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Professional paper

Comparative Study of Antibacterial and Antifungal Effects of Rigid Gas Permeable Contact Lens Disinfecting Solutions

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A B S T R A C T

The aim of this study was to compare antimicrobial efficacy of rigid contact lens disinfecting solutions. We tested five commercially available solutions: Unique pH (Alcon Laboratories), Boston Advance (Polymer Technology Corp.), Nitilens Conditioner GP (Avizor), Total Care (AMO), Boston Simplus (Bausch&Lomb). Their efficacy to disinfect saline solution experimentally contaminated with American Type Culture Collection (ATCC): Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 90028) and Staphylococcus epidermidis (isolated from our laboratory) was tested. All tested solutions reduced concentrations of bacteria and fungi below 1000 CFU/mL (Colony forming unit; reduction by 3 log and 1 log, respectively) after the 8 hours period. Overall, all contact lens care solutions showed good disinfecting activity against tested bacteria and fungi, with more variation in their antifungal than in antibacterial efficacy. Results of our study might be valuable when selecting appropriate solutions for non-compliant contact lens wearers.

***Key words**: rigid gas permeable contact lens, disinfecting solution, antimicrobial, antifungal, multipurpose disinfecting solution efficacy*

Introduction

Over the past 40 years contact lens (CL) have become increasingly popular for the correction of refractive eye errors[1].

In general, CL complication rate depends on patients' compliance with recommended lens care guidelines[2]. Contact lens wear is the major risk factor for microbial keratitis, a potentially vision threatening condition[3]. Contact lens acts as a vector for commensal or potential pathogen microorganisms to by which they adhere and transfer to the ocular surface, colonize the cornea or conjunctiva and consequently cause inflammation or infection[4-6]. The incidence of microbial keratitis among contact lens wearers appears to be increasing. This can be partially explained by the increasing popularity of CL[1]. Reports suggest that all types of care systems can become contaminated, including up to 30% of preserved products[4,7,8]. However, when lens cleaning, rinsing, disinfection and storage instructions are carefully followed, CL contamination can be significantly reduced. Unfortunately, a sizable proportion of CL wearers do not adequately adhere to contact lens care recommendations[2].

Efficient disifnfection solutions have a major role in safe CL wear[7,9]. In the last few decades single purpose solutions have been largerly replaced by the multipurpose solutions (MPS) for cleaning, disinfecting and rinsing rigid gas permeable lenses. Today, around 60% of contact lens wearers use MPS[10]. Although they have simplified cleaning and disinfecting processes, in order to achieve all of the intended tasks, the manufacturers of MPS have to make some compromises. It has been suggested that disinfecting agents used in MPS are less efficient, but have better wetting and comfort abilities[11]. There are still not enough data on the antimicrobial efficacy of these multipurpose solutions, or on the effects of storage conditions on their disinfecting capacities[12].

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The International Organization for Standardization (ISO) has established microbiological requirements and test methods for products and regimens for hygienic management of contact lenses with methodology and acceptance criteria for stand-alone disinfecting solutions (ISO/CD 14729). According to the standard for stand--alone primary acceptance criteria, disinfecting solution must be able to reduce the starting concentration of bacteria (*Serratia marcescens, Pseudomonas aeruginosa* and *Staphylococcus aureus*) by 3 log and fungi (*Fusarium solani* and *Candida albicans*) by 1 log at the minimum disinfection time recommended by the manufacturers[13]. However, two common bacteria, *Staphylococcus epidermidis* and *Escherichia coli,* which are not required by the ISO standards, are often the cause of ocular pathology[14–16].

Therefore, this study investigated relative antimicrobial activity of the commonly used five rigid gas permeable contact lens disinfection solutions and addressed the need for comparing performances of currently available contact lens disinfecting products.

Solutions Unique PH, Nitilens conditioner GP and Total care were now for the first time included in this type of study.

Materials and Methods

We tested the following solutions: Unique pH (Alcon Laboratories), Boston Advance (Polymer Technology Corp.), Nitilens Conditioner GP (Avizor), Total Care (Advanced Medical Optics) and Boston Simplus (Bausch & Lomb) (Table 1). The test solutions were challenged to disinfect saline solution experimentally contaminated with clinical isolates and the standard strains of American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) and *Staphylococcus epidermidis* (isolated from our laboratory).

