The Antibacterial Comparison of 5% and 2.5% Povidone Iodine to 0.01% Hypochlorous Acid Using Corneoscleral Tissue as a Solid-Phase Medium

Regis Kowalski Ι
Roheena Kamyar Ι
Michelle Rhee 2
Alex Mammen Ι
Deepinder Dhaliwal Ι
Eric G Romanowski Ι
Vishal Jhanji Ι
Andrew W Eller Ι

1University of Pittsburgh Medical Center (UPMC), The Charles T. Campbell Ophthalmic Microbiology Laboratory, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; 2The Eye-Bank for Sight Restoration, New York, NY, USA

Purpose: Prophylactic topical antiseptics used to eliminate bacteria on the ocular surface prior to ocular surgery should be both effective and non-irritating. Five percent povidone iodine (PI) is an accepted antiseptic used for prophylaxis. Dilute 2.5% PI and 0.01% hypochlorous acid (HOCl) may be more patient comfortable and equally effective. PI at 5% and 2.5% were compared to HOCl against a battery of bacterial endophthalmitis isolates using corneoscleral tissue as a solid-phase medium to determine antiseptic efficacy.

Methods: Bacteria from 20 cases of endophthalmitis were tested for the elimination of growth against topical 5% PI, 2.5% PI, HOCl, and no antiseptic using donor corneoscleral tissue. The tissue was inoculated with 10^3 colony forming units of bacteria prior to a 3-minute contact time with the antiseptics, placed in liquid growth medium, and monitored for growth at three days. No growth indicated antiseptic treatment success. Differences were analyzed using Chi square (χ^2).

Results: For 20 isolates, 5% PI was comparable to 2.5% PI for preventing bacteria growth (p=0.71), and both were more effective than HOCl (p=0.004). Estimated weighted comparison over a 27-year period indicated that for all bacterial groups, except Streptococcus viridans, 5% PI was equally effective to 2.5% PI for preventing bacterial growth (p=1.0). For Streptococcus viridans, 5% PI was more effective than 2.5% PI (p=0.0001). Both concentrations of PI were more effective than HOCl (p=0.00001).

Conclusion: Five percent PI appears to be optimal as a prophylaxis prior to ocular surgery.

Keywords: povidone iodine, hypochlorous acid, endophthalmitis prophylaxis, intravitreal injection, corneoscleral tissue, antiseptic susceptibility

Introduction

Endophthalmitis after intravitreal injection is a rare but severe complication. Unlike endophthalmitis after cataract surgery, each patient has multiple chances of intraocular infection. Patients often require monthly injections of anti-vascular endothelial growth factor (VEGF) to control age-related macular degeneration (AMD), diabetic macular edema (DME), and retinal vein occlusion (RVO).

Even oral flora spread through speech has been implicated as a vector for intraocular infection. The rate of endophthalmitis after intravitreal injections has been reported by Gregori^3 to be 0.02% to 0.5%, McCannel^5 0.025% (based on 26 culture positive cases out of 105,536 injections), and Merani^5 0.028%. Cheung^6 reported the...
endophthalmitis rate after intravitreal injection of steroids was higher than the intravitreal injection of VEGF. Grzybowski \cite{7} reported that 5.9 million intravitreal injections were administered in the US during 2016.

