

0.01% Hypochlorous Acid as an Alternative Skin Antiseptic: An In Vitro Comparison

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OBJECTIVE Compare the in vitro efficacy of hypochlorous acid 0.01% (HA), povidone iodine 5% (PI), chlorhexidine gluconate 4% (CHG), and isopropyl alcohol 70% (IPA) against common skin microorganisms.

MATERIALS AND METHODS Time-kill studies were conducted against methicillin-susceptible *Staphylococcus aureus* (MSSA) and *Staphylococcus epidermidis* (MSSE), methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE), *Candida albicans*, *Corynebacterium species* (*striatum* and *amycolatum*), *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus capitis*, and *Staphylococcus xylosus*.

RESULTS Methicillin-resistant *S. aureus*: Bactericidal effect was immediate for HA and IPA. For PI and CHG, the effect occurred at 1 and 10 minutes, respectively. Methicillin-resistant *S. epidermidis*: Hypochlorous acid, IPA, and PI had immediate bactericidal effects, whereas CHG required 1 minute. Methicillin-susceptible *Staphylococcus aureus*: All agents had bactericidal effects at 1 minute. *C. species*, *S. pyogenes*, *P. aeruginosa*, and *P. acnes*: All antiseptics demonstrated immediate bactericidal effects. Methicillin-susceptible *Staphylococcus epidermidis* and *S. capitis*: Hypochlorous acid and IPA had immediate effect, whereas PI and CHG required 1 minute. *C. albicans*: Hypochlorous acid, IPA, and PI were immediately bactericidal, whereas CHG required 1 minute. *S. xylosus*: Hypochlorous acid and CHG were immediately bactericidal, whereas IPA and PI required 1 and 2 minutes, respectively.

CONCLUSION In vitro studies of HA 0.01% were observed to have equal or more efficacious antiseptic properties compared with IPA, CHG, and PI. Future studies will be needed to investigate its role in periocular use.

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The human skin microbiome is made up of a myriad of microorganisms that can cause complications ranging from superficial surgical site infections (SSIs) to severe systemic sepsis. Current antiseptic choice varies by anatomic location, with the face and periocular region requiring safety to the ocular surface, while still maintaining a wide antimicrobial spectrum.

Antiseptics are essential for the prevention of SSIs. For this reason, the Center for Diseases Control and the World Health Organization have published guidelines on the use of preoperative antiseptics.^{1,2} One com-

monly used agent is chlorhexidine gluconate 4% (CHG); however, its application for use around the face poses irreversible risks to the cornea, with severe cases requiring corneal transplantation.³ Isopropyl alcohol 70% (IPA), a cheap alternative, is often used for skin antiseptics before catheter insertion or injections.⁴ Its use is limited in the periocular region due to its toxic corneal effects.⁵ Povidone iodine 5% (PI), a safer alternative commonly used in ophthalmic surgery, is limited in its use on patients sensitive to iodine, and also colors the skin on application, requiring additional cleansing after performing the procedure.⁶

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Hypochlorous acid (HA) is naturally produced as part of the cytotoxic myeloperoxidase system in neutrophils.⁷ In vitro, when introduced to a variety of microorganisms, hypochlorous acid causes oxidation of nucleotides, inactivation of cell enzymes, disruption of cell membranes, and rapid cell lysis.^{8–11} Solutions containing HA not only possess effective antimicrobial properties, which have been known since the beginning of the 20th century, but also seem to be well tolerated to continuous use.¹² Hypochlorous acid shows minimal cytotoxic effects, although no studies have been conducted specifically on corneal cells.^{13,14} These unique characteristics may make it an ideal alternative for facial antisepsis before surgery or injections.

The purpose of this time-kill study is to compare the antiseptic properties of a 0.01% HA solution (Avenova; Novabay Pharmaceuticals, Inc., Emeryville, CA) with 3 common skin antiseptics, PI 5% (Betadine; Alcon Laboratories, Inc., Fort Worth, TX), CHG 4% (Hibiclens; Mölnlycke Health Care US, Norcross, GA), and IPA 70% (Swan; Vi-Jon, One Swan Drive, Smyrna, TN).

