Comparison of Hand Hygiene Procedures for Removing
*Bacillus cereus* Spores

TEPPEI SASAHARA*, SHUNJI HAYASHI, KOICHI HOSODA, YUJI MORISAWA, AND YOSHIKAZU HIRAI

Department of Infection and Immunity, School of Medicine, Jichi Medical University,
3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

Received 9 September, 2013/Accepted 16 December, 2013

*Bacillus cereus* is a spore-forming bacterium. *B. cereus* occasionally causes nosocomial infections, in which hand contamination with the spores plays an important role. Therefore, hand hygiene is the most important practice for controlling nosocomial *B. cereus* infections. This study aimed to determine the appropriate hand hygiene procedure for removing *B. cereus* spores. Thirty volunteers’ hands were experimentally contaminated with *B. cereus* spores, after which they performed 6 different hand hygiene procedures. We compared the efficacy of the procedures in removing the spores from hands. The alcohol-based hand-rubbing procedures scarcely removed them. The soap washing procedures reduced the number of spores by more than 2 log. Extending the washing time increased the spore-removing efficacy of the washing procedures. There was no significant difference in efficacy between the use of plain soap and antiseptic soap. Handwashing with soap is appropriate for removing *B. cereus* spores from hands. Alcohol-based hand-rubbing is not effective.

Key words: *Bacillus cereus* / Bacterial spore / Hand hygiene / Handwashing / Alcohol-based hand-rubbing.

Hand hygiene is one of the most important practices for preventing patient-to-patient transmission of microorganisms. Current hand hygiene guidelines recommend alcohol-based hand-rubbing as a routine practice, because it results in the removal of microorganisms from hands effectively, conveniently, and quickly (Boyce et al., 2002 : Cookson et al., 2009 : Pittet et al., 2009 : Pratt et al., 2007). However, several bacterial species form spores that are highly resistant to alcohol, and therefore alcohol-based hand-rubbing may have no effect in preventing nosocomial transmission of spore-forming bacteria (Russell, 1990). This problem has not been sufficiently addressed to date.

*Bacillus cereus* is a spore-forming gram-positive rod that causes various infectious diseases, such as food poisoning, bacteremia, meningitis, pneumonia, and endocarditis (Bottone, 2010). *B. cereus* spores are ubiquitous in various environments including hospitals (Ohsaki et al., 2007 : Sasahara et al., 2011), and thus health care workers’ hands are often contaminated with the spores (Van Der Zwet et al., 2000). In addition, it has been reported that hand contamination with *B. cereus* can cause nosocomial infections (Sasahara et al., 2011 : Van Der Zwet et al., 2000). Given these facts, appropriate hand hygiene procedures are needed for removing *B. cereus* spores from hands. In the present study, we compared the efficacy of several hand hygiene procedures (Table 1) in removing *B. cereus* spores from hands.

Thirty healthy volunteers (15 men and 15 women, aged between 20 and 54 years, mean 29.1 years) participated in this study, and informed consent was obtained from all subjects prior to participation. The skin of all subjects’ hands was in normal condition. This study was approved by the institutional ethics committee of Jichi Medical University.

One standard strain (*B. cereus* ATCC 10876) and 4 clinical strains of *B. cereus* were used as the test microorganisms for this study. The clinical strains were obtained from blood samples of patients with *B. cereus* bacteremia. The *B. cereus* strains were inoculated on
isms adhering to the subjects' hands, they washed their hands before the examinations with a plain liquid soap (Kao Co.) for more than 60 seconds. Hands were also thoroughly disinfected with an alcohol-based liquid sanitizer (Kenei Pharma. Co. Ltd.). The washed and disinfected hands were experimentally contaminated by spreading 2.5 mL of a test microorganism suspension on each hand, and then the subjects performed each of the 6 hand hygiene procedures listed in Table 1. Subjects did not perform hand hygiene procedures in the control experiment. Subsequently, hand contamination with the test microorganism was assessed using the glove juice method (FDA, 1974; FDA, 1978), and the degree of contamination was expressed as CFU per hand. The efficacy of each hand hygiene procedure was determined by log₁₀ reduction of hand contamination between the control and each hand hygiene procedure. Data were statistically analyzed using SPSS version 19 (IBM Co. Ltd., Somers, NY, USA). The efficacy of the hand hygiene procedures was compared using the analysis of variance (ANOVA) and Tukey's test. P < 0.05 was considered significant.