Ophthalmologist has turned to antiseptics instead of antibiotics for surgical prophylaxis, which includes intravitreal prophylaxis, because of the potential of acquired resistance due to frequent antibiotic application.\cite{8} The prophylaxis consensus prior to intravitreal injection and other ocular surgeries is the application of the antiseptic, 5% povidone iodine (PI), to the ocular surface and the clearance of the eyelid and lashes from the injection site.\cite{9,10} PI is a composite of elemental iodine, hydrogen iodine, and povidone.\cite{11} It is an antibacterial broad-spectrum, in which free iodine is the active component, and it has minimal residual activity. It can be an irritant when left on tissue surfaces for a long time. The stinging and burning nature of 5% PI has prompted retinal specialists, especially in cases of multiple intravitreal injections, and other ocular surgeons to seek other effective prophylactic measures. This includes lower dilutions of PI and 0.01% hypochlorous acid (HOCl). It has been reported that PI at lower concentrations may be as active as higher concentrations due to the release of free iodine.\cite{12,13} Hypochlorous acid is a strong oxidizing agent used in the hospital setting as a cleaning and disinfecting agent.\cite{15} At a neutral pH of 7.0, it is non-toxic and leaves no residual. (Infection Control Today.com/view/hypochlorous-acid-definitive-terminal-cleaning-hospital-environment) (Dimmitt D, June 9, 2014) (accessed 01/14/2021). HOCl has been marketed (NovaBay Pharmaceuticals Inc, Emeryville, CA) (www.avenova.com/hypochlorous/acid) for eyelid hygiene and eye health to reduce bacteria that may cause blepharitis and exasperate dry eye. It has no indication for prophylaxis and contact with the ocular surface.

In two previous in vitro studies, we compared 5% PI and 0.01% HOCl. In the Chronister study,\cite{16} we found 5% PI to be more effective than HOCl (Sterilid®) (HOCl spray foam that contains tea tree oil). Using a liquid formulation of hypochlorous acid, Klocek\cite{17} demonstrated similar activity between 5% PI and 0.01% HOCl (Avenova®), but the bactericidal effect by 0.01% HOCl was reduced from 2 to 1 minute. In an eyelid study by Gonzalez\cite{18} on volunteers, with 5% PI applied to one eyelid and 0.01% HOCl (Avenova®) applied to the other eyelid, the reduction of bacterial flora was equivalent. It must be noted that Kanclerz compared 10% PI to HOCl for cataract surgery prophylaxis. They found conjunctiva lavage with 10% PI decrease bacterial load and HOCl did not. HOCl was more comfortable to the patient and no endophthalmitis was noted for either treatment group.\cite{19}

The present unique study utilizes corneoscleral tissue to evaluate antiseptic susceptibility on a solid phase medium. The corneoscleral tissue would be a better reality assimilation of the ocular surface than bacterial dispersion in a liquid medium. The antiseptics would be tested against a panel of bacterial endophthalmitis isolates. The design of the study was to eliminate bacteria and not demonstrate a reduction, because dead bacteria do not cause infection. We hypothesize that 5% PI, 2.5% PI and HOCl will prevent equally bacterial growth within the recommended time frame of 3 minutes (Betadine packet insert®). Analysis will include a projected weighted comparison over a 27-year period.

**Methods**

**Bacterial Endophthalmitis Isolates**

Twenty bacterial endophthalmitis isolates were tested for this study: 1) *Staphylococcus aureus* (Sa) E904, 2) coagulase negative *Staphylococcus* (CNS) E820, E923, E922, and E 920, 3) *Enterococcus faecalis* (Ef) E913, 4) *Streptococcus viridans* group (Sv) (also referred to some as *viridans Streptococcus*) E927, E926, E919, and E910, 5) Beta-hemolytic Streptococcus E819 (Beta-Strep, ) 6) *Pseudomonas aeruginosa* (Pa) E915, 7) *Serratia marcescens* (Sm) E886, 8) *Bacillus cereus* (Bc) E776, 9) Methicillin Resistant *Staphylococcus aureus* (MRSA) E897, 10) *Streptococcus pneumoniae* (Sp) E891, E845, E737, E709, and 11) *Haemophilus influenzae* (Hi) E789. Single isolates of the 11 groups were initially tested. Antiseptic resistance was noted for the single isolates of CNS, Sv, and Sp. It was decided to test three additional isolates for these groups to determine whether there was consistent resistance. All isolates were stocked in a clinical tissue bank, de-identified for patient identification, and were used to support antibiotic susceptibility validation and laboratory certification. The 20 isolates were selected from a collection of 689 isolates that included intraocular infection due to intravitreal infection, surgery, trauma, and an endogenous route.\cite{20} The number of endophthalmitis cases due to intravitreal injection could not be precisely deduced.

