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Major Article

Hand hygiene using a new hand-cleansing formulation without sanitizers: Effect on *Staphylococcus aureus* removal and recovery of properties against skin damage

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Biofilm
Potassium oleate
The surface evaluation of scaliness

Background: *Staphylococcus aureus* is known to form a biofilm and colonize on damaged skin of the hands. We investigated changes in the quantity of *S aureus* on the hands and changes in skin damage when using a hand-cleansing formulation with potassium oleate but without a sanitizer (formulation A), which is highly effective in removing *S aureus* biofilm and causes minimal skin damage.

Material and Methods: The participants (14 medical staff members) used 2 types of hand-cleansing formulations (formulations A and B), each for 4 weeks. *S aureus* of the hands was cultured from swab samples on agar plates. Surface of hands was measured using an ultraviolet light microscope.

Results and Discussion: The quantity of *S aureus* after using formulation A for 4 weeks was $10^{1.08 \pm 0.05}$ CFU/mL, a statistically significant decrease from the quantity of *S aureus* ($10^{1.59 \pm 0.19}$ CFU/mL) just before use ($P = .029$). Also, dryness of hand surfaces decreased. With formulation B, the quantity of *S aureus* did not significantly change from before to after use ($P > .05$). This presumably occurs because formulation A gently removes *S aureus* biofilm.

Conclusions: Formulation A removed *S aureus* from the hands of participants, and skin damage on the hands improved.

Health care-associated infections prolong patient hospitalization, increase health care costs, and increase mortality rates.[1] The incidence of *Staphylococcus aureus* infections, a leading cause of health care-associated infections, is particularly high, and prevention is important because of a high rate of antibiotic resistance.[1] In general, microorganisms are likely to be transmitted by medical staff to patients by hand contact.[2] This means that hand hygiene is important in preventing infections. Various measures, including increased compliance with hand hygiene protocol using alcohol-based handrub solutions, has been somewhat effective.[3] However, despite the use of hand hygiene protocol, outbreaks of *S aureus* infection on damaged hand skin have been reported,[4-6] and disinfection and removal of *S aureus* on damaged hand skin has reportedly been difficult.[7]

Therefore, disinfection and removal of colonized *S aureus* from damaged skin is a challenge, and this colonized *S aureus* can be a major problem in medical settings. More effective hand hygiene is necessary to remove colonized *S aureus*, but because skin damage can persist as a result of alcohol-based hand hygiene, further *S aureus* colonization is likely, thus creating a repetitive cycle.[8-11] Damaged skin is also a hindrance to hand hygiene, and if required hand hygiene measures are not implemented, the risk of *S aureus* and other bacterial infections via hand contact increases.[12]

In a previous study, we confirmed the possibility of the presence of a *S aureus* biofilm on the surface of the hands of medical

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personnel. Therefore, as a factor associated with colonization of *S aureus* on damaged skin of the hands, we turned our attention toward this *S aureus* biofilm. In other words, we hypothesized that if *S aureus* biofilm can be removed while causing minimal hand skin damage, *S aureus* can effectively be removed and hand skin damage itself can be improved. In the current study, we enrolled medical staff members and investigated changes in the quantity of *S aureus* on their hands and changes in skin damage when using a hand-cleansing formulation (formulation A) that is highly effective in removing *S aureus* biofilm, yet caused minimal damage to the skin of the hands.

MATERIALS AND METHODS

Hand-cleansing formulations used in this study

- Formulation A: Foam-type formulation containing potassium oleate as the chief component, and potassium acyl glycinate and 1,3-butylene glycol, but without a sanitizer. (This product is to be launched by Kao Corporation, Tokyo, Japan).
- Formulation B: Foam-type formulation containing potassium laurate as the chief component, and isopropyl methyl phenol as a sanitizer (commercially available).

Cell viability

Cell viability[13] was calculated using a 3-dimensional skin model of reconstructed human epidermis (LabCyte EPI-MODEL, Japan Tissue Engineering Co Ltd, Aichi, Japan) to predict skin damage from the hand-cleansing formulations. Other than a formulation exposure time of 10 minutes to the LabCyte EPI-MODEL, the procedure and method for calculating cell viability were performed according to the manufacturer's instruction.

