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Original article

Antibacterial efficacy of stable sodium hypochlorite

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Abstract

The bactericidal efficacy of stable sodium hypochlorite (s-SH, AirRish) was evaluated through *in vitro* testing against planktonic solutions of *Staphylococcus aureus* and *Staphylococcus epidermidis*. After an exposure time of 5 minutes viable colony forming units of both species fell below the limit of detection with both 100 ppm and 200 ppm s-SH, a greater than 6 log10 reduction ($p < 0.001$). Additionally, the antimicrobial effect of s-SH was also examined in hand sanitization applications. In a 60s hand rub, 200 ppm s-SH achieved a 96% reduction in hand CFUs, of similar effect as a typical alcohol sanitizer ($p = 0.28$). s-SH maintained considerable bactericidal efficacy at the tested concentrations.

KEY WORDS: stable sodium hypochlorite, antibacterial, sanitization, *Staphylococcus aureus*

Introduction

Frequent handwashing is of vital importance for the prevention of illness and reducing the spread of communicable disease. However, frequent handwashing with detergents damages the protective proteins of the stratum corneum, removes beneficial lipids from the skin, and may trigger contact dermatitis [1]. Irritation and desiccation from repeated handwashing causes cracked and broken skin that is ultimately more vulnerable to infection in spite of the employed hygienic practice. For repeated everyday use, sanitizing hand rubs are an effective method for hand sanitization that produce less irritation [2]. Alcohol based sanitizing solutions are highly effective at eliminating pathogens, but cause significant desiccation [3] without the addition of emollients. Alcohols such as isopropanol and n-propanol may also perturb keratinocyte functioning and cause irritation [4]. Ethanol does not typically cause skin irritation, but can cause erythema in individuals with dysfunctional aldehyde dehydrogenase [5]. Additionally, during periods of high demand, manufacturing and supply chains may struggle to meet high demand for sanitizers, as occurred during the early stages of the ongoing SARS-CoV-2 pandemic. There is a need for a varied supply of safe, non-irritating, and effective hand sanitizing agents.

Sodium hypochlorite (NaClO) is a chemical compound with broad antimicrobial activity that is used for cleaning and deodorization in hospital, food processing, and household environments. NaClO also sees use in a broad variety of contexts, including water sanitization and dental procedures, such as endodontic treatment.

NaClO rapidly degrades at high temperatures and under exposure to ultraviolet light, oxygen, and contact with organic contaminants. These drawbacks complicate the long-term storage and practical use of NaClO. However, a new formulation of a stable sodium hypochlorite (s-SH) solution has been developed which is better able to withstand degradation and extends the shelf life of s-SH when stored in a cool, dark environment. S-SH is used as an industrial surface sanitizer at concentrations of 1,000 and 2,000 ppm where its antimicrobial efficacy is well established.

One hundred ppm and 200 ppm formulations have been developed for use in personal hand and skin sanitization, and non-industrial surface sanitization. Recently we evaluated the skin safety of 200 ppm s-SH in a 24-hour closed path test [6], and observed no adverse effects of skin contact with s-SH. The bactericidal efficacy of these lower concentration formulations requires confirmation. In this study we evaluated the bactericidal efficacy of 100 and 200 ppm s-SH.

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Methods

Test Materials

One hundred ppm and 200 ppm S-SH was provided by AirRish Co., Ltd. (Osaka, Japan). An alcohol solution was used as a positive control, consisting of 63.5% ethanol and 10.5% isopropanol (Yamazen; Osaka).

In Vitro Bactericidal Test

Overnight stock of *Staphylococcus aureus* (ATCC 12600) and *Staphylococcus epidermidis* (ATCC14990) was prepared in Tryptic Soy Broth (Becton, Dickinson, and Company; MD, USA) and incubated for 24 hours at 37 °C with 250 rpm shaking. 100 µL aliquots of bacterial stock were inoculated into 9.9 mL of 100 ppm s-SH, 200 ppm s-SH, alcohol, or 0.1 M phosphate buffer (pH 7.4) as a control. Solutions were vortexed and incubated at room temperature (~25 °C) for 5 minutes before neutralization and serial dilution in 0.1 M phosphate buffer. 100 µL aliquots of dilutions were plated on Tryptic Soy Agar (Becton, Dickinson, and Company; MD, USA) in triplicate and colony counting was performed after 72 hours of incubation at 37 °C.