TABLE 1. Hand hygiene procedures and their protocols

| Hand hygiene procedure                | Protocol                                                                 |
|---------------------------------------|--------------------------------------------------------------------------|
| Handwashing procedures                |                                                                          |
| Water-only washing                    | Hands were washed under running water for 30 seconds.                    |
| 60-second plain soap washing          | Hands were washed with 3 mL of a plain liquid soap (Kao Co.) for 60 seconds and then rinsed under running water for 30 seconds. |
| 30-second plain soap washing          | Hands were washed with 3 mL of a plain liquid soap (Kao Co.) for 30 seconds and then rinsed under running water for 30 seconds. |
| 30-second antiseptic soap washing     | Hands were washed with 3 mL of an antiseptic liquid soap (Kao Co.) for 30 seconds and then rinsed under running water for 30 seconds. |
| Alcohol-based hand-rubbing procedures |                                                                          |
| Rubbing with an alcohol-based liquid   | Hands were rubbed together with 3 mL of an alcohol-based liquid sanitizer (Kenei Pharma. Co. Ltd.) for 30 seconds. |
| Rubbing with an alcohol-based gel      | Hands were rubbed together with 3 mL of an alcohol-based gel sanitizer (GOJO Inds. Inc.) for 30 seconds. |

A volume of 3 mL of each product was applied to the hand, according to previous studies. (Larson et al., 1987; Sugawara et al., 2010)

One standard strain (Escherichia coli ATCC 11229) and 4 fecal strains of E. coli were also used for this study. The fecal strains were obtained from healthy adults. The strains were inoculated on nutrient agar plates (Nissui Pharma. Co. Ltd.), and then the plates were incubated at 37°C for 7 days. Thereafter, the bacterial cells were harvested from the plates and suspended in sterile saline. The suspensions, which contained vegetative forms and spores, were heated at 65°C for 30 minutes to kill vegetative forms. The spore suspensions were adjusted to concentrations of approximately 10⁶ colony-forming units (CFU) per mL with sterile saline.

A plain liquid soap (Kao Co., Tokyo, Japan) and an antiseptic liquid soap containing triclosan (Kao Co.) were used for handwashing. An alcohol-based liquid sanitizer (76.9-81.4% v/v ethanol: Kenei Pharma. Co. Ltd., Osaka, Japan) and an alcohol-based gel sanitizer (76.9-81.4% v/v ethanol: GOJO Inds. Inc., Akron, Ohio, USA) were used for hand-rubbing.

In order to minimize the effect of extant microorganisms adhering to the subjects' hands, they washed their hands before the examinations with a plain liquid soap (Kao Co.) for more than 60 seconds: hands were also thoroughly disinfected with an alcohol-based liquid sanitizer (Kenei Pharma. Co. Ltd.). The washed and disinfected hands were experimentally contaminated by spreading 2.5 mL of a test microorganism suspension on each hand, and then the subjects performed each of the 6 hand hygiene procedures listed in Table 1. Subjects did not perform hand hygiene procedures in the control experiment. Subsequently, hand contamination with the test microorganism was assessed using the glove juice method (FDA, 1974; FDA, 1978), and the degree of contamination was expressed as CFU per hand. The efficacy of each hand hygiene procedure was determined by log₁₀ reduction of hand contamination between the control and each hand hygiene procedure. Data were statistically analyzed using SPSS version 19 (IBM Co. Ltd., Somers, NY, USA). The efficacy of the hand hygiene procedures was compared using the analysis of variance (ANOVA) and Tukey's test. P < 0.05 was considered significant.

