tvst

Article

Susceptibility of Contact Lens-Related *Pseudomonas aeruginosa* Keratitis Isolates to Multipurpose Disinfecting Solutions, Disinfectants, and Antibiotics

Mahjabeen Khan[1], Fiona Stapleton[1], and Mark Duncan Perry Willcox[1]

[1] School of Optometry and Vision Science, University of New South Wales, Sydney, Australia

Correspondence: Fiona Stapleton, Level 3, Rupert Myers Building North Wing, School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales 2052, Australia. e-mail: f.stapleton@unsw.edu.au

Received: September 20, 2019
Accepted: December 29, 2019
Published: April 9, 2020

Keywords: susceptibility; minimum inhibitory concentration; minimum bactericidal concentration; synergy; fractional inhibitory concentration

Citation: Khan M, Stapleton F, Willcox MDP. Susceptibility of contact lens-related *Pseudomonas aeruginosa* keratitis isolates to multipurpose disinfecting solutions, disinfectants, and antibiotics. Trans Vis Sci Tech. 2020;9(5):2, https://doi.org/10.1167/tvst.9.5.2

Purpose: This study analyzed the susceptibilities of 17 contact lens (CL)-related keratitis isolates of *Pseudomonas aeruginosa* from Australia to antibiotics, multipurpose contact lens disinfecting solutions (MPDS), and disinfectants through minimum inhibitory (MIC) and minimum bactericidal concentrations.

Methods: Antibiotics included ciprofloxacin, levofloxacin, gentamicin, tobramycin, piperacillin, imipenem, ceftazidime, and polymyxin B. The MPDS OPTI-FREE PureMoist, Complete RevitaLens OcuTec, Biotrue, and Renu Advanced Formula and the constituent disinfectants; alexidine dihydrochloride, polyquaternium-1, polyaminopropyl biguanide, and myristamidopropyl dimethylamine (Aldox) were analyzed. The combined susceptibility of disinfectants based on the MPDS formulation was assessed through fractional inhibitory concentration.

Results: All isolates were susceptible to levofloxacin and gentamicin, 2/17 were resistant to ciprofloxacin; 1/17 was resistant to tobramycin, piperacillin, and polymyxin; and 3/17 were resistant to ceftazidime whereas 12/17 were resistant to imipenem. Of the four MPDSs, for Renu Advanced Formula 8/17 strains have an MIC $\leq$ 11.36 for OPTI-FREE PureMoist 14/17 strains have an MIC $\leq$ 11.36% for Complete RevitaLens 9/17 strains have an MIC $\leq$ 11.36, and for Biotrue 5/17 strains have MIC = 11.36. All strains were killed by 100% MPDS. At the concentrations used in the MPDSs, individual disinfectants were not active. From three tested isolates, no synergy was found in dual combinations of disinfectants. However, synergy was found for triple combination of disinfectants for three tested strains.

Conclusions: Australian CL-related isolates of *P aeruginosa* were susceptible to most antibiotics. There was variability in susceptibility to different MPDS. Individual disinfectant excipients had limited activity. The combination of the disinfectants showed synergy, antagonism, and no interaction.

Translational Relevance: This study will help to choose better preventive and treatment measures for microbial keratitis.

Introduction

Contact lenses have been used for decades for refractive, cosmetic, and therapeutic purposes. Although contact lenses have optical and vocational benefits, they are associated with certain complications. Corneal infection is rare but is the most severe complication of contact lens wear, occurring in around 4 per 10,000 wearers per year,[1] and can cause visual loss in 10% to 15% cases.[1] *Pseudomonas aeruginosa* is the most commonly isolated bacterium from contact lens-related microbial keratitis.[2] This may be due to its strong adhesion to contact lenses and contact lens cases compared with other microorganisms.[3] *P aeruginosa* can also develop biofilms on these surfaces,[4] which facilitates persistence of the organism.[5]

Contact lens multipurpose disinfecting solutions (MPDS) are used to minimize the numbers of bacteria on lenses for the safe use of daily wear contact

lenses. Daily wear is the most common wear schedule for contact lens wearers in many countries.[6] However, there are reports that bacteria can become resistant to these disinfectants,[7] which raises concerns about the effectiveness of these solutions. Resistance to disinfecting solutions may be due to inherent resistance associated with the cytotoxic phenotype of *P aeruginosa*,[8] the surface charge of the bacterial cell,[9] or expression of outer membrane proteins such as OprR.[10] Harboring *qac* genes[11] may confer resistance to disinfectants, and although this has been shown with ocular isolates of *Staphylococcus aureus*,[12] this has not been seen in a limited number of strains of *P aeruginosa* evaluated.[11] *Qac* genes can occur on class 1 integrons along with genes for antibiotic resistance; this raises concern of cotransfer of these genes amongst bacterial populations.