Methods

All bacteria tested were from ocular isolates from conjunctiva or socket infections of patients cultured at Bascom Palmer Eye Institute during a 4-month period (November 2016 through February 2017). These organisms were chosen as representative of common skin flora and included: methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA and MSSA, respectively), *Staphylococcus epidermidis* (MRSE and MSSE, respectively), *Staphylococcus xylosum*, *Staphylococcus capitis*, *Corynebacterium striatum*, *Corynebacterium amycolatum*, *Streptococcus pyogenes*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Each organism was streaked onto a 5% sheep blood agar plate (Remel; Thermo Fisher Scientific, Waltham, MA) and incubated at 36 to 37°C for 24 to 48 hours. Sufficient colonies of each isolate were inoculated into sterile saline and adjusted to a 0.5 McFarland standard (1.8×10^8 CFU/mL) in accordance with Clinical Laboratory Standards Institute. Ten microliters (10 μ L/mL) from each standard suspension was inocu-

lated onto 5% sheep blood agar to confirm CFU/mL count and serve as a control.

A 1-mL aliquot from each of the individual organism suspensions was added to 1 mL of each antiseptic and sampled at 0, 1, 2, 5, 10, 15, and 30 minutes using a 10- μ L calibrated loop. Control suspensions and samples were inoculated onto 5% sheep blood agar plates in triplicates for each time point and incubated at 36 to 37°C in CO₂ for 48 hours for aerobic organisms, or 96 hours in an anaerobic pouch (Gas-Pak EZ pouch; Becton, Dickinson and Company, Franklin Lakes, NJ) for anaerobic organisms. Immediately after incubation, the number of colony forming units (CFUs) was counted and the CFU/mL was calculated. A 3-log reduction or 99.9% killed was selected as in vitro efficacy or bactericidal end point. The tester was aware of the antiseptic agent used during the testing, which was not conducted in a blinded manner. Repeat testing was required for CHG to confirm the variability in results.

Isolate identifications were confirmed using a combination of an automated system (Vitek 2; BioMerieux, Durham, NC) and commercial biochemical kits (BioMerieux, Durham, NC, and Remel, Waltham, MA).

Results

Antiseptic in vitro efficacy was found to be variable against the tested organisms. Figure 1A–H summarize the in vitro efficacy of the 4 antiseptics.

Organisms

Methicillin-resistant *S. aureus*: A 3-log reduction was demonstrated on immediate contact for HA and IPA against MRSA. At 0 minute, HA and IPA were more bactericidal than PI. After the first minute, the bactericidal effect was equivalent (at 1, 2, 5, 10, 15, and 30 minutes). Chlorhexidine gluconate demonstrated 99.9% kill at 2 minutes, whereas 15 minutes were required to eliminate all CFUs.

Methicillin-susceptible *S. aureus*: All 4 antiseptics were bactericidal at 1 minute for the MSSA isolate. None of the 4 antiseptics demonstrated immediate growth inhibition for MSSA.