Hand contamination with the test microorganism was quantified using the glove juice method (FDA, 1974; FDA, 1978). The subjects' hands were inserted into sterile polyvinyl chloride gloves (JMS Co. Ltd., Tokyo, Japan), and then the plates were incubated at 37°C for 7 days. Thereafter, the bacterial cells were harvested from the plates and suspended in sterile saline. The suspensions, which contained vegetative forms and spores, were heated at 65°C for 30 minutes to kill vegetative forms. The spore suspensions were adjusted to concentrations of approximately 10⁶ colony-forming units (CFU) per mL with sterile saline.

One standard strain (Escherichia coli ATCC 11229) and 4 fecal strains of E. coli were also used for this study. The fecal strains were obtained from healthy adults. The strains were inoculated on nutrient agar plates (Nissui Pharma. Co. Ltd.), and then the plates were incubated at 37°C for 12 hs. The bacterial cells were suspended in sterile saline, and the suspensions were adjusted to concentrations of approximately 10⁶ CFU/mL.

A plain liquid soap (Kao Co., Tokyo, Japan) and an antiseptic liquid soap containing triclosan (Kao Co.) were used for handwashing. An alcohol-based liquid sanitizer (76.9-81.4% v/v ethanol: Kenei Pharma. Co. Ltd., Osaka, Japan) and an alcohol-based gel sanitizer (76.9-81.4% v/v ethanol: GOJO Inds. Inc., Akron, Ohio, USA) were used for hand-rubbing.
PROCEDURES FOR REMOVING *B. CEREUS* SPORES

**FIG. 1.** Spore-removing efficacy of hand hygiene procedures. Thirty subjects participated and 1 standard strain (*B. cereus* ATCC 10876) was used. The efficacy of the hand hygiene procedures is expressed as log10 reductions of *B. cereus* spores adhering to the subjects’ hands. Data are represented as mean ± standard deviation for the 30 subjects. A, 30-second water-only washing; B, 60-second plain soap washing; C, 30-second plain soap washing; D, 30-second antiseptic soap washing; E, hand-rubbing with an alcohol-based liquid; F, hand-rubbing with an alcohol-based gel. Means with the same symbol do not differ, and different symbols indicate significant differences at *P* < 0.05.

Japan) containing 50 mL of sampling solution (0.04% KH2PO4, 1.01% Na2HPO4, and 0.10% Triton X-100). The gloved hands were uniformly massaged for 1 minute. Solutions were collected from the gloves and diluted 10-fold with sterile saline, and the diluents were inoculated onto agar medium plates. Mannitol Egg Yolk Polymyxin (MYP) agar plates (Becton Dickinson and Co., Franklin Lakes, NJ, USA) and deoxycholate-hydrogen sulphide-lactose (DHL) agar plates (Becton Dickinson and Co.) were used for *B. cereus* and *E. coli* culture, respectively. The plates were incubated at 37°C for 12 hs, and then the numbers of colonies on the plates were counted. Mannitol-negative, lecinthinasenegative, large, flat, and granular colonies on MYP agar plates were identified as *B. cereus* (Mossel et al., 1967). Red colonies on DHL agar plates were identified as *E. coli* (Kim et al., 1998).

We first evaluated the spore-removing efficacy of the 6 hand hygiene procedures (Table 1) with 30 subjects using 1 *B. cereus* strain. The 30 subjects contaminated their hands with spores of the standard *B. cereus* strain (*B. cereus* ATCC 10876), and then the efficacy of the hand hygiene procedures was evaluated as described above. The results of this experiment are summarized in Fig. 1. The alcohol-based hand-rubbing procedures removed few *B. cereus* spores from the subjects’ hands. However, all the handwashing procedures effectively reduced the number of *B. cereus* spores on the hands, although efficacy varied widely among the subjects. Comparing the efficacy of the different handwashing procedures, the water-only washing was significantly inferior to the soap washing procedures (*P* < 0.01), and the 60-second soap washing was significantly superior to the 30-second soap washing (*P* < 0.01). There was no significant difference in efficacy between the plain soap washing and the antiseptic soap washing (*P* = 0.97).