The bacteria were grown on the blood agar plate while *Candida albicans* on the Emmons agar plate. Using the physiological saline, the microbial suspensions were adjusted to contain 1.0×10^8 colony-forming units per millilitre (CFU/mL) bacteria and 1.0×10^6 CFU/mL fungi. The appropriate volume of the disinfection solution (10 mL A-B; 2 mL C-E) and 2 mL of the physiological saline were inoculated with the appropriate volume of the microbial suspension (100 μL in A-B; 20 μL in C-E and in the physiological saline) to achieve a final concentration of 1.0×10^6 CFU/mL bacteria and 1.0×10^4 CFU/mL fungi. This concentration was the first one in the series of dilutions (1.0×10^6 or 1.0×10^4 to 1.0×10^1) with which the plates were inoculated separately four times. The mixtures of the disinfection solution and the microbial suspension were stored at the ambient temperature, which was 25±1 °C. After 8-hour incubation (overnight disinfection period), appropriate disinfectant neutraliser was applied and the plates were inoculated. The blood and the Mueller-Hinton agar plates were used for identification of the bacterial grow and the Emmons agar plate for identification of fungal growth. The agar plates were cultured at 35±2 °C for 24–72 hours. In addition, sterility control of disinfection solutions (100 μL of each solution were seeded in the blood and Mueller-Hinton agar plate) and microbial growth control (100 μL of each microbial suspension in a series of dilutions 1.0×10^8 or 1.0×10^6 to 1.0×10^1 CFU/mL were seeded in the blood or Emmons agar plate) were performed.

Results

All study solutions reduced microorganism concentrations below 1000 CFU/mL (concentrations of bacteria and fungi reduced by 3 log and 1 log, respectively). However, there were differences in their disinfecting efficacy (Figure 1).

Solution A containing Polyquad preservative (PQ-1) showed excellent microorganism reduction efficacy against *Staphylococcus aureus*, *Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans* (concentration <10 CFU/mL), but less efficacy against *Escherichia coli* (<100 mCFU/mL, Figure 1).

Solution B containing Polyaminopropyl biguanide (PAPB), Chlorhexidine gluconate and Ethylenediaminetetraacetic acid (EDTA) as disinfecting agents showed excellent efficacy against all bacteria tested (all below

TABLE 1
FORMULATIONS OF THE RGP CONTACT LENS DISINFECTION SOLUTIONS

RGP lens solution	Code used in study	Manufacturer	Active ingredients
Unique PH	A	Alcon	Antimicrobial 0.001%, EDTA 0.01%; AL 12355 polymer system, Polyethylene glycol, PQ-1
Boston Advance	B	Polymer corp.	PAPB 0.0005%; Chlorhexidine gluconate 0.003%; EDTA 0.05%
Nitilens conditioner GP	C	Avizor	PHMB 0.0002%, EDTA 0.1%
Total care	D	AMO	PHMB 0.006%; Lauryl quaternised protein 0.085%; EDTA 0.127%
Boston Simplus	E	Bausch& Lomb	PAPB 0.0005%

EDTA – Ethylenediaminetetraacetic acid; PAPB – Polyaminopropyl biguanide; PHMB – Polyhexamethylene biguanide = Polixetonium chloride = Polyhexanide; PQ-1 – Polyquad preservative

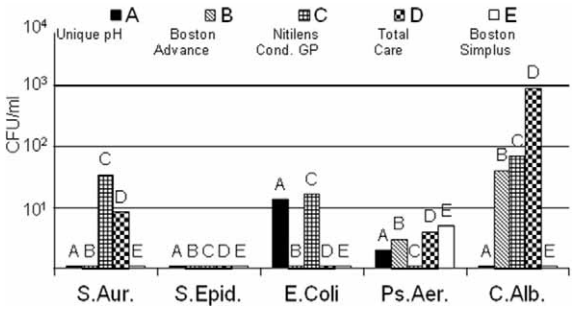

Fig. 1. Number of colony-forming units per mililiter (CFU/mL) of bacteria and fungi remained after 8-hour disinfection period for study solutions. Thick horizontal line represents 3 log and 1 log reduction criteria for bacteria and fungi, respectively.

<10 CFU/mL), but was not equally effective against *Candida albicans* (<100 CFU/mL).