Various antibiotics are used for the treatment of microbial keratitis, but emerging resistance to the antibiotics[13] from the possession of inherent and acquired resistance mechanisms is increasing.[14] Emerging resistance of ocular isolates of *P aeruginosa* has been reported internationally[15] with variation in their resistance profile to antimicrobials.[16] Resistance may not only be associated with the possession of *qac* genes, but also with genes conferring virulence traits such as *exoU* and *exoS*.[17,18] Inherent resistance mechanisms include low membrane permeability, expression of efflux pumps, production of antibiotic-inactivating enzymes, and mutation of resistance genes.[19] Acquired resistance occurs when genes conferring resistance are inserted into mobile genetic elements such as integron and transposons,[20] which can then migrate around bacterial populations. The severity of infections caused by *P aeruginosa* and its ability to acquire resistance and virulence genes, giving it the potential to resist almost all antibiotic classes, increases the concerns about *P aeruginosa* infections.[19] In the management of corneal infection, despite topical administration of antibiotics resulting in high tissue concentrations, poor clinical outcomes may occur partly from antibiotic resistance.[21] The consequences of keratitis caused by multiple-drug resistant *P aeruginosa* can be severe and vision threatening given the limited choice of effective antimicrobials.[22]

There is limited information available on the antimicrobial and disinfectant susceptibility patterns of clinical ocular isolates of *P aeruginosa* in Australia. Earlier studies have often used standard strains[23] or only a limited numbers of clinical isolates.[11] Therefore, the aim of this study was to investigate the sensitivities of ocular isolates of *P aeruginosa* to various antibiotics and MPDSs.

Table 1. Strains of *Pseudomonas aeruginosa* Recovered from Microbial Keratitis

P aeruginosa Isolates	Source	Year of Isolation
115	Cornea	2004
116	Cornea	2004
121	Contact lens	2005
123	Cornea	2005
124	Cornea	2005
126	Cornea	2005
127	Cornea	2005
129	Cornea	2005
155	Cornea	2006
162	Cornea	2006
165	Cornea	2001
169	Cornea	2006
174	Cornea	2006
176	Contact lens	2004
179	Cornea	2006
181	Cornea	2006
182	Cornea	2004

Materials and Methods

P aeruginosa Isolates

Strains of *P aeruginosa* isolated from contact lens-related microbial keratitis (either from corneal scrapes or contact lenses) from Queensland, Australia, between the years 2001 to 2006 were retrieved from the culture collection of the School of Optometry and Vision Science, University of New South Wales, Sydney, Australia (Table 1). The strains were stored at -80°C and revived on nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, UK). Isolates were then inoculated into Mueller-Hinton broth (Oxoid Ltd.) and grown at 37°C for 18 to 24 hours. The optical density of the bacterial suspension was adjusted 0.1 (1×10^8 CFU/mL) at 660 nm using a spectrophotometer (FLUOstar Omega, BMG LABTECH, Germany).[24]

Susceptibility to Multipurpose Disinfecting Solutions

Susceptibility of the bacterial strains to four commercially available MPDS; OPTI-FREE PureMoist (Alcon, Fort Worth, TX, USA), Complete RevitaLens OcuTec (Abbot Medical Optics, Hangzhou ZJ, China), and Biotrue and Renu Advanced Formula (Bausch + Lomb, Rochester, NY, USA) (Table 2) was measured using a previously described method.[24] In

Table 2. Multipurpose Disinfecting Solutions

MPDS	Manufacturer	Disinfectants	Surfactants	Other Ingredients
Opti-Free PureMoist	Alcon, Fort Worth, TX, USA	Polyquaternium-1 10 ppm, Aldox 6 ppm	Tectronic 1304, polyoxyethylene-polyoxybutylene copolymer	Sodium citrate, sodium chloride, boric acid, aminomethyl-propanol, sorbitol, ethylenediaminetriacetic acid
Complete RevitaLens OcuTec	Abbot Medical Optics, Hangzhou ZJ, China	Alexidine dihydrochloride 1.6 ppm, polyquaternium-1 3 ppm	Tetronic 904	Sodium citrate, sodium chloride, boric acid, sodium borate decahydrate, ethylenediaminetriacetic acid
Biotrue	Bausch +Lomb, Rochester, NY, USA	PAPB (PHMB) 1.3 ppm, polyquaternium-1 1 ppm	Poloxamine, sulfobetaine	Sodium chloride sodium borate, boric acid, ethylenediaminetriacetic acid, hyaluronan
Renu Advanced	Bausch +Lomb, Rochester, NY, USA	PAPB (PHMB) 0.5 ppm, polyquaternium-1 1.5 ppm, alexidine 2 ppm	Poloxamine, poloxamer 181	Sodium chloride, boric acid, sodium borate, ethylenediaminetriacetic acid, diglycine

Aldox, myristamidopropyl dimethylamine; PAPB, polyaminopropyl biguanide and is synonymous with PHMB (polyhexamethylene biguanide).