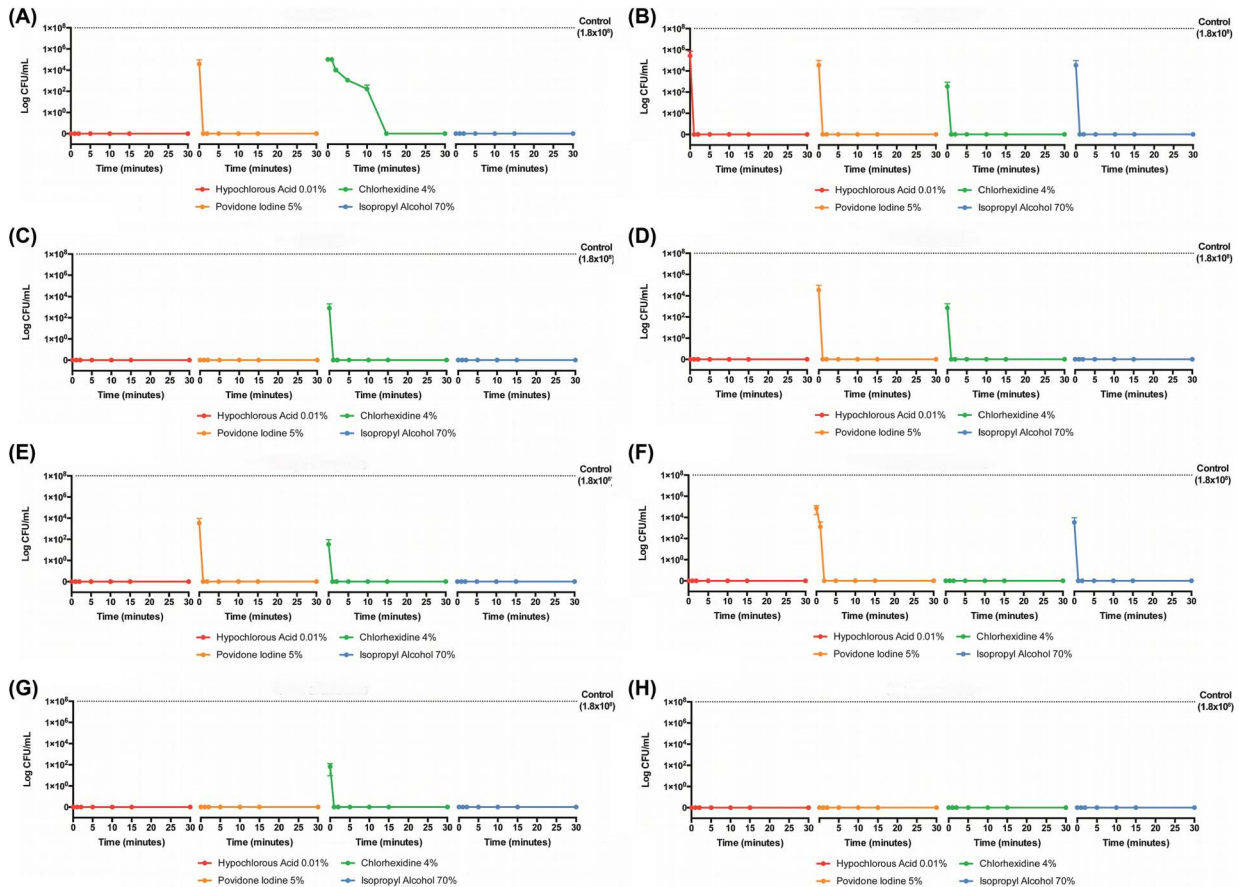


Figure 1. In vitro efficacy of common antiseptics against (A) methicillin-resistant *Staphylococcus aureus* (B) methicillin-susceptible *Staphylococcus aureus* (C) methicillin-resistant *Staphylococcus epidermidis* (D) methicillin-susceptible *Staphylococcus epidermidis* (E) *Staphylococcus capitis* (F) *Staphylococcus xylosus* (G) *Candida albicans* (H) *Propionibacterium acnes*, *Corynebacterium striatum*, *Corynebacterium amycolatum*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*. CFU, colony forming unit.

Methicillin-resistant *S. epidermidis*: All antiseptics were bactericidal with immediate exposure. Chlorhexidine gluconate eliminated all CFUs at 1 minute.

Methicillin-susceptible *S. epidermidis*: Hypochlorous acid and IPA inhibited all bacteria growth at 0 minute. Povidone iodine and CHG reached 99.9% kill at 1 minute.

***S. capitis*:** Hypochlorous acid and IPA eliminated all CFUs on immediate exposure. Although CHG and PI were bactericidal at 0 minutes, they eliminated all CFUs at 1 minute.

***S. xylosus*:** Hypochlorous acid and CHG have an immediate growth-inhibiting effect; whereas IPA was bactericidal at 0 minute and eliminated all CFUs at 1 minute. Povidone iodine demonstrated efficacy at 1

and 2 minutes, respectively. The results for these 2 coagulase-negative staphylococci species were distinct from *S. epidermidis*.

***C. albicans*:** Hypochlorous acid, IPA, and PI inhibited all bacterial growth at 0 minute. Chlorhexidine gluconate was bactericidal at 0 minute and has the same results at 1 minute.

For both types of *Corynebacterium species* (*striatum* and *amycolatum*), *Streptococcus pyogenes*, *P. aeruginosa*, and *P. acnes*, all 4 antiseptics exhibited immediate bactericidal effects at 0 minute.

Antiseptics

Hypochlorous acid 0.01%: Hypochlorous acid eliminated all bacterial colonies at 0 minute against most of

the microorganisms tested. With *MSSA*, the total colony reduction occurred at 1 minute.

Isopropyl alcohol 70%: Isopropyl alcohol had immediate effects at 0 minute for almost all microorganisms tested. With *MSSA* and *S. xylosus*, the total colony reduction occurred at 1 minute.

Povidone iodine 5%: Povidone iodine showed total colony reduction on *MRSE*, *C. albicans*, *C. striatum* and *amycolatum*, *S. pyogenes*, *P. acnes*, and *P. aeruginosa* on immediate exposure. At 1 minute, PI had a bactericidal effect on *MRSA*, *MSSA*, *MSSE*, and *S. capitis*. At 2 minutes, PI was effective against *S. xylosus*.