For testing, the bacterial isolates were retrieved from frozen stocks (−80°C) and grown overnight on trypticase soy agar plates supplemented with 5% sheep’s blood.
The following day, the bacterial isolates were suspended in 5 mL of saline to a 0.5 McFarland standard which corresponded to a growth of approximately $1 \times 10^8$ CFU/mL. Colony counts were determined by plating serial dilutions of the suspension. The dilutions were $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$. The $10^{-3}$ dilution would contain approximately $1 \times 10^5$ colony forming units (CFU) per mL of bacteria. A 0.01 mL (10 µL) sample of this would contain $10^3$ CFU of bacteria. This was the inoculum aliquoted to the corneal tissue in this experiment.

Human Corneoscleral Tissue
Excess corneoscleral donor tissues (rims) were used to test susceptibility in this study as a solid phase instead of using liquid media. In general, the rims were excess tissue received from corneal surgeons after keratoplasty surgery and not from the eye bank. Corneoscleral rims were cultured for microbial contamination after keratoplasty by placing the rims in 10 mL of enriched thioglycollate broth (BBL™, Becton, Dickinson and Co., Sparks, MD). After 5 days of incubation at 37°C, the culture-negative rims were removed from the enriched thioglycollate broth and soaked in 50 mL of PBS (phosphate buffered saline) for 72 hours to elute any inhibitory factors (ie gentamicin as part as Optisol-GS®). The rims were stored at −80°C for research purposes in a clinical tissue bank. In addition, whole corneas deemed not suitable for surgical use, but biologically safe research tissue, were donated by the Eye Bank (The CORE Eye Bank of Pittsburgh Pennsylvania). These corneas were also cultured for bacteria and fungi, eluted in 50 mL of PBS for 72 hours, and stored at −80°C. It must be re-emphasized that we only controlled for inhibitory factors that could inhibit bacterial growth.

Antiseptics
Five percent povidone iodine (pH= 4.0) (Betadine, Alcon, Fort Worth, TX) (Lot #KWB002) and 0.01% hypochlorous acid (pH=3.78) (Avenova, NovaBay Pharmaceuticals, Emeryville, CA) (Lot # 57653) were purchased for this study. Five percent PI was diluted 1:2 in phosphate buffer saline (pH 7.2, Gibco, Grand Island, NY) to make 2.5% PI (pH=5.34).

Experimental Protocol
The retrospective study did not require an Institutional Review Board/Ethics committee approval because direct patient contact and personal information were not involved. The CORE Eye Bank of Pittsburgh Pennsylvania has given permission for the research use of these excess corneal tissues. Figure 1 is a diagram detailing the experimental steps for the elimination of bacteria attached to corneoscleral tissue by topical antiseptics.

1. Twenty donor corneal rims were retrieved from −80°C, thawed, quartered, and placed in 4 wells (marked a-d) of a 6 well multi-well plate. Each bacterial group initially was only tested once due to the paucity of corneal rims. Four isolates of CNS, Sv, and Sp were tested to assure susceptibility consistency.

2. $10^3$ CFU of bacteria in a 10 µL volume was placed on each quarter of corneal tissue.

3. Treatment for each quarter rim was as follows:
   “a” was the untreated control (no antiseptic),
   “b” was treated with 5% PI,
   “c” was treated with 2.5% PI, and
   “d” was treated with 0.01% HOCl.

4. Treatment was administered topically with 3 sprays to cover the tissue. The experiment was staggered to allow 3 minutes contact time for the untreated control and three antiseptics. Avenova® spray bottles were used for all three antiseptic preparations.

5. After 3 minutes, the treated tissues were transferred to 10 mL of growth medium: Mueller Hinton broth (Sa, CNS, Pa, Sm, Bc, MRSA), Mueller Hinton broth with 3% lysed horse blood cells (Sv, Sp, Ef, Beta-Strep), or Haemophilus test medium (Hi) depending on the organism.

6. Observance of growth was monitored for 3 days and growth in liquid medium was confirmed on trypti-case soy agar supplemented with 5% sheep blood (Hi was confirmed on chocolate agar).

7. Growth in comparison to untreated control was denoted as failure of treatment to eliminate bacteria. No growth indicated treatment success.