brief, each MPDS was serially diluted in phosphate-buffered saline (NaCl 80 g/L, Na_2HPO_4 11.5 g/L, KCl 2 g/L, and KH_2PO_4 2 g/L, pH = 7.2) to obtain final concentrations of 90.9%, 45.45%, 22.72%, 11.36%, 5.68%, and 2.84%. The serially diluted MPDS (200 µL) was added to wells of a microtiter plate and a 20 µL bacterial suspension was added to achieve a final concentration of 1×10^5 CFU/mL. The plates were incubated for 18 to 24 hours at 37°C. Growth turbidity was measured using a spectrophotometer (FLUOstar Omega, BMG LABTECH, Germany) at 660 nm to obtain the minimum inhibitory concentration (MIC). MIC was taken as the dilution of MPDS with no visible growth. To measure the minimum bactericidal concentration (MBC), viable counts were performed (on nutrient agar plates incubated at 37°C for 18 to 24 hours) from wells at the MIC and the two next lower dilutions of MPDS. The MBC was the concentration of MPDS that gave 99.99% (3 log units) bacterial killing.[25,26]

Inhibition of *P aeruginosa* by Disinfectants

Polyaminopropyl biguanide (PAPB; Novachem Pty Ltd Heidelberg West, VIC, Australia), polyquaternium-1 (Toronto Research Chemicals Inc. Toronto, ON, Canada), myristamidopropyl dimethylamine (Aldox; Toronto Research Chemicals Inc. Toronto, ON, Canada), and alexidine dihydrochloride (Cayman Chemicals, Ann Arbor, MI, USA) were used. Disinfectants were prepared as 10X stock solutions in phosphate-buffered saline. Dilutions ranging between 1% and 0.0000390% were used such that the concentration of the disinfectants present in the MPDSs were in the range tested. Two hundred microliters of disinfectant and 20 µL of the bacterial cells (final concentration of 1×10^5 CFU/mL) were incubated in 96-well microtiter plates for 18 to 24 hours at 37°C to determine the MIC (as described previously). Viable plate count was performed as described to elucidate the MBC of each disinfectant.

Fractional Inhibitory Concentration of Components of MPDS by Checkerboard Method

Three isolates (123, 127, and 155) were selected because of the variation in their MICs to different disinfectants (higher and lower MIC value for different disinfectants). Interactions between disinfectants were analyzed through a modified checkerboard method.[27]

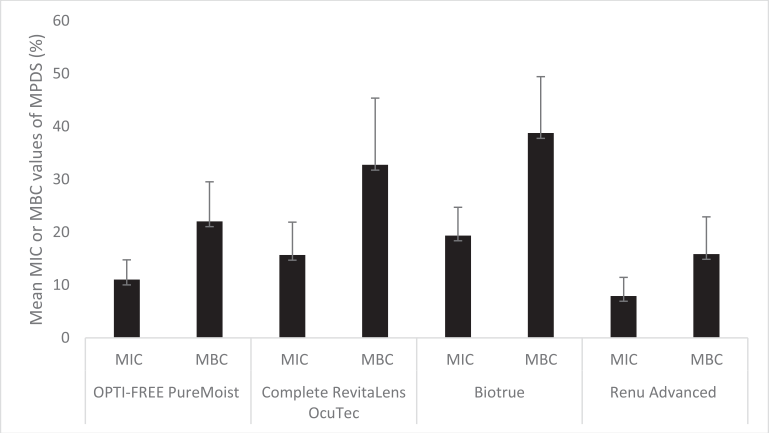

Figure 1. MICs and MBCs of the different MPDS used in the study (mean ± standard deviation).

The dual combinations tested were selected from those used in the composition of each MPDS (Table 2). The triple combination of polyquaternium-1, polyhexamethylene biguanide (PHMB), and alexidine (present in Renu Advanced) was tested with a small modification to the checkerboard assay.[28] Briefly, the three disinfectants were diluted in three different directions in order of increasing concentration in the 96-well microtiter plate. So, the three disinfectants were combined in different concentrations in the wells (Fig. 1). In the 3-dimensional assay, 11 dilution steps of disinfectant A, 7 dilution steps of disinfectant B, and 6 dilution steps of disinfectant C were tested. The experiment was repeated three times, changing the position of disinfectants to check different combinations of all the three disinfectants in the Renu Advanced formula.