We subsequently evaluated the spore-removing efficacy of the procedures with 1 subject using 5 *B. cereus* strains (*B. cereus* ATCC 10876 and the 4 clinical strains). The results of this experiment are summarized in Fig. 2. This experiment yielded results similar to those of the above experiment with all strains.

We also evaluated the efficacy of the hand hygiene
procedures against *E. coli* contamination. This experiment was conducted with 1 subject using 5 *E. coli* strains. The subject contaminated his hands with each of the 5 *E. coli* strains (*E. coli* ATCC 11229 and the 4 fecal strains), and then the efficacy of the hand hygiene procedures was evaluated. The results of this experiment are summarized in Fig. 3. All hand-rubbing and handwashing procedures effectively removed *E. coli* from the subject’s hands. The hand-rubbing procedures drastically reduced the number of *E. coli* on the hands, with an efficacy that was clearly superior to that of the handwashing procedures.

Hand hygiene procedures, including handwashing and alcohol-based hand-rubbing, are the most important practices for preventing nosocomial infections (Boyce et al., 2002; Cookson et al., 2009; Pittet et al., 2009; Pratt et al., 2007). Handwashing has been recognized as an important hand hygiene procedure for many years, and thus it has been promoted in many medical facilities (Ojajärvi, 1980). However, hands can be washed only at handwashing stations, and this can be inconvenient in busy clinical settings. In comparison, alcohol-based hand-rubbing does not require handwashing stations, and thus it can be performed anytime and anywhere. In addition, alcohol-based hand-rubbing can reduce microorganism contamination faster and more effectively than handwashing (Girou et al., 2002; Guilhermetti et al., 2010; Ojajärvi, 1980). For these reasons, health care workers currently tend to rely only on hand-rubbing, and neglect handwashing (Boyce et al., 2002; Karabay et al., 2005).

The efficacy of alcohol-based hand-rubbing depends on the bactericidal activity of alcohol sanitizers. However, bacterial spores are highly resistant to alcohol (Russell, 1990), and therefore it is possible that alcohol-based hand-rubbing may fail to prevent nosocomial infections caused by spore-forming bacteria. This is an important pitfall in infection control. Therefore, appropriate hand hygiene procedures for removing bacterial spores are required. *B. cereus* is one of the most important pathogenic spore-forming bacteria (Bottone, 2010). In the present study, we evaluated the efficacy of different hand hygiene procedures in removing *B. cereus* spores from hands.

There are several types of alcohol-based hand sanitizers available, including solution, gel, and foam sanitizers. In this study, we evaluated the efficacy of alcohol-based hand-rubbing using solutions and gel sanitizers: neither sanitizer was effective in removing *B. cereus* spores from hands. These results demonstrate that alcohol-based hand-rubbing cannot prevent nosocomial transmission of *B. cereus* spores via hands.

The efficacy of handwashing in removing *B. cereus* spores was also evaluated in this study. All the handwashing procedures removed *B. cereus* spores from hands effectively. All the soap washing procedures reduced the number of spores by more than 2 log$_{10}$ CFU per hand, although the 30-second water-only washing reduced numbers by only 1 log$_{10}$ CFU per hand. These results demonstrate that soap washing procedures are appropriate for removing *B. cereus* spores from hands. In addition, we evaluated the influence of washing time on spore removal in the soap washing procedures. The 60-second washing removed more bacterial spores than the 30-second washing, which suggests that extending washing time can increase the efficacy of handwashing. Therefore, health care workers should be conscious that thorough and extended handwashing increases the efficacy of the practice. In this study, both plain soap and antiseptic

![Fig. 3](image_url)
 soap were used for handwashing, but there was no significant difference in the efficacy between the soaps. Thus, the addition of antiseptic chemicals to soap has no value in reducing hand contamination with *B. cereus* spores.