8. Positive-growth after antiseptic treatments were tabulated in Table 1. The number of isolates, percent incidence, and number per year were calculated for a 27-year period from 1993 to 2019 (clinical bank of the Charles T. Campbell Ophthalmic Laboratory). The total number of bacterial isolates for the 27
years was also determined. We calculated the percent incidence by dividing the isolate incidences by the total number (689). By dividing the isolate incidence by 27, we calculated the number of isolates per year for each bacterial group.

9. Differences in antiseptic treatment were analyzed with Chi square testing ($\chi^2$) [https://www.socscistatistics.com/tests/chisquare/default2.aspx] (accessed July 6, 2021) for the 20 isolates.

10. The estimated number of positive tests after antiseptic treatment failure was determined from the weighted incidence of each bacterial group for the period 1993 to 2019 (n=689). These are tabulated in Table 2. The positive numbers between the antiseptic treatment groups were analyzed with Chi square testing ($\chi^2$).

Results

Table 1 details a comparison of 5% and 2.5% PI antibacterial efficacy to 0.01% HOCl using bacteria isolated from endophthalmitis attached to corneoscleral tissue. Five percent PI was comparably effective to 2.5% PI for preventing bacteria growth ($\chi^2$, p=0.71). Both 5% and 2.5% PI were more effective in preventing bacterial growth than 0.01% HOCl ($\chi^2$, p=0.004).

Of the bacteria that comprised of 4 isolates each, CNS (n=4), 5% PI (4 of 4) and 2.5% PI (4 of 4) were more effective for preventing bacterial growth than HOCl (1 of 4). For Sv (n=4), 5% PI (3 of 4) was more effective for preventing bacterial growth than 2.5% PI (1 of 4) and HOCl (1 of 4). For Sp (n=4), 5% PI (4 of 4) and 2.5% PI (4 of 4) were more effective for preventing bacterial growth than HOCl (1 of 4).

Both concentrations of PI and HOCl did not prevent the growth of Beta-Strep (isolated after an intravitreal injection), Ef, and Bc. Based on an intravitreal injection infection rate of 0.00025 and a yearly endophthalmitis infection probability of approximately 0.5 per year (Table 1), the infection rate for each of these bacteria would be 1 per 10,000 per year (this is based on endophthalmitis cases, not the total number of intravitreal injections). In other words, out of 10,000 endophthalmitis cases in any one year, there is only a single chance that it would be by Beta-Strep, Ef, or Bc.

Table 2 details a weighted comparison of 5% and 2.5% PI to 0.01% HOCl based on bacteria isolated from endophthalmitis over a 27-year period (1993–2019). For all bacterial groups except Sv, 5% PI was equally effective to 2.5% PI for preventing bacteria ($\chi^2$, p=1.0). Both 5% and 2.5% PI were more effective than 0.01% HOCl ($\chi^2$, p=0.00001). For Sv, 5% PI was more effective than 2.5%
PI for reducing growth ($\chi^2$, p=0.0001). The probability of a Sv endophthalmitis based on a 0.00025 infection rate and an estimate yearly rate of 2.26 would indicate 5.65 infections per 10,000. In contrast, CNS would be 39.25 per 10,000.

**Discussion**

Antibiotic susceptibility testing for systemic treatment is based on the concentrations of antibiotic in the blood serum. There are no standards for topical treatment or intravitreal injection. Testing is performed in liquid medium with varying concentrations of antibiotic to determine an inhibitory concentration and not a bactericidal concentration. This dispersion of bacteria in antibiotic supplemented medium requires 24 hours of incubation time. This indirect approach is cost-effective and is the standard to estimate in vitro to in vivo correlation in a timely manner, but this does not necessarily represent the true