Fifty microliters of each disinfectant were used to give a total volume of 150 µL in every well. The concentration for each disinfectant ranged between 16× MIC to 0.24× MIC. Bacterial inocula were prepared as described previously and the plates were incubated for 18 to 24 hours at 37°C to determine the combined MIC. For the evaluation of the type of the interaction between different disinfectants the fractional inhibitory concentration index (FICI) was calculated using the formula[27]:

$$\mathrm{FICI}_{A/B} = \frac{\mathrm{MIC}_{A(\mathrm{combination})}}{\mathrm{MIC}_{A(\mathrm{alone})}} + \frac{\mathrm{MIC}_{B(\mathrm{combination})}}{\mathrm{MIC}_{B(\mathrm{alone})}}$$

For triple disinfectants, the FIC of disinfectant C was added to the above equation.[28]

$$\mathrm{FICI}_{A/B/C} = \frac{\mathrm{MIC}_{A(\mathrm{combination})}}{\mathrm{MIC}_{A(\mathrm{alone})}} + \frac{\mathrm{MIC}_{B(\mathrm{combination})}}{\mathrm{MIC}_{A(\mathrm{alone})}} + \frac{\mathrm{MIC}_{C(\mathrm{combination})}}{\mathrm{MIC}_{A(\mathrm{alone})}}$$

Synergy was defined when the FICI was ≤0.5, no interaction when the FICI was >0.5 but <4, and antagonism when the FICI was >4.[29,30]

Antibiotic Susceptibility Testing

Susceptibility of antibiotics was assessed using MIC and MBC, performed following the standard protocol described by Clinical and Laboratory Standards Institute.[31] The antibiotics used were ciprofloxacin, levofloxacin, gentamicin, ceftazidime (Sigma-Aldrich, USA), polymyxin B (Sigma-Aldrich, Denmark) tobramycin, piperacillin (Cayman Chemical Company, USA) and imipenem (LKT Lab Inc., USA). The concentrations tested ranged from 5120 µg/mL to 0.25 µg/mL. The different concentrations of antibiotics were achieved by diluting in Mueller-Hinton broth in the 96-well plate.

The MIC for each antibiotic was determined in 96 wells plates with 100 µL serially diluted antibiotics and 100 µL of the bacterial inocula with a final concentration of 1×10^5 CFU/mL per well incubated 37°C for 18 to 24 hours. Antibiotics were diluted with Mueller-Hinton broth and bacterial cells were diluted with fresh media. The MIC and MBC were measured as described for MPDS previously. Interactions of strains with antibiotics can be described as susceptible or resistant based on Clinical and Laboratory Standards Institute[31] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018) breakpoints. There are no standards for interpreting topical ocular treatment or efficacy with contact lens solutions, but the serum standards can be used if it is assumed that the antibiotic concentrations in the ocular tissue and contact lens solutions are equal or greater than the

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (% of Original) of MPDS

Strains of *P aeruginosa*	OPTI-FREE PureMoist (%)		Complete RevitaLens OcuTec (%)		Biotrue (%)		Renu Advanced Formula (%)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
115	11.36	22.72	11.36	45.45	11.36	22.72	5.68	11.36
116	5.68	11.36	11.36	22.72	11.36	22.72	11.36	22.72
121	22.72	45.45	22.72	45.45	22.72	45.45	11.36	22.72
123	11.36	22.72	22.72	45.45	22.72	45.45	11.36	22.72
124	11.36	22.72	22.72	45.45	22.72	45.45	11.36	22.72
126	11.36	22.72	11.36	22.72	22.72	45.45	11.36	22.72
127	5.68	11.36	5.68	11.36	11.36	22.72	11.36	22.72
129	11.36	22.72	11.36	22.72	22.72	45.45	11.36	22.72
155	11.36	22.72	11.36	22.72	11.36	22.72	5.68	11.36
162	11.36	22.72	11.36	22.72	22.72	45.45	5.68	11.36
165	11.36	22.72	22.72	45.45	22.72	45.45	5.68	11.36
169	5.68	11.36	11.36	22.72	22.72	45.45	5.68	11.36
174	11.36	22.72	11.36	22.72	11.36	22.72	2.84	5.68
176	11.36	22.72	22.72	45.45	22.72	45.45	5.68	11.36
179	11.36	22.72	11.36	22.72	22.72	45.45	5.68	11.36
181	11.36	22.72	22.72	45.45	22.72	45.45	11.36	22.72
182	11.36	22.72	22.72	45.45	22.72	45.45	1.42	2.84

antibiotic concentrations that can be attained in the serum.[32]

Comparison Among Antibiotics, MPDS, and Disinfectants

As mentioned, interactions of strains with antibiotics can be described as susceptible or resistant, but there are no such definitions for MPDS or individual disinfections. Therefore, for MPDS, strains with MIC greater than 10% were categorized as resistant. The 10% cutoff for MPDS is arbitrary and it cannot be used as a standard for reference for any other study. For disinfectants, those strains having MIC above what was present in the respective MPDS were considered resistant (if the disinfectant was present in more than one MPDS at different concentrations, its mean concentration was taken for this analysis).

