

Accepted Manuscript

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PII: S0091-6749(19)30069-7

DOI: <https://doi.org/10.1016/j.jaci.2019.01.009>

Reference: YMAI 13840

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 8 November 2018

Revised Date: 5 December 2018

Accepted Date: 7 January 2019

Please cite this article as: Sawada Y, Tong Y, Barangi M, Hata T, Williams MR, Nakatsuji T, Gallo RL, Dilute bleach baths used for treatment of atopic dermatitis are not antimicrobial *in vitro*, *Journal of Allergy and Clinical Immunology* (2019), doi: <https://doi.org/10.1016/j.jaci.2019.01.009>.

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Dilute bleach baths used for treatment of atopic dermatitis are not antimicrobial *in vitro*

Short title: Bleach baths are not antimicrobial

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Conflict of Interest: RLG is a consultant and has equity interest in MatriSys Bioscience and Sente Inc. and a cofounder of MatriSys Bioscience. The rest of the authors declare that they have no relevant conflicts of interest

Acknowledgments: none

Funding: RLG, TH and TN are supported by the Atopic Dermatitis Disease Network (ADRN) U19 AI117673. RLG, TN and MRW are also supported by NIH R01AR06781. YS is supported by a JSPS Overseas Research Fellowships.

Capsule summary: Soaking in a dilute solution of household bleach (.005%) is not an effective antibacterial treatment despite its common use to suppress *S. aureus* colonization on the skin of patients with atopic dermatitis.

Keywords: Bleach bath, antibacterial, atopic dermatitis, *S. aureus*

Abbreviations: *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; CFU, colony forming units; TSB, tryptic soy broth; agr, accessory gene regulator; YFP, yellow fluorescent protein. NS, not significant

To the Editor:

Colonization of the skin of patients by *S. aureus* is considered a risk for skin infection and an exacerbating factor in atopic dermatitis¹. Due to the negative effects associated with *S. aureus* colonization, clinicians commonly seek methods to eradicate colonization by *S. aureus*. The use of a diluted bleach solution (sodium hypochlorite, NaOCl 0.005%) is often used for this purpose, with conflicting reports of its efficacy to decrease severity of inflammation or eradicate *S. aureus* on the skin²⁻⁴. In this study, we sought to directly test the assumption that NaOCl, at the concentrations recommended for bleach baths, is an effective antibacterial agent.

Three laboratory strains of *S. aureus* (USA300, Newman, and Sanger 252) were initially selected for this study. To test survival on a nutrient rich surface, bacterial colony growth was initiated by plating 2×10^3 colony forming units (CFU) of each strain on 100cm² TSB agar plates, then submerging each plate in various concentrations of bleach for 15 minutes at 37 °C. 24 hrs after this treatment, direct colony counting showed no significant difference in the survival of *S. aureus* exposed to between 0% and 0.01% NaOCl in water (**Figure 1A**). A bactericidal effect of dilute bleach against these strains of *S. aureus* was only evident at concentrations above 0.03%, a concentration of bleach that is cytotoxic to human cells much greater than should be used clinically.

The unexpected observation that the concentrations of NaOCl used in bleach baths were not antibacterial against *S. aureus* prompted us to further explore the role of other variables on bacterial survival in defined laboratory culture conditions. Two *S. epidermidis* strains (1475 and ATCC12228), representing another abundant bacterial species found on the skin, and a biofilm forming strain (1457) and a non-biofilm former (ATCC12228), were next tested on agar as done for *S. aureus*. These *S. epidermidis* strains were also not killed by clinically relevant concentrations of NaOCl in water (**Figure 1B**). Next, to test if culture system or source of the household bleach influenced these results, *S. aureus* USA300 was grown in TSB broth at 37°C for 24 hours. The source of household bleach had no effect. Similar to growth on agar, bacterial survival was not inhibited at the clinically used concentration (0.005%) of NaOCl (**Figure 1C**). Furthermore, since the bacterial growth phase can determine sensitivity to antibiotic agents, with bacteria in a growth phase (log phase growth) often showing greater sensitivity than stationary phase bacteria⁵, we also tested the sensitivity of *S. aureus* USA300 in log phase growth compared to bacteria at the stationary phase. No difference in sensitivity to bleach was observed under these conditions (**Figure 1D**). Taken together, we conclude that the concentration of

NaOCl recommended for clinical use in bleach baths does not inhibit the survival or growth of *S. aureus* or *S. epidermidis* under laboratory conditions.