Solution C containing polyhexamethylene biguanide (PHMB) and EDTA showed good efficacy against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, as the concentration of bacteria was below 10 CFU/mL, whereas it was less effective against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (<100 CFU/mL).

Solution D containing PHMB, EDTA and Lauryl quaternised protein showed good antibacterial activity (all bacteria below 10 CFU/mL), with the exception of *Candida albicans* (<900 CFU/mL)

Solution E containing PAPB showed excellent efficacy against all microorganisms tested (all below 10 CFU/ mL).

Overall, solution E demonstrated the greatest disinfection efficacy (all below 10 CFU/mL), as well as excellent activity against clinical strains of *P. aeruginosa* and maximum antifungal activity.

From the selected test organisms *Staphylococcus epidermidis* was found to be most sensitive and *Candida albicans* was found to be most resistant to the disinfection solutions used.

All of the five test solutions in this study provided a reduction greater than 3.0 logarithmic reduction against tested bacteria and fungi, with more variation in their antifungal than in antimicrobial efficacy. The mean log reduction of concentrations of microorganism for each of the rigid gas permeable (RGP) contact lens solutions after 8-hour disinfection time is shown in Table 2.

Discussion and Conclusion

In order to achieve the safest contact lens wear, lens care systems must be potent enough to destroy harmful microorganisms, while at the same time should not be damaging the cornea[6,13]. Therefore, the manufacturers should always balance the ability of solutions to retain a broad spectrum of antimicrobial activity while allowing for only minimal toxicity. These requirements are even more difficult to be achieved by multipurpose solutions[11,12].

In this study we tested 5 different contact lens disinfecting solutions containing different disinfecting agents: two containing PHMB, one containing PAPB, another PAPB in combination with Chlorhexidine gluconate, and one Polyquad preservative, four solutions contained EDTA.

The observed antimicrobial efficiency of Boston Advance and Boston Simplus was consistent with other studies[18–20]. To the extent of our knowledge, there are no reports on studies investigating Unique PH, Nitilens conditioner GP and Total care antimicrobial activity.

All study solutions reduced microorganism concentrations below 1000 CFU/mL (concentrations of bacteria and fungi reduced by 3 log and 1 log, respectively), and therefore met the requirements of the ISO stand-alone primary criteria for disinfecting solutions. However, there were differences in their disinfecting efficacy.

Although not required by ISO Guidelines, *Staphylococcus epidermidis* is one of the most common bacteria in the eyes of lens wearers[13,14]. Likewise, disinfecting activity against *Escherichia coli* is not required in the ISO Guidelines, but it commonly contaminates contact lens accessories stored in bathrooms[14]. We believe that contact lens solution disinfecting activity should be extended to as much as possible resistant as well as common microbial species. Therefore, both *Escherichia coli* and *Staphylococcus epidermidis*, were also tested in our study.

Disinfection time in our study was 8 hours, which is defined as overnight disinfection. Some manufacturers address that minimal disinfection period can be even shorter, but we believe that 8-hour disinfection period is appropriate as it resembles everyday life situations considering that lens wearers usually do not wear lenses overnight, as we observed from our clinical practice.

All solutions were the most effective against *Staphylococcus epidermidis*. The fact that *Staphylococcus epi-*

TABLE 2
MEAN LOG REDUCTION AFTER 8-HOUR OVERNIGHT DISINFECTION

Test solution	*Stapyococus aureus*	Stapylococus epidermidis	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
A	>5.0	>5.0	4.8	>5.0	>3.0
B	>5.0	>5.0	>5.0	>5.0	2.3
C	4.7	>5.0	4.8	>5.0	2.1
D	>5.0	>5.0	>5.0	>5.0	1.1
E	>5.0	>5.0	>5.0	>5.0	>3.0

dermidis was not standardized, but was isolated from our laboratory might provide an explanation for these results. However, considering that *Staphylococcus epidermidis* was isolated from our laboratory, our results add »real life« experience to this experiment. As we live surrounded by unstandardized bacteria, the efficacy of a disinfecting solution should be measured by its efficacy to kill most microorganisms that could be present[17]. Therefore, we believe our results are rather interesting.

