



## Original article

## Contamination of healthcare workers' hands with bacterial spores



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## ABSTRACT

*Clostridium* species and *Bacillus* spp. are spore-forming bacteria that cause hospital infections. The spores from these bacteria are transmitted from patient to patient via healthcare workers' hands. Although alcohol-based hand rubbing is an important hand hygiene practice, it is ineffective against bacterial spores. Therefore, healthcare workers should wash their hands with soap when they are contaminated with spores. However, the extent of health care worker hand contamination remains unclear. The aim of this study is to determine the level of bacterial spore contamination on healthcare workers' hands. The hands of 71 healthcare workers were evaluated for bacterial spore contamination. Spores attached to subject's hands were quantitatively examined after 9 working hours. The relationship between bacterial spore contamination and hand hygiene behaviors was also analyzed. Bacterial spores were detected on the hands of 54 subjects (76.1%). The mean number of spores detected was 468.3 CFU/hand (maximum: 3300 CFU/hand). Thirty-seven (52.1%) and 36 (50.7%) subjects were contaminated with *Bacillus subtilis* and *Bacillus cereus*, respectively. Nineteen subjects (26.8%) were contaminated with both *Bacillus* species. *Clostridium difficile* was detected on only one subject's hands. There was a significant negative correlation between the hand contamination level and the frequency of handwashing ( $r = -0.44$ ,  $P < 0.01$ ) and a significant positive correlation between the hand contamination level and the elapsed time since last handwashing ( $r = 0.34$ ,  $P < 0.01$ ). Healthcare workers' hands may be frequently contaminated with bacterial spores due to insufficient handwashing during daily patient care.

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## 1. Introduction

Healthcare workers' hands can transmit pathogenic microorganisms between patients in medical facilities; therefore, healthcare workers are always required to keep their hands clean [1–3]. Alcohol-based hand rubbing is the most common routine hand hygiene practice, can be easily performed anywhere at any time, and removes pathogenic microorganisms from hands effectively and quickly [4,5]. However, alcohol-based hand rubbing is not enough to prevent hand contamination by every microorganism.

For example, bacteria that form bacterial spores (i.e. *Clostridium* species and *Bacillus* spp.) are highly resistant to alcohol disinfectants [6]. Therefore, alcohol-based hand rubbing is ineffective against contamination with bacterial spores [7–10]. Spore-forming bacteria such as *Clostridium difficile*, *Bacillus cereus*, and *Bacillus subtilis* can cause hospital infections and nosocomial outbreaks [11–17]. Therefore, healthcare workers should wash their hands with soap when their hands are contaminated with these bacteria [7–10]. However, many healthcare workers think that hand contamination with spore-forming bacteria is less important than contamination with other pathogenic microorganisms, such as *Staphylococcus aureus*, *Enterococcus* spp., or gram-negative bacteria. Furthermore, the extent of healthcare worker hand contamination with bacterial spores remains unclear. Therefore, in this study, we evaluated the level of bacterial spore contamination on healthcare

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workers' hands. We also analyzed the relationship between bacterial spore contamination and hand hygiene behaviors.

## 2. Materials and methods

This study was conducted from April 01 to August 31 2013 in the emergency medicine ward of Jichi Medical University Hospital, a tertiary hospital with 1132 beds. Various types of emergency patients, including trauma patients, have been admitted to this ward. Seventy-one healthcare workers (61 nurses, 10 doctors) were invited to participate in this evaluation. Because this evaluation was conducted as a part of an infection control and hand hygiene education campaign in this ward, most of the nurses and the doctors on duty participated in this evaluation. Informed consent was obtained from all subjects. This study was approved by the institutional ethics committee of Jichi Medical University (No. 15-152).

Bacterial spores attached to subject's hands were quantitatively evaluated after 9 working hours. The hand hygiene behavior (i.e. the frequency of alcohol-based hand rubbing, the elapsed time between last alcohol-based hand rubbing and sampling, the frequency of handwashing, and the elapsed time between last handwashing and sampling) of each subject during the past 9 working hours was assessed by study examiners. Staff on duty was included in the evaluation. Subjects counted the frequency of alcohol-based hand rubbing or handwashing, and they recorded the last time of alcohol-based hand rubbing or handwashing on their own. Then they reported the findings of their hand hygiene behavior to the examiners. Two members of our infection control team acted as the examiners. GOJO™ (GOJO Industries, Inc., Akron, OH, USA) and Purerubbing™ (Air Liquide Co., Paris, France) were used for alcohol-based hand rubbing at our hospital.