Meanwhile, we also evaluated the efficacy of the hand hygiene procedures in removing *E. coli*. The alcohol-based hand-rubbing procedures drastically reduced *E. coli* numbers in our study, and the efficacy of the handwashing procedures was inferior to that of the alcohol-based hand-rubbing procedures. These results support the notion that alcohol-based hand-rubbing is the most appropriate procedure for removing non-spore-forming bacteria from hands (Boyce et al., 2002; Cookson et al., 2009; Pittet et al., 2009; Pratt et al., 2007).

Several nosocomial outbreaks of *B. cereus* infections have been reported (Balm et al., 2012; Van Der Zwaan et al., 2000). Contamination of health care workers’ hands with *B. cereus* spores played important roles in these outbreaks. We also had an outbreak of *B. cereus* infections in 2006 (Sasahara et al., 2011). During this outbreak, our hospital staff had routinely rubbed their hands with alcohol-based sanitizers before and after patient care, which however did not improve the control of this outbreak. In contrast, they tended to neglect handwashing. Our study reveals that handwashing removes spores from hands effectively, but that alcohol-based hand-rubbing does not. These results suggest that the above outbreaks were linked to recent trends in hand hygiene practice, which overstate the importance of hand-rubbing at the expense of handwashing. *Clostridium difficile* is also an important spore-forming species that can cause nosocomial infections (McFarland et al., 1989). The incidence of nosocomial *C. difficile* infections has increased worldwide in the past decade (Freeman et al., 2010). Alcohol-based hand-rubbing was not effective for removing *C. difficile* spores from hands (Jabbar et al., 2011; Oughton et al., 2009). Both their and our results suggest that recent hand hygiene practices, which mainly consist of alcohol-based hand-rubbing, are not effective in preventing nosocomial infections with spore-forming bacteria.

In conclusion, handwashing with soap is appropriate for removing *B. cereus* spores from hands. In contrast, alcohol-based hand-rubbing is not effective. The importance of handwashing should be revaluated in order to control nosocomial infections due to spore-forming bacteria.

**REFERENCES**

Balm, M. N., Jureen, R., Teo, C., Yeoh, A. E., Lin, R. T., Dancer, S. J., and Fisher, D. A. (2012) Hot and steamy: outbreak of *Bacillus cereus* in Singapore associated with construction work and laundry practices. *J. Hosp. Infect.*, 81, 224-230.

Bottone, E. J. (2010) *Bacillus cereus*, a volatile human pathogen. *Clin. Microbiol. Rev.*, 23, 382-398.

Boyce, J. M., Pittet, D., Healthcare Infection Control Practices Advisory Committee, and HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force (2002) Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America. *MMWR Recomm. Rep.*, 51 (RR-16), 1-45.

Cookson, B., Mathai, E., Allegranzi, B., Pessoa-Silva, C. L., Bagheri Nejad, S., Schneider, A., Tschopp, C., Wendt, C., and Pittet, D. (2009) Comparison of national and subnational guidelines for hand hygiene. *J. Hosp. Infect.*, 72, 202-210.

FDA (1974) Effectiveness testing of surgical hand scrub (glove juice test). *Federal Register*, 39, 33137-33138.

FDA (1978) Effectiveness testing of surgical hand scrub (glove juice test). *Federal Register*, 43, 1242-1243.

Freeman, J., Bauer, M. P., Baines, S. D., Corver, J., Fawley, W. N., Goorhuis, B., Kuijper, E. J., and Wilcox, M. H. (2010) The changing epidemiology of *Clostridium difficile* infections. *Clin. Microbiol. Rev.*, 23, 529-549.

Girou, E., Loeau, S., Legrand, P., Oppein, F., and Brun-Buisson, C. (2002) Efficacy of handrubbing with alcohol-based solution versus standard handwashing with anti-septic soap: randomised clinical trial. *BMJ*, 325, 362.

Guilhermetti, M., Marques Wiirzler, L. A., Castanheira Facio, B., da Silva Furlan, M., Campo Meschial, W., Bronnaro Tognim, M. C., Botelho Garcia, L., and Luiz Cardoso, C. (2010) Antimicrobial efficacy of alcohol-based hand gels. *J. Hosp. Infect.*, 74, 219-224.