Statistical Analysis

The data were statistically analyzed using the Statistical Package for the IBM SPSS v25 (IBM Corp., Armonk, NY, USA). Differences between the distribution of the MICs of the bacterial isolates to MPDS and disinfectants were evaluated using Friedman's two-way analysis of variance. Briefly, mean ranks were calculated for each MPDS and disinfectant (a higher rank equates to a lower level of efficacy and vice versa). *P* values less than 0.05 were considered as significant. Based on a significant difference in the analysis of variance test, post hoc pairwise comparisons were conducted to identify the differences between the disinfectants and MPDSs.

Results

Multipurpose Solution Susceptibilities

Contact lens-related isolates of *P aeruginosa* showed variations in their susceptibility to Renu Advanced Formula, OPTI-Free PureMoist, Complete RevitaLens OcuTec, and Biotrue, exhibiting different MIC and MBC levels to each of the MPDS (Table 3). When all four MPDSs were used at 100% concentration, they all reduced the bacterial growth to below the limit of detection (i.e., no bacteria grew on the agar plates). However, at other dilutions, there were differences in MICs and MBCs between the MPDSs. In general, the MBC of each MPDS was equivalent to twice its MIC. Overall, Renu Advanced formula had the lowest average MIC (7.9%) and MBC (15.8%),

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Disinfectants

Strains of *Pseudomonas aeruginosa*	PAPB (PHMB) (ppm)		Alexidine (ppm)		Polyquaternium-1 (ppm)		Aldox (ppm)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
115	22.72	45.45	2.84	5.64	5.64	11.36	727.27	1454.54
116	45.45	90.9	2.84	5.64	11.36	22.72	727.27	1454.54
121	45.45	90.9	2.84	5.64	5.64	11.36	727.27	1454.54
123	45.45	90.9	2.84	5.64	11.36	22.72	90.9	181.81
124	22.72	45.45	2.84	5.64	11.36	22.72	90.9	181.81
126	22.72	45.45	2.84	5.64	5.64	11.36	181.81	363.63
127	45.45	90.9	2.84	5.64	2.84	5.64	90.9	181.81
129	45.45	90.9	2.84	5.64	5.64	11.36	45.45	90.9
155	22.72	45.45	2.84	5.64	11.36	22.72	22.72	45.45
162	22.72	45.45	1.41	2.84	5.64	11.36	181.81	363.63
165	22.72	45.45	5.64	11.36	5.64	11.36	90.9	181.81
169	45.45	90.9	2.84	5.64	5.64	11.36	181.81	363.63
174	22.72	45.45	0.70	1.41	2.84	5.64	90.9	181.81
176	22.72	45.45	0.70	1.41	2.84	5.64	90.9	181.81
179	22.72	45.45	2.84	5.64	1.41	2.84	90.9	181.81
181	22.72	45.45	2.84	5.64	5.64	11.36	181.81	363.63
182	45.45	90.9	2.84	5.64	5.64	11.36	90.9	181.81

Aldox, myristamidopropyl dimethylamine; PAPB, polyaminopropyl biguanide.

followed by OPTI-FREE PureMoist (average MIC 11.02, MBC 22.05), Complete RevitaLens OcuTec (average MIC 15.7%, MBC 32.7%), and ,Biotrue (average MIC 19.37%, MBC 38.7%; Fig. 1).

A significant difference among MPDS types was found ($P = 0.0313$; OPTI-FREE PureMoist vs. RevitaLens, $P \leq 0.0001$; OPTI-FREE PureMoist vs. Biotrue, $P \leq 0.0001$; RevitaLens OcuTec vs. Renu Advanced formula and $P \leq 0.0001$; Biotrue vs. Renu Advanced Formula) except for OPTI-FREE PureMoist vs. Renu Advanced Formula ($P = 0.25$) and RevitaLens OcuTec versus Biotrue ($P = 0.12$).

Inhibition of *P aeruginosa* by Disinfectants

Analysis of the disinfectants in the MPDS individually showed that all the disinfectants gave higher MICs and MBCs than the concentrations in the dilutions of MPDS (Table 4), indicating that in isolation the disinfectants were less active against *P aeruginosa* than when they were formulated into MPDS. For example, OPTI-FREE with Aldox and polyquaternium-1 was effective even when the concentrations of these were reduced to 6 and 10 ppm, respectively, upon diluting the MPDS. Generally, the MBC of each disinfectant was double the MIC. Overall, alexidine had the lowest mean MIC (2.66 ppm) and MBC (5.31 ppm) followed by polyquaternium-1 (mean MIC = 6.2, MBC = 12.5) and the PAPB (mean MIC = 32 ppm; mean MBC = 64 ppm). Aldox had the highest mean MIC (217 ppm) and MBC (435 ppm) among all the disinfectants (Fig. 2).