Skin Sanitization Test

Skin microflora samples were collected from the skin of the hand via swabbing. Sterile cotton sample collection swabs were dipped in extraction buffer (0.15 M NaCl and 0.01% Tween20) and firmly brushed 10 times against a 4 cm^2 section of the palm and immediately plated. Sanitizing hand rub was performed based on a modified protocol of the European Standard [7]. Roughly 3 mL of sanitizing solution was applied to the hands and rubbed into the skin with a hand washing motion for 60 seconds. Post-sanitization, an additional sample swab was collected as previously described. Plates were incubated for 24 hours at 37 °C before colony counting was performed. Plates were incubated again for an additional 48 hours to observe any slow growing colonies. Control swabs were dipped in extraction buffer and exposed to the air of the testing environment before plating.

Results

In Vitro Bactericidal Effect

All test solutions demonstrated strong bactericidal activity against planktonic *S.aureus* and *S.epidermidis* (***Fig.1***). After exposure to sanitizing solutions, bacterial stock of an initial concentration of ~4×10^8 CFU/mL (*S. aureus*) and ~6×10^8 CFU/mL (*S. epidermidis*) experienced a total loss of cell viability. No colonies were observed at any dilution, with a minimum detection level of 100 CFU/mL, corresponding to a reduction of at least 6 $\log_{10}$ compared to control. With an exposure time of 5 minutes, s-SH showed equal *in vitro* bactericidal efficacy to the alcohol rub mix at both 100 ppm and 200 ppm concentrations.

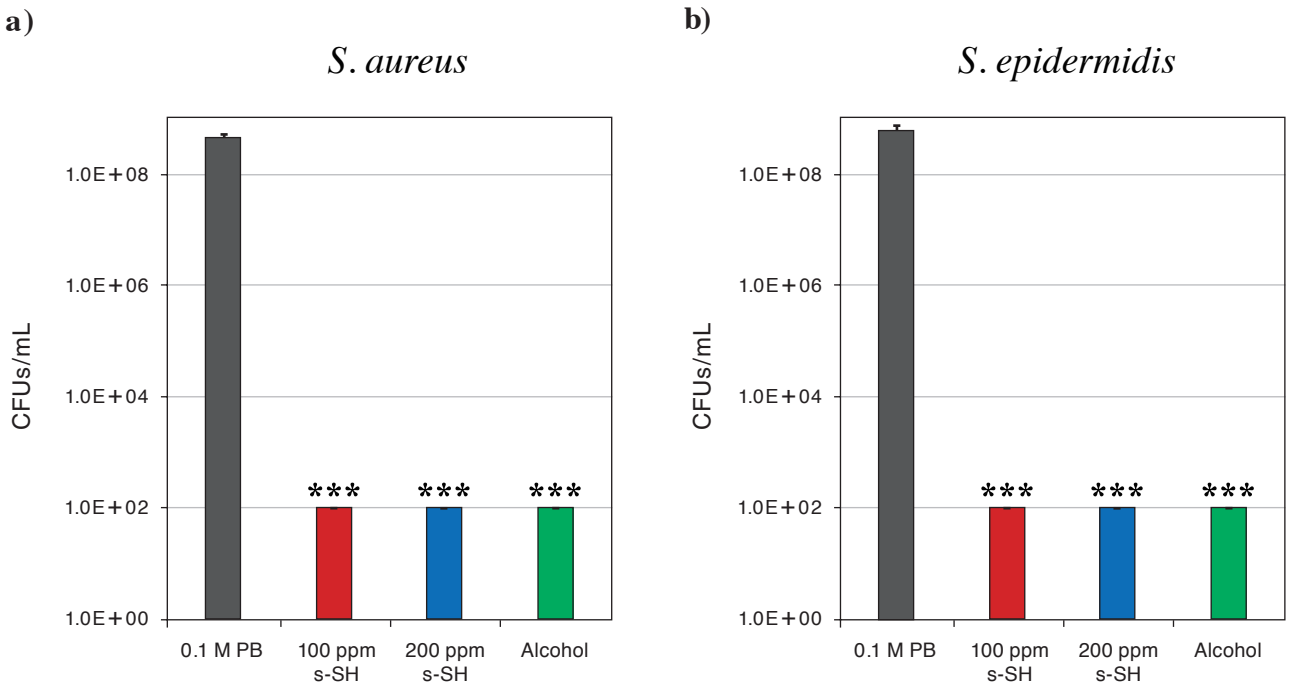

Fig.1. ***In vitro*** **bactericidal efficacy.**

Cell viability of *S. aureus* (**a**) and *S. epidermidis* (**b**) after 5-minute exposure to sanitizing solutions. 1.0E+02 CFUs/mL (100 CFU/mL) is the theoretical minimum detection limit, although no viable colonies were observed. n = 3, mean ± SD. Statistical difference calculated by Student's t-test, *** $p < 0.001$. CFUs, colony forming unit; SD, standard deviation.