S. aureus growing on agar or in nutrient-rich broth does not accurately model conditions on the skin. The epidermis has a complex 3-dimensional structure composed of skin folds and skin appendages such as sebaceous glands, eccrine glands, and hair follicles. The composition of the epidermal surface may also influence the capacity of bleach baths to act as an antimicrobial. To examine this, 1×10^6 CFU of *S. aureus* USA300 were applied to explants of pigskin for 15 minutes at room temperature, the skin was then submerged in a range of NaOCl concentrations for 15 minutes to simulate immersion in a bleach bath. Immediately after this treatment the surviving CFU were measured. Similar to the results in defined cultures, 0.005% NaOCl had no significant bactericidal effect on *S. aureus* compared with water alone (**Figure 1E**). Therefore, these results suggest that bleach bath has no antibacterial action against *S. aureus* on skin.

In our final experiment, we assessed if NaOCl might have a beneficial therapeutic effect against *S. aureus* by influencing expression of virulence functions of bacteria rather than directly killing them. The agr quorum-sensing system plays a central role in the regulation of *S. aureus* virulence by controlling the expression of toxins that can cause epidermal damage and skin inflammation^{6, 7}. To test the action of NaOCl on agr activity, an agr-YFP reporter strain of *S. aureus* was examined during exposure to bleach for 24 hrs in TSB at 37°C. A bleach bath solution of 0.005% showed no significant effect on agr activity compared with water (**Figure 1F**). These results show the *S. aureus* agr quorum-sensing system is also not inhibited during bleach bath treatment.

Bleach baths have been reported by clinicians and patients to be associated with improvement of inflammation in atopic dermatitis³, and reported to reduce colonization that could result in deep tissue infections². It has been a common assumption that the recommendation of inclusion of 1/4 to 1/2 cup of 6% household bleach in a bathtub full of water (40 gallons) is an effective method to reduce bacterial load on the skin, and that clinical effects may related to this presumed antibacterial activity. However, our study clearly demonstrates that bleach baths have no direct bactericidal activity against *S. aureus* or *S. epidermidis*. To obtain an antibacterial effect as previously reported⁸, bleach concentrations must be at least 0.03%, higher than is safe for the skin or that has been proposed for clinical use. Therefore, a benefit from use of bleach bath cannot be attributed to the direct killing or inhibition of *S. aureus*.

These observations are not intended to directly support or refute the potential benefits of bleach bath therapy, only to dispel the false conclusion that it is a form of antimicrobial therapy. Indeed, as a recent meta-analysis concluded that water baths alone can significantly decrease the severity of atopic dermatitis⁴, beneficial effects may be due to actions other than directly on the skin microbiome. With improved disease the bacterial burden may indirectly decrease due to enhanced innate antimicrobial functions. The laboratory strains tested here were selected as reflective of clinical *S. aureus* strains on AD but may not represent the sensitivity of all strains. It is also conceivable that bleach baths may be directly anti-inflammatory on the skin. A previous study has reported that NaOCl can oxidize cysteine residues of the inhibitor of Nuclear Factor- κ B (NF- κ B), inhibit NF- κ B activity, and improve skin disease in mice⁹. However, the observations of our study show that a bleach bath does not directly inhibit *S. aureus*. This information should be considered in the interpretation of future studies of topical bleach baths.

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Figure 1. 0.005% bleach is not antimicrobial.

(A) Colony forming units (CFUs) of 3 strains of *S. aureus* growing on agar when exposed to bleach. (B) The survival of 2 strains of *S. epidermidis* growing on agar after exposure to bleach. (C) The survival of *S. aureus* growing in TSB solution after exposure to different household bleach solutions (Pure Bright[®], Clorox[®], and Waxie[®]). (D) *S. aureus* survival when growing at log phase or stationary phase during exposure to bleach. (E) *S. aureus* surviving on pig skin after exposure to bleach (F) *S. aureus* agr reporter activity (RFU) and survival (CFU) after exposure to bleach. Results are mean $\pm$ SD. *= $p < 0.05$ by Student's t test. All data are representative one of three independent experiments. The data of (A-C) were evaluated by three measurements made in parallel in each experiment.

Figure 1