Bacterial spores were recovered from both of the subjects' hands by using the glove juice method [18,19]. The subjects' hands were inserted into sterile polyvinyl chloride gloves (JMS, Tokyo, Japan) containing 25 mL of sampling solution (0.04% KH<sub>2</sub>PO<sub>4</sub>, 1.01% Na<sub>2</sub>HPO<sub>4</sub>, and 0.10% Triton X-100), and the gloved hands were massaged for 1 min. Solutions were collected from the gloves and heated at 80 °C for 20 min to eradicate vegetative form microorganisms other than bacterial spores. The solutions were then 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) were used for *Bacillus* spp., and cycloserine-cefoxitin-fructose agar (CCFA) plates (Becton Dickinson and Co.) were used for *C. difficile*. After inoculation, the MYP agar plates were incubated at 37 °C under aerobic conditions for 12 h, and the CCFA plates were incubated at 37 °C under anaerobic conditions for 48 h. The numbers of colonies on the plates were then counted. Mannitol-positive, lecithinase-negative, large, yellow, wrinkle-surfaced colonies on MYP agar plates were identified as *B. subtilis*, and Mannitol-negative, lecithinase-positive, large, flat, granular colonies on MYP agar plates were identified as *B. cereus* [20,21]. Colonies that were yellow, circular with a slightly filamentous edge, lipase-negative, lecithinase-negative, and had a flat to low umbonate in profile and a ground-glass appearance on CCFA plates were identified as *C. difficile* [22].

The contamination of subjects' hands with transient bacteria other than spore-forming bacteria was also evaluated. Gram-negative bacteria and *Enterococcus* spp. were recovered from the hands using the glove juice method as described above. The solutions containing bacteria were diluted 10-fold with sterile saline without heating, and the diluents were inoculated onto agar medium plates. MacConkey agar plates (Becton Dickinson and Co.) were used for gram-negative bacteria, and Enterococcosel agar plates (Becton Dickinson and Co.) were used for *Enterococcus* spp.

After inoculation, these agar plates were incubated at 37 °C under aerobic conditions for 12 h, and the numbers of colonies on the plates were counted. Species identification of the colonies was determined using an automated system for microbial identification (Vitek 2 system; SYSMEX bioMerieux Co. Ltd., Tokyo, Japan).

All analyses were performed using SPSS Statistics software (IBM SPSS Statistics version 19 for Windows; IBM Corp, NY, USA). A Student's t-test was used to compare two groups (nurses and doctors) of data. The significance threshold was set at 0.05. We assessed the correlations between the degree of hand contamination and hand hygiene behavior using a Pearson product–moment correlation coefficient. The minimum detection threshold for bacterial sampling in this study was 25 CFU/hand.

## 3. Results

The level of bacterial spore contamination found on the hands of the 71 subjects is shown in Fig. 1. Bacterial spores were detected on the hands of 54 subjects (76.1%) and the mean number of bacterial spores was 468.3 CFU/hand (maximum: 3300 CFU/hand). In addition, the hands of all doctors were contaminated with bacterial spores. The hands of 37 subjects (52.1%) were contaminated with *B. subtilis* (mean: 307.0 CFU/hand, maximum: 3000 CFU/hand), the hands of 36 subjects (50.7%) were contaminated with *B. cereus* (mean: 138.5 CFU/hand, maximum: 1000 CFU/hand), and the hands of 19 subjects (26.8%) were contaminated with both species of *Bacillus*. Meanwhile, *C. difficile* was detected on only one subject's hands (1.4%) and the contamination level was low (50 CFU/hand). There was no significant difference between the bacterial spore contamination level on the hands of the nurses and the hands of the doctors ( $P = 0.27$ ).

Gram-negative bacteria and *Enterococcus* spp. were detected on only 14 subjects' hands (19.7%). *Pseudomonas* spp. were detected on nine (13.7%, mean: 155.6 CFU/hand), *Escherichia coli* on three (4.2%, Mean 41.7 CFU/hand), *Klebsiella oxytoca* on one (1.4%, 325 CFU/hand), and *Enterococcus faecalis* on one (1.4%, 300 CFU/hand).

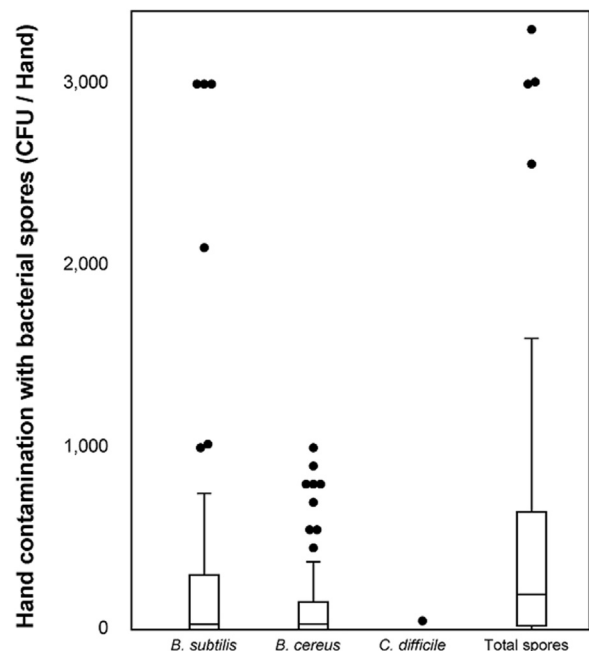


Fig. 1. Bacterial spore hand contamination among healthcare workers. Data are displayed as a box-and-whisker plot and include the smallest value observed, lower quartile, median, upper quartile, and largest value observed. Black points indicate outliers or single points.

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