique and a modified washing technique to remove *Clostridium difficile* from hands

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Background: The efficacy of the World Health Organization (WHO)–recommended handwashing technique against *Clostridium difficile* is uncertain, and whether it could be improved remains unknown. Also, the benefit of using a structured technique instead of an unstructured technique remains unclear.

Methods: This study was a prospective comparison of 3 techniques (unstructured, WHO, and a novel technique dubbed WHO shortened repeated [WHO-SR] technique) to remove *C difficile*. Ten participants were enrolled and performed each technique. Hands were contaminated with 3×10^6 colony forming units (CFU) of a nontoxigenic strain containing 90% spores. Efficacy was assessed using the whole-hand method. The relative efficacy of each technique and of a structured (either WHO or WHO-SR) vs an unstructured technique were assessed by Mann-Whitney *U* test and Wilcoxon signed-rank test.

Results: The median effectiveness of the unstructured, WHO, and WHO-SR techniques in $\log_{10}$ CFU reduction was 1.30 (interquartile range [IQR], 1.27-1.43), 1.71 (IQR, 1.34-1.91), and 1.70 (IQR, 1.54-2.42), respectively. The WHO-SR technique was significantly more efficacious than the unstructured technique ($P = .01$). Washing hands with a structured technique was more effective than washing with an unstructured technique (median, 1.70 vs 1.30 $\log_{10}$ CFU reduction, respectively; $P = .007$).

Conclusions: A structured washing technique is more effective than an unstructured technique against *C difficile*.

Clostridium difficile infections (CDIs) cause significant morbidity and mortality.[1] Patients with CDI shed spores in their environment that can contaminate health care workers' hands.[2] Consequently, the World Health Organization (WHO) recommends performing handwashing rather than hand rubbing with an alcohol-based solution after contact with a patient with CDI, especially in the context of outbreaks.[3] The organization also recommends a standardized structured technique to ensure efficacy and reproducibility.[3] However, even though the efficacy of this technique to remove various bacteria such as *Escherichia coli* has been largely demonstrated, its efficacy to remove *C difficile* spores is unknown.

Furthermore, despite being the hand cleansing method of choice against *C difficile*, handwashing remains relatively inefficient to remove spores from contaminated hands.[4-10] Several studies have shown that an unstructured handwashing technique (ie, washing without following a standardized protocol) is associated with only a 0.78-2 $\log_{10}$ reduction in *C difficile* load.[4-10] By comparison, hand hygiene with alcohol-based formulations is associated with a 4-5 $\log_{10}$ reduction in *E coli*.[3] Whether the performance of a structured technique would confer any benefit over an unstructured technique remains unknown. Also, whether the efficacy of the WHO handwashing technique to remove *C difficile* could be improved remains to be determined. Consequently, we performed a

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prospective study (1) to evaluate the efficacy of the WHO-recommended technique and (2) to evaluate the efficacy of 2 additional handwashing methods: an unstructured method, and a method that involves the performance of a modified WHO technique (dubbed the WHO shortened repeated [WHO-SR] technique). Preliminary exploratory studies have suggested that this method may be superior to the WHO technique, and it was therefore included in the current prospective study.

METHODS

This study is a prospective crossover study assessing the efficacy of 3 techniques to remove a nontoxigenic strain of *C difficile* from the hands of healthy volunteers. Each participant performed the 3 techniques (unstructured, WHO, and WHO-SR) starting with the unstructured technique. The order of the second and third technique (WHO or WHO-SR) was determined randomly. Participants had never learned any formal handwashing technique prior to this study, did not have any skin condition, and did not wear jewelry. Procedures in any given patient were at least 72 hours apart to prevent a carryover effect. Teaching of the WHO and WHO-SR techniques was conducted after the unstructured handwash. The following variables were controlled for each trial: volume of soap (3 mL per wash episode), water flow (4 L/min), and water temperature (40°C ± 1°C). Each trial used the same sink, brand of nonantimicrobial soap (Aquaress; Deb Group, Denby, U.K.), and hand drying paper (Decor; Cascades, Kingsey Falls, QC, Canada). The study was approved by the institutional research ethics committee.