Chlorhexidine gluconate 4%: Chlorhexidine gluconate had an immediate effect against *C. striatum* and *amycolatum*, *S. pyogenes*, *P. acnes*, *P. aeruginosa*, and *S. xylosus*. At 1 minute, a full bactericidal effect was observed against *MRSE*, *MSSA*, *MSSE*, *S. capitis*, and *C. albicans*. Surprisingly, CHG required 2 minutes for a 99.9% kill of *MRSA* and 15 minutes for a total colony reduction. Repeat testing of this outlier yielded identical results.

Discussion

This in vitro study compares 4 commonly used antiseptics and their susceptibilities. The authors' results revealed a 99.9% kill for all evaluated organisms by 2 minutes for 3 of the 4 antiseptics. The only exception was CHG 4% against *MRSA*, which required 15 minutes to have equivalent effects.

Chlorhexidine gluconate 4% and PI 5% appeared to have the most varied efficacy to the organisms tested. Chlorhexidine gluconate binds to the bacterial or fungal cell membrane, destabilizing the structural integrity of the wall, causing cell death.¹⁵ However, PI acts by protein and DNA halogenation, blocking the electron chain transport, and disrupting intracellular enzymes and the cell membrane, eventually resulting in cell death.¹⁶ Although both antiseptics show immediate bactericidal effects to the numerous organisms listed in Figure 1, these antiseptics had a slower time-kill against *MRSA*, *MSSA*, *MSSE*, and *S. capitis*. Uniquely, CHG 4% was the only antiseptic to have slower time-kill against *MRSE* and *C. albicans*,

requiring 1 minute for complete bactericidal/fungicidal effects.

Hypochlorous acid 0.01% had an immediate microbicidal effect on all organisms evaluated when compared with the 3 commonly used skin antiseptics. This highlights its use as a potential alternative skin cleanser. The authors' results show that when compared with PI 5% and CHG 4%, HA 0.01% had equal or more immediate time-kill response to every microorganism tested. Although IPA 70% had a more immediate time-kill response compared with either CHG 4% or PI 5%, it had equivalent limitations to HA 0.01% against *MSSA*, and limited effects against *S. xylosus*.

Interestingly, one would expect *MRSA* or *MSSA* to have similar in vitro findings. Instead, the authors see a wide variability between the bactericidal effects of HA, IPA, and CHG against these organisms. Although both HA and IPA were immediately active against *MRSA*, both antiseptics required 1 minute to enact their respective effects against *MSSA*. The most prominent example was in the case of CHG, where a full colony reduction of *MSSA* took 1 minute versus 15 minutes in the case of *MRSA*.

These results have been observed in other studies and have been attributed to genes that encode for efflux-mediated resistance to CHG and the time-kill comparisons against endophthalmitis isolates of staphylococcal aureus isolates.^{15,17} By extrapolating these findings, one would expect intraspecies susceptibility differences between a variety of antiseptics. In vivo testing from Novabay Pharmaceuticals Inc. revealed that HA continued bacterial load of the lower eyelid skin completely after 20 minutes with various gram-positive, anaerobes, and gram-negative organisms; however, interesting a new bacteria flora over population.¹⁸

In practice, the use of PI is limited, especially in aesthetic facial filler injections due to its discoloring properties and tendency to hide fine rhytides. With these limitations, some clinicians prefer the use of CHG to PI due to its colorless nature. A recent multicenter randomized controlled study also demonstrated that CHG was superior to PI in preoperative skin cleansing.¹⁹ However, CHG is limited not only by its toxicity to the

corneal surface, causing severe keratitis with minimal exposure, but also by its antiseptic action against MRSA. Thus, the authors' *in vitro* results show that HA may have potential as a periocular and facial skin antiseptic and alternative surgical preparation.

Limitations to this study include the introduction of possible bias in antiseptic plating because the tests were not conducted in a blinded manner. The authors attempted to reduce any observed bias by using calibrated loops and standardizing each step of their time-kill study. Another possible limitation is that each time-kill solution for an organism was created only once and subsequently plated. The authors attempted to limit this source of error by inoculating each time point in triplicates and repeating the time-kill study for any agent that appeared as an outlier. This was performed in the case of CHG and MRSA, which on repeat testing, showed reproducible and identical time-kill results.