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Untreated</th>
<th>5% Povidone Iodine</th>
<th>2.5% Povidone Iodine</th>
<th>0.01% Hypochlorous Acid</th>
<th>Isolates 1993–2019</th>
<th>Percent Incidence 1993–2019</th>
<th># Per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coag Negative Staph E877</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>424</td>
<td>61.5%</td>
<td>15.7</td>
</tr>
<tr>
<td>Coag Negative Staph E923</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Coag Negative Staph E922</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Coag Negative Staph E920</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>80</td>
<td>11.6%</td>
<td>2.96</td>
</tr>
<tr>
<td>Staphylococcus aureus E904</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Streptococcus viridans E927</td>
<td>POS</td>
<td>Neg</td>
<td>POS</td>
<td>Neg</td>
<td>61</td>
<td>8.8%</td>
<td>2.26</td>
</tr>
<tr>
<td>Streptococcus viridans E926</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Streptococcus viridans E919</td>
<td>POS</td>
<td>Neg</td>
<td>POS</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Streptococcus viridans E910</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MRSA E897</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>NEG</td>
<td>29</td>
<td>4.2%</td>
<td>1.07</td>
</tr>
<tr>
<td>Streptococcus pneumoniae E891</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>26</td>
<td>3.8%</td>
<td>0.96</td>
</tr>
<tr>
<td>Streptococcus pneumoniae E845</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Streptococcus pneumoniae E737</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Streptococcus pneumoniae E709</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Beta hemolytic Streptococcus E819</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>15</td>
<td>2.2%</td>
<td>0.55</td>
</tr>
<tr>
<td>Enterococcus faecalis E913</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>14</td>
<td>2.0%</td>
<td>0.52</td>
</tr>
<tr>
<td>Bacillus cereus E776</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>14</td>
<td>2.0%</td>
<td>0.52</td>
</tr>
<tr>
<td>Serratia marcescens E886</td>
<td>POS</td>
<td>NEG</td>
<td>neg</td>
<td>POS</td>
<td>10</td>
<td>1.4%</td>
<td>0.37</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa E915</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>9</td>
<td>1.3%</td>
<td>0.33</td>
</tr>
<tr>
<td>Haemophilus influenzae E789</td>
<td>POS</td>
<td>Neg</td>
<td>Neg</td>
<td>POS</td>
<td>7</td>
<td>1.0%</td>
<td>0.26</td>
</tr>
<tr>
<td>Percent Positive</td>
<td>20 of</td>
<td>4 of 20</td>
<td>6 of 20 30%</td>
<td>15 of 20 75%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Notes:** Bacteria – These were representative bacteria isolated from endophthalmitis for this study. Other bacterial genus and species were isolated, but not tested. The E followed by a number indicates the endophthalmitis isolate number from the clinical bank collection. “POS” indicates positive-growth in liquid medium and treatment failure. “neg” indicates no growth in liquid medium, and treatment success. 5% povidone iodine was equally effective to 2.5% povidone iodine for preventing bacteria growth ($\chi^2$, p=0.71). Both 5% ($\chi^2$, p=0.0005) and 2.5% ($\chi^2$, p=0.004) povidone iodine were more effective for eliminating bacteria than 0.01% hypochlorous acid.
realism of treatment. A more accurate approach to test susceptibility would be to use a solid medium to simulate an infection in tissue (i.e., skin, cornea). The anti-infective would be applied to the tissue with attached bacteria at different time frames to determine efficacy of treatment. This method of clinical susceptibility would be near impossible for each patient.

In the present study, corneoscleral tissue (representing the ocular surface) was used as a solid medium; bacteria from endophthalmitis were tested; antiseptics were tested for efficacy; and a 3-minute time-period was the contact period. The limitation of this model was the paucity of corneoscleral tissue. Corneal rims are firstly offered to research within our department. Fortunately, the experience of the first author (RPK) allowed single testing with confidence in the results. The bacteria selected were based on frequent causes of endophthalmitis (CNS, *Streptococcus* species, Sa) and bacteria that produced severe endophthalmitis (Be, Ef, Gram-negative bacteria). Longer contact times with antiseptics may be advantageous, but this may not be practical for the ophthalmologist who is administering an intravitreal injection; plus, the antiseptics may be uncomfortable to the patient. Our study does not include the antibacterial effect of the host defense of the ocular surface, which may be noteworthy, but not a variable in this report.