The comparative activities of each disinfectant based on the MIC and MBC were significantly different from each other ($P \leq 0.001$; alexidine vs. PAPB, $P \leq 0.001$; alexidine vs. Aldox, $P \leq 0.001$; polyquaternium-1 vs. PHMB, $P = 0.047$; polyquaternium-1 vs. Aldox ($P \leq 0.001$) except between Alexidine and polyquaternium-1 ($P = 0.505$) and PHMB and Aldox ($P = 0.278$). By Friedmann's two-way analysis of variance, alexidine was ranked lowest having the lowest MIC and therefore the highest antimicrobial activity. This was followed by polyquaternium-1 > PHMB > Aldox.

Fractional Inhibitory Concentration of Components of MPDS

The combinations tested were selected based on their presence in the MPDSs. In dual combinations, none of the disinfectants showed synergistic activity.

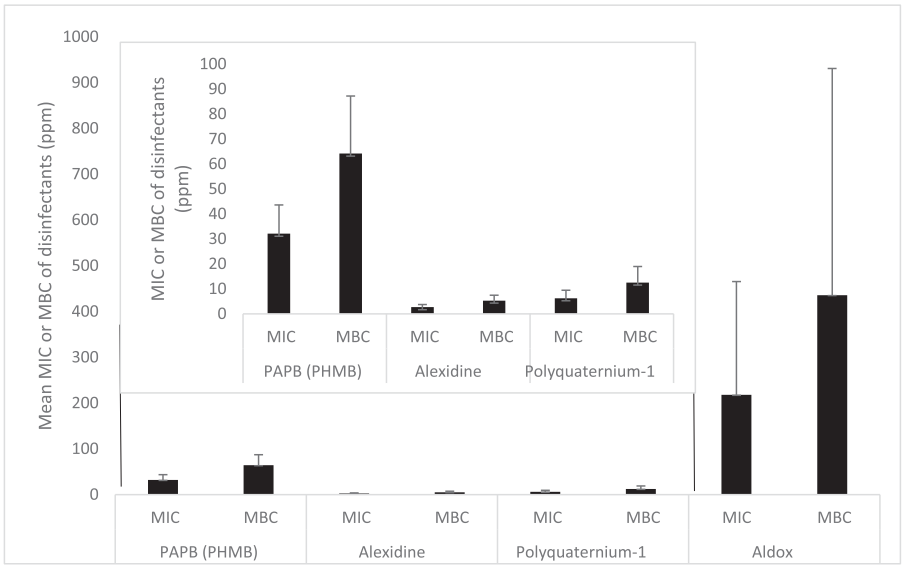

Figure 2. MICs and MBCs for disinfectants used in the study (mean ± standard deviations). The insert shows a magnified view of the MIC and MBC of the disinfectants PAPB, alexidine, and polyquaternary-1, which have lower MIC/MBCs than Aldox and so are not easily visible on the original graph.

No interactions between the disinfectants were found for isolate 127 except for the triple combination of the disinfectants. For isolate 123, antagonism was found between polyquad-1 and PAPB; for isolate 155, antagonism occurred between polyquad-1 and alexidine or polyquad-1 and PAPB (Table 5). For the triple combination, synergy (FICI $\leq$ 0.5) occurred with all the isolates.

Antibiotic Susceptibilities

Table 6 summarizes the MIC and MBC levels of the strains. All the tested isolates were susceptible to gentamicin and levofloxacin. For tobramycin, polymyxin B, and piperacillin, 94% of CL (Contact Lens) isolates were susceptible. For ciprofloxacin 88% and ceftazidime, 82% of CL isolates were susceptible. Susceptibility to imipenem was 29%. Strain 127 was

Table 5. Fractional Inhibitory Concentration

Pseudomonas aeruginosa strains	Disinfectants Combination	MIC in the Combination (ppm)	Checkerboard FICI	Interpretation
P.aer 123	Polyquad-1+Aldox	5.64 + 90.9	1.5	No interaction
	Polyquad-1+PAPB	45.45 + 90.9	6	Antagonism
	Polyquad-1+Alexidine	5.64 + 5.64	2.5	No interaction
	Polyquad-1+PAPB+Alexidine	0.78 + 2.84 + 0.78	0.36	Synergy
P.aer 127	Polyquad-1+Aldox	2.84 + 11.36	1.125	No interaction
	Polyquad-1+PAPB	4.5 + 90.9	3.6	No interaction
	Polyquad-1+Alexidine	2.5 + 2 .84	1.2	No interaction
	Polyquad-1+PAPB+Alexidine	0.0097 + 5.64 + 0.39	0.25	Synergy
P.aer 155	Polyquad-1+Aldox	11.36 + 45.45	3	No interaction
	Polyquad-1+PAPB	45.45 + 90.9	8	Antagonism
	Polyquad-1+ALX	45.45 + 5	16.4	Antagonism
	Polyquad-1+PAPB+Alexidine	0.39 + 2.84+ 0.78	0.4	Synergy

Combined MIC is the that of each disinfectant when tested in combination with other disinfectants.