Skin Sanitization

Immediately prior to sanitizing hand rub, sampled skin showed a mean microbial count of 178.4 ± 127.3 CFUs. After application and washing, all solutions demonstrated a substantial decrease of skin colony forming units (***Fig. 2***). The 100 ppm s-SH hand rub resulted in an 81% reduction of CFUs, significantly less than 200 ppm s-SH at 96% ($p < 0.01$) and the alcohol solution at 98% ($p < 0.01$). The efficacy of 200 ppm s-SH and the alcohol solution did not significantly differ ($p = 0.28$)

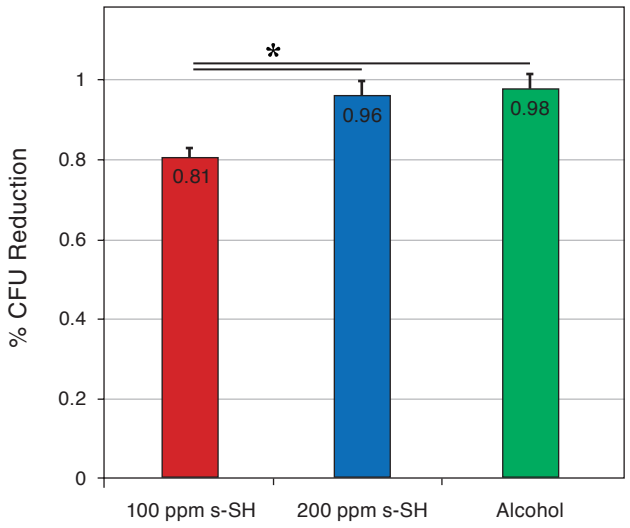

Fig. 2. **Reduction of CFUs after sanitizing hand rub.**

Percent reduction in colony forming units after 60-second hand rub with 3 mL of sanitizing solution. n = 3, mean ± SD. Statistical difference calculated by Student's t-test, * $p < 0.01$. CFUs, colony forming units; SD, standard deviation.

Discussion

Mechanism of Microbicidal Action

NaClO has a long history of use as a cleaning and disinfection agent. It's microbicidal activity is well established, although the mechanism of these effects has been less well understood until recent years. NaClO damages lipids, DNA, and inhibits essential enzyme activity [8]. In solution NaClO forms hypochlorous acid (HClO) at varying concentrations depending on pH, which is the primary microbicidal agent. HClO is permeable to bacterial membranes and readily denatures essential bacterial proteins, leading to the formation of large intracellular aggregates [9].

Skin Sanitization Experiments

Resident microbial content of the skin before testing was considerably variable between tests, and the antimicrobial effects of the sanitizing solutions were more readily apparent against skin with higher microbial content, where a larger decrease in CFUs was capable of being observed. Skin with lower baseline microbial content at the time of testing resulted in a smaller reduction of CFUs, despite low final CFU counts (*e.g.*, 3 CFU decreasing to 1 after washing). Samples with extremely low baseline CFUs (below 10) were excluded from analysis.

Due to the ongoing SARS-CoV-2 pandemic, stricter than usual hygiene and hand sanitization standards are being adhered to, which may explain some of the lower than typical CFU counts on pre-sanitization skin. For the same reason, repeated use of desiccating alcohol-based hand rubs and frequent hand washing have increased the incidence of dry and broken skin [10]. Therefore, direct contamination of hands with bacterial broth prior to sanitization (which may have better demonstrated efficacy against higher bacterial concentrations) was avoided due to of the risk of infection via open wounds on the skin of the hands. Thus, the sanitization effects were tested against resident hand microbes only.

Conclusions

Stable sodium hypochlorite at concentrations of 100 ppm and 200 ppm is safe for use on the skin and demonstrates strong antibacterial activity. Exposure to s-SH in planktonic liquid solution caused a total loss of cell viability in both *S. aureus* and *S. epidermis* within 5 minutes of contact time. In skin sanitization applications, s-SH significantly reduced the amount of colony forming units on the skin of the hands, and efficacy did not significantly differ from the alcohol-based solution at a concentration of 200 ppm. It was suggested that this test product may be a disinfectant with a sterilizing action equivalent to that of alcohol products.

Conflict of interest disclosure statement

Research support was provided by AirRish Corporation.

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