Endophthalmitis after intravitreal injection is a rare event, but our data indicate the best antiseptic to prevent infection is 5% PI. Except for Sv, 2.5% PI was just as effective as 5% PI. HOCl was less effective as an antiseptic to prevent bacterial growth in the present study, but HOCl may provide prophylaxis in those that are allergic to PI.

Bacteria such as Be, Ef, and Beta-Strep may not be eliminated with PI and HOCl, but fortunately these are very rarely the causative agents in post-surgical endophthalmitis. It appears that both PI and HOCl have less activity to the *Streptococci* groups and *Bacillus* species that have cell wall components that inactivate free iodine and resist oxidation. These bacteria can have

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**Table 2** Weighted Comparison of 5% and 2.5% Povidone Iodine Antibacterial Activity to 0.01% Hypochlorous Acid Based on Bacteria Isolated from Endophthalmitis Over a 27-Year Period (1993–2019). Corneoscleral Tissue Was Used as a Solid-Phase Medium to Assimilate the Ocular Surface. Prevention of Bacterial Growth After Antiseptic Application Indicated Success of the Antiseptic

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Isolates (1993–2019)</th>
<th>No Antiseptic</th>
<th>5% Povidone Iodine</th>
<th>2.5% Povidone Iodine</th>
<th>0.01% Hypochlorous Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase Negative Staphylococcus*</td>
<td>424</td>
<td>424</td>
<td>0</td>
<td>0</td>
<td>318 (0.75 of 424)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>80</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus viridans*</td>
<td>61</td>
<td>61</td>
<td>15 (0.25 of 61)</td>
<td>46 (0.75 of 61)</td>
<td>46 (0.75 of 61)</td>
</tr>
<tr>
<td>Methicillin-Resistant Staphylococcus aureus</td>
<td>29</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumoniae*</td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>20 (0.75 of 26)</td>
</tr>
<tr>
<td>Beta hemolytic Streptococcus</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Bacillus</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9</td>
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<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Percent Positive</td>
<td>689</td>
<td>100% (689 of 689)</td>
<td>8.4% (58 of 689)</td>
<td>13.0% (89 of 689)</td>
<td>66% (453 of 689)</td>
</tr>
</tbody>
</table>

Notes: Asterisk indicates that 4 representative isolates each were tested for growth by coagulase negative *Staphylococcus*, *Streptococcus viridans*, and *Streptococcus pneumoniae*. Parenthesis indicates the estimated percent positive of total number of isolates in the 27-year period 1993–2019 based on the testing of the 4 isolates from Table 1. For all bacterial groups except *Streptococcus viridans*, 5% povidone iodine was equally effective to 2.5% povidone iodine for preventing bacteria ($\chi^2$, p=1.0). For *Streptococcus viridans*, 5% povidone iodine was more effective than 2.5% povidone iodine for reducing growth ($\chi^2$, p=0.0001). Both 5% ($\chi^2$, p=0.00001) and 2.5% povidone iodine ($\chi^2$, p=0.00001) were more effective than 0.01% hypochlorous acid.

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varying peptidoglycan and cell wall structures, and these bacteria frequently form capsules for antibiotic protection.

We reject our original hypothesis because both 5% PI and 2.5% PI were more effective than HOCl for preventing bacterial growth from corneoscleral tissue. Our model should be considered to evaluate other anti-infectives for preventing bacterial growth on ocular tissues.

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Disclosure
Dr Michelle Rhee reports grants from Ocular Therapeutix, is a Medical Advisor Board member for NovaBay, and consultant for The Eye-Bank for Sight Restoration, outside the submitted work. Dr Deepinder Dhaliwal reports personal fees from Kala, personal fees from Trefoil, personal fees from Haag Streit, personal fees from Novartis, personal fees from Allergan, personal fees from Novartis, personal fees from Allergan, personal fees from Staar Surgical, grants from Noveome, grants from Kowa, outside the submitted work. The authors have no current “Significant Conflict of Interests” to disclose for the completion of this study as determined by the Office of Research, University of Pittsburgh, Pittsburgh, PA, USA.

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