Aldox, myristamidopropyl dimethylamine; ALX, alexidine; FICI, fractional inhibitory concentration index (synergy: FICI $\leq$0.5; no interaction: 0.5 $<$ FICI $\leq$ 4; and antagonism: FICI $>$ 4); MIC, minimum inhibitory concentration; PAPB, polyaminopropyl biguanide; Polyquad-1, polyquaternium-1.

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for Contact Lens-Related Keratitis *P aeruginosa* Isolate

Antibiotics Breakpoints	Ciprofloxacin µg/mL, ≤1, 2, ≥4		Levofloxacin µg/mL, ≤2, 4, ≥8		Gentamicin µg/mL, ≤4, 8, ≥16		Tobramycin µg/mL, ≤4, 8, ≥16		Piperacillin µg/mL, ≤16		Imipenem µg/mL, ≤2, 4, ≥8		Ceftazidime µg/mL, ≤8, 16, ≥32		Polymyxin B µg/mL, ≤2, 4, ≥8	
Strains of *P aeruginosa*	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
115	0.25 (S)	0.5	0.5 (S)	1	1 (S)	1	0.25 (S)	1	8 (S)	16	4 (I)	8	8 (S)	16	0.25 (S)	0.25
116	0.25 (S)	0.5	0.5 (S)	1	0.5 (S)	1	0.25 (S)	1	8 (S)	8	4 (I)	4	2 (S)	2	0.5 (S)	0.5
121	0.25 (S)	0.5	1 (S)	1	0.5 (S)	2	2 (S)	4	2 (S)	4	8 (R)	8	2 (S)	2	1 (S)	1
123	1 (S)	1	1 (S)	1	0.25 (S)	0.5	4 (S)	4	8 (S)	16	4 (I)	8	2 (S)	2	1280 (R)	1280
124	1 (S)	2	0.5 (S)	1	0.5 (S)	2	0.25 (S)	1	4 (S)	8	8 (R)	16	2 (S)	4	1 (S)	2
126	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	0.25 (S)	0.5	8 (S)	16	8 (R)	16	128 (R)	256	1 (S)	2
127	1 (S)	2	0.25 (S)	1	2 (S)	4	32 (R)	128	4 (S)	16	4 (I)	8	128 (R)	256	0.5 (S)	1
129	0.25 (S)	1	0.25 (S)	1	0.25 (S)	0.5	0.25 (S)	0.25	4 (S)	8	4 (I)	8	2 (S)	4	0.25 (S)	0.5
155	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	4 (S)	4	4 (S)	4	2 (S)	2	2 (S)	4	0.25 (S)	0.5
162	0.5 (S)	1	0.5 (S)	1	0.25 (S)	0.5	0.25 (S)	1	8 (S)	8	4 (S)	4	2 (S)	4	0.25 (S)	0.5
165	1 (S)	1	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	1	8 (S)	8	16 (R)	16	2 (S)	4	0.25 (S)	0.5
169	2 (I)	4	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	4 (S)	8	2 (S)	4	1 (S)	2	0.25 (S)	0.25
174	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	1 (S)	2	8 (R)	16	0.5 (S)	2	0.25 (S)	0.5
176	0.5 (S)	1	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	4 (S)	8	2 (S)	8	2 (S)	4	0.25 (S)	0.5
179	2 (I)	4	0.25 (S)	0.5	0.25 (S)	0.5	0.5 (S)	0.5	4 (S)	8	2 (S)	4	1 (S)	2	0.25 (S)	0.5
181	1 (S)	4	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	32 (R)	64	4 (I)	8	16 (R)	32	0.5 (S)	1
182	1 (S)	2	0.25 (S)	0.5	0.25 (S)	0.5	0.25 (S)	0.5	4 (S)	8	8 (S)	16	1 (S)	2	0.25 (S)	0.5

R, resistant; S, susceptible.

Table 7. Heat Map for the Comparative Susceptibilities of Antibiotics and Disinfectants for *P aeruginosa* Isolates

P. aeruginosa isolates	CIP	LEVO	GN	TOB	PIP	IMI	CEFTA	POLY-B	OPTI	REV	BIO	RENU	PAPB	Polyquad-1	Alexidine	Aldox
115	S	S	S	S	S	I	S	S	R	R	R	S	R	R	R	R
116	S	S	S	S	S	I	S	S	S	R	R	R	R	R	R	R
121	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R
123	S	S	S	S	S	I	S	R	R	R	R	R	R	R	R	R
124	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R
126	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R
127	S	S	S	S	S	I	R	S	S	R	R	R	R	S	R	R
129	S	S	S	S	S	I	S	S	R	R	R	R	R	R	R	R
155	S	S	S	S	S	S	S	S	R	R	R	S	R	R	R	R
162	S	S	S	S	S	S	S	S	R	R	R	S	R	R	R	R
165	S	S	S	S	S	R	S	S	R	R	R	S	R	R	R	R
169	I	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R
174	S	S	S	S	S	R	S	S	R	R	R	S	R	S	S	R
176	S	S	S	S	S	S	S	S	R	R	R	S	R	S	R	R
179	I	S	S	S	S	S	S	S	R	R	R	S	R	S	R	R
181	S	S	S	S	R	I	R	S	R	R	R	R	R	R	R	R
182	S	S	S	S	I	R	S	S	R	R	R	S	R	R	R	R