The authors' results show that HA exhibits a more rapid time-kill response compared with both PI and CHG, and a similar efficacy to IPA. In surgery, the time between preparation and initial cut is typically several minutes, allowing for the bactericidal effects of all 4 agents to set in. Rather, during facial injections or minor procedures, penetration of the skin by an instrument may occur within 1 minute of the preparation, arguing for the use of a bactericidal agent with immediate effects.

Taken together, these results suggest that HA 0.01% has the potential to be an equivalent, if not superior, alternative for antiseptics before procedures ranging from aesthetic injections to surgery. Limitations to the clinical use of HA 0.01% include recent evidence which suggests inhibited bactericidal action of HA in the presence of organic solvents.²⁰ Although the Food and Drug Administration has approved products containing HA for use on the eyelids and periocular skin, no study proves its safety on corneal epithelial cells. Thus, further studies need to be conducted to evaluate its efficacy and safety in a clinical setting.

References

1. Mangram AJ, Horan TC, Pearson ML, Silver LC, et al. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control

- and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control* 1999;27:97–132; quiz 133–134; discussion 196.
2. Leaper DJ, Edmiston CE. World Health Organization: global guidelines for the prevention of surgical site infection. *J Hosp Infect* 2017;95:135–6.
3. Steinsapir KD, Woodward JA. Chlorhexidine keratitis: safety of chlorhexidine as a facial antiseptic. *Dermatol Surg* 2017;43:1–6.
4. Hibbard JS. Analyses comparing the antimicrobial activity and safety of current antiseptic agents: a review. *J Infus Nurs* 2005;28:194–207.
5. Mac Rae SM, Brown B, Edelhofer HF. The corneal toxicity of presurgical skin antiseptics. *Am J Ophthalmol* 1984;97:221–32.
6. Lopez Saez MP, de Barrio M, Zubeldia JM, Prieto A, et al. Acute IgE-mediated generalized urticaria-angioedema after topical application of povidone-iodine. *Allergol Immunopathol (Madr)* 1998;26:23–6.
7. Babior BM. The respiratory burst of phagocytes. *J Clin Invest* 1984;73:599–601.
8. Albrich JM, McCarthy CA, Hurst JK. Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. *Proc Natl Acad Sci U S A* 1981;78:210–4.
9. Clark RA. Modulation of the inflammatory response by the neutrophil myeloperoxidase system. *Adv Exp Med Biol* 1982;141:207–16.
10. Slivka A, LoBuglio AF, Weiss SJ. A potential role for hypochlorous acid in granulocyte-mediated tumor cell cytotoxicity. *Blood* 1980;55:347–50.
11. Clark RA, Szot S. The myeloperoxidase-hydrogen peroxide-halide system as effector of neutrophil-mediated tumor cell cytotoxicity. *J Immunol* 1981;126:1295–301.
12. Whitfield N. Surgical skills beyond scientific management. *Med Hist* 2015;59:421–42.
13. Kim HJ, Lee JG, Kang JW, Cho HJ, et al. Effects of a low concentration hypochlorous acid nasal irrigation solution on bacteria, fungi, and virus. *Laryngoscope* 2008;118:1862–7.
14. Crew JR, Thibodeaux KT, Speyrer MS, Gauto AR, et al. Flow-through instillation of hypochlorous acid in the treatment of necrotizing fasciitis. *Wounds* 2016;28:40–7.
15. Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? *J Antimicrob Chemother* 2012;67:2547–59.
16. Rackur H. New aspects of mechanism of action of povidone-iodine. *J Hosp Infect* 1985;6:13–23.
17. Klocek MSMA, Dhaliwal DK, Kowalski RP. Time-kill comparison of povidone iodine to hypochlorous acid against endophthalmitis isolates of staphylococci. Presented at the Ocular Microbiology and Immunology Group, Chicago, Illinois, October 14, 2016.
18. Stroman DW, Mintun K, Epstein AB, Brimer CM, et al. Reduction in bacterial load using hypochlorous acid hygiene solution on ocular skin. *Clin Ophthalmol* 2017;11:707–14.
19. Darouiche RO, Wall MJ Jr, Itani KM, Otterson MF, et al. Chlorhexidine-alcohol versus povidone-iodine for surgical-site antiseptics. *N Engl J Med* 2010;362:18–26.
20. Eryilmaz M, Palabiyik IM. Hypochlorous acid—analytical methods and antimicrobial activity. *Trop J Pharm Res* 2013;12:123–6.

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