According to our results, Boston Advance showed the highest antibacterial activity against all bacteria tested. This might be attributed to the fact that it contains two antimicrobial agents, PAPB and Chlorhexidine gluconate, which might provide a feasible explanation.

To the best of our knowledge, this is among the first studies assessing Boston Advance lens care solution for antibacterial and antifungal activity.

As disinfecting agents, Boston Advance contains both PAPB and Chlorhexidine gluconate, while Boston Simplus contains only PAPB. Both of the latter solutions show excellent antimicrobial efficacy, although Boston Simplus seem to be more effective against *Candida albicans*. Although having strong antimicrobial activity, patients preferred Boston Simplus to Boston Advance, especially when evaluated for comfort, unaided daytime vision, care and handling[18].

Unique pH, containing PQ-1, a biocide used commercially in contact lens disinfecting solution, induces cytoplasmic membrane damage to bacteria and plasma membrane damage to C. albicans, which results in K+ leakage from the bacteria and C. albicans, and has good activity against both bacteria and fungi[21,22].

Nitilens Conditioning GP contains biguanide-based antimicrobial agents, PHMB, while Total care contains PHMB in higher concentration, as well as Lauryl quaternised protein. These antimicrobial agents contain cationic active sites that have the ability to lyse microbial cellular membranes by electrostatic interaction. PHMB is a polymer with 6 to 14 active sites, showing antimicrobial activity for gram-positive and gram-negative bacteria[16]. Both solutions showed similar antibacterial activity against all microorganisms tested, although Nitilens Conditioning GP showed somewhat lower efficacy against *E. Coli*, and Total Care showed less efficacy against *Candida albicans*. It is possible that besides solution components, other solution qualities, e.g. viscosity and ionic balance of the solution, contribute significantly to the overall antimicrobial activity[24,25].

EDTA, which is very common ingredient in lens care solutions, removes Mg^{2+}, influences the cell envelope, and consequently destroys Gram-negative bacteria.

There is one limitation which should be acknowledged. Antimicrobial activity was tested against microorganisms in suspension. As shown in a recent study, microorganisms adhering to the surface of the lens case may be more difficult to eliminate[26].

Overall, all contact lens care solutions showed good antimicrobial activity against all bacteria and fungi. Whilst we noted small variations in their antimicrobial activity, considerable variation in their antifungal activity was found. That fact might become of clinical relevance among non-compliant patients. A solution that showed better antimicrobial efficacy could possibly provide higher safety for non-compliant patients by minimizing the risk of eye infections.

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Disclosures

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KOMPARATIVNA STUDIJA ANTIBAKTERIJSKIH I ANTIGLJIVIČNIH SVOJSTAVA TEKUĆINA ZA DEZINFEKCIJU POLUTVRDIH KONTAKTNIH LEĆA

S A Ž E T A K

Cilj našeg istraživanja je bila usporedba antibakterijskih i antigljivičnih svojstava pet tekućina za dezinfekciju polutvrdih kontaktnih leća. Testirali smo sljedeće tekućine: Unique pH (Alcon Laboratories), Boston Advance (Polymer Technology Corp.), Nitilens Conditioner GP (Avizor), Total Care (Advanced Medical Optics), Boston Simplus (Bausch & Lomb). Testirali smo njihovu efikasnost u dezinfekciji fiziološke otopine kontaminirane sljedećim sojevima mikroorganizama American Type Culture Collection (ATCC): Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 90028) i Staphylococcus epidermidis (soj izoliran iz našeg laboratorija). Sve testirane tekućine smanjile su koncentraciju bakterija i gljivica ispod 1000 CFU/mL (Colony forming unit; smanjenje 3 log i 1 log od početne koncentracije) nakon 8-satnog dezinfekcijskog razdoblja. Međutim, postoje razlike u njihovoj dezinfekcijskoj učinkovitosti. Rezultati naše studije pokazuju kako sve testirane tekućine imaju dobar dezinfekcijski učinak protiv testiranih bakterija i gljivica, pokazujući nešto veće međusobne razlike antigljivične nego antibakterijske učinkovitosti. Uočene razlike učinkovitosti tekućina za dezinfekciju leća mogle bi doći do izražaja kod nesuradljivih nositelja kontaktnih leća, te bi rezultati ovog istraživanja mogli biti značajni kod odabira adekvatne tekućine za nesuradljive pacijente.