Handwashing techniques

For the unstructured technique, participants were allowed to wash their hands as they would at home without any specific requirements. They were neither monitored nor timed. For the WHO technique, participants followed each step recommended by the WHO, including wetting of hands (10 seconds), soaping (20 seconds), rinsing (15 seconds), and drying of hands (15 seconds) (Fig 1).[3] The entire procedure lasted 60 seconds. For the WHO-SR technique, participants performed the entire WHO technique over 30 seconds rather than 60 seconds, and repeated the technique twice in a row, so that the total amount of time spent performing each step (wetting, soaping, rinsing, and drying) and the entire procedure were the same as the WHO technique (60 seconds) (Fig 2). The underlying hypothesis for this WHO-SR technique was that 2 consecutive but shorter handwashes would be more effective than a single, longer handwash by avoiding water-soap saturation with epidermal material (skin cells and microbes).

Preparation of seeding solution

We prepared a standardized seeding solution containing a high proportion of *C difficile* spores using a protocol adapted from Hasan et al.[11] Spores were prepared by inoculating brain-heart infusion broths with a nontoxigenic strain (ATCC 700059; ATCC, Rockville, MD). The broths were then incubated in an anaerobic chamber at 36°C for 7-10 days and were gently mixed once daily. Starting on day 7, we periodically assessed the level of sporulation by direct examination with malachite green staining. Once the proportion of spores reached 70%, the broths were centrifuged thrice at 4,500 *g* for 15 minutes, followed by decantation of the supernatant and washing of the residual pellets with a 30-mL solution of phosphate-buffered saline (PBS) and 0.1% Triton X-100 surfactant (Dow Chemical Canada ULC, Varennes, Canada). These pellets were then suspended in tubes containing 5 mL of PBS and 10% glycerol and enriched by heat-shock immersion in a water bath at 65°C for 10 minutes. Finally, the tubes were pooled together and were supplemented with PBS and 10% glycerol solution to reach a final solution of 210 mL. The final solution was aliquoted into seeding tubes of 7 mL and stored at –80°C.

We quantified the spore load of 3 randomly selected aliquots by serial 10-fold dilutions onto prereduced cycloserine cefoxitin fructose agars with horse blood and taurocholate as described previously.[12] The proportion of spores was determined by direct microscopy with malachite green staining. The 3 samples yielded a concentration of 1.0×10^6 colony forming units (CFU)/mL with 90% spores.

Assessment of handwashing techniques efficacy

Efficacy of handwashing was assessed using a modified whole-hand test method (ASTM E1174-13).[13] Briefly, this test method involves the artificial contamination of the entire surface of both hands with a known inoculum of *C difficile* followed by recovery by elution and quantification of remnant spores from the skin after the handwashing technique under study has been performed. Each assessment involved 2 contamination procedures with 3 mL of seeding solution. The first contamination procedure was followed by recovery of cells by elution and served as a baseline value. The second procedure involved the performance of the handwashing technique under study after seeding but prior to elution. Elution was performed by placing the subject's hands in nonpowdered latex gloves (AMD Ritmed Premium, Lachine, Canada) filled with 75 mL PBS plus Triton X-100 elution solution. Hands were then massaged for 60 seconds, and 5 mL of the solution were sampled for *C difficile* quantification by serial dilution on cycloserine cefoxitin fructose agars with horse blood and taurocholate agar plates.

The efficacy of the washing techniques to reduce *C difficile* loads on hands was determined by computing the arithmetic difference between the number of *C difficile* cells recovered after the performance of the washing technique under study and the number of *C difficile* cells that was recovered from contaminated hands that did not undergo handwashing (baseline).

Statistical analysis

The efficacy of each technique to remove *C difficile* was expressed in CFU reduction on a logarithmic scale ($\log_{10}$ CFU reduction) as a median and interquartile range (IQR). The relative efficacy of each technique was compared using the Wilcoxon signed-rank test for paired samples. To analyze the benefit of performing a structured technique instead of an unstructured technique, we regrouped the WHO and WHO-SR techniques and compared their efficacy with the unstructured technique using the Mann-Whitney *U* test. A *P* value <.05 was considered to indicate statistical significance. All data were analyzed using SPSS version 20.0 statistical software (SPSS, Chicago, IL).

RESULTS

Ten participants were recruited (6 men and 4 women) between June and August 2012. A total of 60 hand contaminations were performed (30 for baseline and 30 for handwashing techniques). The global efficacy of each technique is shown in Figure 3. The median effectiveness (IQR) of the unstructured, WHO, and WHO-SR techniques was 1.30 (IQR, 1.27-1.43), 1.71 (IQR, 1.34-1.91), and 1.70 (IQR, 1.54-2.42), respectively. Overall, washing hands with a structured technique (either WHO or WHO-SR technique) was more effective than washing with an unstructured technique to remove *C difficile* (median, 1.70 vs 1.30 $\log_{10}$ CFU reduction, respectively; $P = .007$). When comparing each of these methods, the WHO-SR technique