S aureus *biofilm removal rate*

For the biofilm formation, Tryptic Soy Broth No. 2 (Sigma-Aldrich Co LLC, St Louis, MO) was used for preculture, then 0.15 mL *S aureus* (NBRC13276) solution from the same medium, diluted to an optical density at 600 nm of 0.1, was added to the wells of 96-well plates.[14,15] These were cultured at 37°C for 48 hours. To measure biofilm removal, 0.2 mL of each hand-cleansing formulation was placed in contact with the formed biofilm at room temperature for 1 minute. The quantity of residual biofilm was measured by staining with 0.1% crystal violet solution, decolorizing with ethanol, and measuring absorbance at 570 nm. The *S aureus* biofilm removal rate was calculated as shown below. Tryptic Soy Broth No. 2 without *S aureus* was used as a blank. Saline was used as a control.

Biofilm removal rate

The rate of biofilm removal was determined by the equation (%) = (C – test) / (C – B) × 100, where B = absorbance of blank, C = absorbance of control, and Test = absorbance after using test hand-cleansing formulations.

Hand-cleansing formulation: Commissioned test

Participants

A survey was conducted among 63 medical staff members at Geriatric Health Services Facility "Cosmos" on July 14, 2014. *S aureus* was isolated from swab samples of the hands in 8 participants, and 6 participants responded that they were concerned about hand roughness (skin damage) during the winter. Testing was performed in these 14 participants. The participants were divided into 2 groups (A and B) for testing, based on which floor they staffed. All participants met the following conditions during the test period: no current use of drugs such as steroids during the study period, not pregnant or nursing, and no change in brand or frequency of use of any hand products during this time.

Setting and design

Figure 1 shows the test schedule. The test was conducted between November 4, 2014, and March 9, 2015. This was a prospective, crossover, double-blind commissioned test. Formulations A and B were each used for 4 weeks. To ensure that the hand conditions of the participants were the same before use of formulations A and B, a 2-week controlling period was established. To eliminate any carryover effect between formulations A and B, the time required for stratum corneum turnover was considered, and a 6-week washout period was established. During the controlling and washout periods, formulation C, which is regularly used at Geriatric Health Services Facility "Cosmos", was used. Formulation C is a liquid-type commercially available product containing potassium laurate as its chief component and isopropyl methyl phenol. At –2, 0, 2, and 4 weeks of using formulations A and B, hand roughness (ie, skin damage) and the quantity of *S aureus* and aerobic bacteria from swab samples were assessed. Testing was performed 20 minutes after the participants had washed their hands using the specified formulation and hands had been placed at rest. This study was approved by an institutional review board of Geriatric Health Services Facility "Cosmos," and the procedure of this study conformed to the principles of the Declaration of Helsinki.

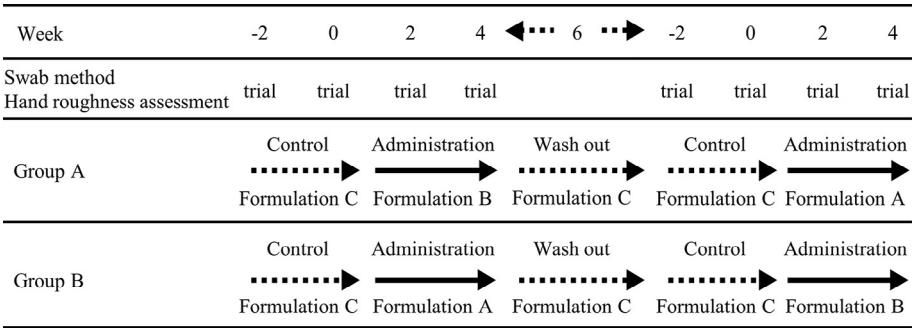

Fig 1. Test schedule for evaluation of hand-cleansing formulations. Solid arrows denote the duration of use and dashed arrows denote the controlling and washout periods. Formulations indicated under each arrow were used during that period.