Jabbar, U., Leischner, J., Kasper, D., Gerber, R., Sambol, S. P., Parada, J. P., Johnson, S., and Gerdin, D. N. (2010) Effectiveness of alcohol-based hand rubs for removal of *Clostridium difficile* spores from hands. *Infect. Control Hosp. Epidemiol.*, 31, 565-570.

Karabay, O., Sencan, I., Sahin, I., Alpteker, H., Ozcan, A., and Oksuz, S. (2005) Compliance and efficacy of hand rubbing during in-hospital practice. *Med. Princ. Pract.*, 14, 313-317.

Kim, Y. B., Okuda, J., Matsumoto, C., Morigaki, T., Asai, N., Watanabe, H., and Nishibuchi, M. (1998) Isolation of an *Escherichia coli* O157 : H7 strain producing Shiga toxin 1 but not Shiga toxin 2 from a patient with hemolytic uremic syndrome in Korea. *FEMS Microbiol. Lett.*, 166, 43-48.

Larson, E. L., Eke, P. I., Wilder, M. P., and Laughon, B. E. (1987) Quantity of soap as a variable in handwashing. *Infect. Control*, 8, 371-375.

McFarland, L. V., Mulligan, M. E., Kwok, R. Y., and Stamm, W. E. (1989) Nosocomial acquisition of *Clostridium difficile* infection. *N. Engl. J. Med.*, 320, 204-210.

Mossel, D. A., Koopman, M. J., and Jongerius, E. (1974) Enumeration of *Bacillus cereus* in foods. *Appl. Microbiol.*, 15, 650-653.

Ohsaki, Y., Koyano, S., Tachibana, M., Shibukawa, K., Kuroki, M., Yoshida, I., and Ito, Y. (2007) Undetected *Bacillus* pseudo-outbreak after renovation work in a teaching hospital. *J. Infect.*, 54, 617-622.

Ojalärvi, J. (1980) Effectiveness of hand washing and disin-
fection methods in removing transient bacteria after patient nursing. J. Hyg. (Lond.), 85, 193-203.
Oughton, M. T., Loo, V. G., Dendukuri, N., Fenn, S., and Libman, M. D. (2009) Hand hygiene with soap and water is superior to alcohol rub and antiseptic wipes for removal of Clostridium difficile. Infect. Control Hosp. Epidemiol., 30, 939-944.
Pittet, D., Allegranzi, B., Boyce, J., and World Health Organization World Alliance for Patient Safety First Global Patient Safety Challenge Core Group of Experts (2009) The World Health Organization Guidelines on Hand Hygiene in Health Care and their consensus recommendations. Infect. Control Hosp. Epidemiol., 30, 611-622.
Pratt, R. J., Pellowe, C. M., Wilson, J. A., Loveday, H. P., Harper, P. J., Jones, S. R., McDougall, C., and Wilcox, M. H. (2007) epic2 : National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. J. Hosp. Infect., 65 (Suppl 1), S1-64.
Russell, A. D. (1990) Bacterial spores and chemical sporidical agents. Clin. Microbiol. Rev., 3, 99-119.
Sasahara, T., Hayashi, S., Morisawa, Y., Sakihama, T., Yoshimura, A., and Hirai, Y. (2011) Bacillus cereus bacteremia outbreak due to contaminated hospital linens. Eur. J. Clin. Microbiol. Infect. Dis., 30, 219-226.
Sugawara, E., Kobayashi, H., Kajiura, T., Hiejima, Y., Sogawa, Y., Endo, H., and Takeuchi, C. (2010) Comparison of various Hand hygiene methods for the Spores contaminate on hands. J. Healthcare-associated infect. (In Japanese), 2, 37-40.
Van Der Zwet, W. C., Parlevliet, G. A., Savelkoul, P. H., Stoof, J., Kaiser, A. M., Van Furth, A. M., and Vandenbroucke-Grauls, C. M. (2000) Outbreak of Bacillus cereus infections in a neonatal intensive care unit traced to balloons used in manual ventilation. J. Clin. Microbiol., 38, 4131-4136.