S Susceptible I Intermediate R Resistant

Aldox, myristamidopropyl dimethylamine; BIO, Biotrue; CEFTA, ceftazidime; CIP, ciprofloxacin; GN, gentamicin; IMI, imipenem;

LEVO, levofloxacin; OPTI, OPTI-FREE PureMoist; PAPB, polyaminopropyl biguanide; PIP, piperacillin; POLYB, polymyxin B; polyquad-1, polyquaternium-1; RENU, Renu Advanced Formula; REV, RevitaLens OcuTec; TOB, tobramycin.

Green: Susceptible; Yellow: Intermediate; Red: Resistant

a multidrug-resistant strain, resistant to two different classes of antibiotic (the aminoglycoside tobramycin and the beta-lactam ceftazidime). Two isolates, strains 126 and 181, were resistant to two different beta-lactams. One isolate, 123, was resistant to polymyxin B with MIC and MBC values of 1280 µg/mL.

Comparison Among Antibiotics, MPDS, and Disinfectants

Table 7 shows the comparison of susceptibilities of antibiotics with MPDS and disinfectants. Many isolates that were susceptible to antibiotics were correspondingly not susceptible to disinfectants.

Discussion

This study reports the in vitro susceptibilities of *P aeruginosa* strains isolated from contact lens-related keratitis in Australia to various antimicrobials. The study has demonstrated that strains of *P aeruginosa* had different susceptibilities to MPDS, but all strains were susceptible to all the MPDS when they were used at 100% concentrations, indicating good activity overall for the MPDS against *P aeruginosa* isolates. The MIC for the disinfectants in the MPDS when tested alone were mostly higher than the concentrations of the disinfectants in the MPDS, yet combinations of the disinfectants found in different MPDSs did not show synergy, suggesting that it is the whole MPDS formulation that results in high antimicrobial activity.

There was a reduction in activity of MPDSs upon dilution (i.e., diluted MPDSs do not completely kill *P aeruginosa* strains compared with their 100% concentration). In use, this may result in dilution, drying, or topping off the MPDSs. This was repeated following an outbreak of CL-related *Fusarium keratitis* attributed to performance of the MPDS ReNu with MoistureLoc.[33] The data in the current investigation reinforce the need to instruct daily wear contact lens users in the proper use of MPDS and to avoid topping off.

The finding that Renu Advanced was associated with the lowest MICs and MBCs is perhaps not surprising given that this MPDS contains three different disinfectants. Renu Advanced contains alexidine as its primary disinfectant, which is an efficient disinfectant against bacteria[34] and against the biofilms formed by bacteria.[35] In the current study, alexidine had the lowest MIC of any other disinfectant. Another disinfectant present in Renu Advanced is PAPB (PHMB),

which has also been proven to be effective against bacteria,[36] particularly *P aeruginosa*[37], although in the current study PAPB was less effective than alexidine or polyquaternium-1. Polyquaternium-1 is the third component disinfectant of Renu Advanced and has been shown to have significant activity against *P aeruginosa*.[38] Even though the Renu Advanced formula was highly effective against *P aeruginosa,* the individual disinfectants were not effective at the concentration in Renu Advanced. However, this tri-disinfectant system was the only formulation to show synergy between the disinfectants which may have contributed to the overall better activity of this product.

The next most effective MPDS was OPTI-FREE PureMoist. This contrasts with the results in another study that compared OPTI-FREE PureMoist and Biotrue, with both MPDSs having similar results.[39] OPTI-FREE PureMoist contains polyquaternium-1, which showed good activity when used alone, as well as aldox. Interestingly, aldox had a relatively high MIC and MBC (i.e., low activity). Aldox is believed to be more effective against fungi.[38] The difference in activity of OPTI-FREE PureMoist compared with the individual disinfectants when used in combination was particularly marked with strain 155, which showed a high level of antagonism between polyquaternium-1 and aldox. This further reinforces the effect of the whole formulation on overall antimicrobial activity. The addition of the antimicrobial ethylenediaminetriacetic acid and surfactants (both known to be antimicrobial)[40] likely resulted in the relatively high antimicrobial activity of OPTI-FREE PureMoist.

Complete RevitaLens OcuTec had a lower efficacy than OPTI-Free PureMoist, although in a previous study[41] both the MPDSs showed similar levels of efficacy. Biotrue has been shown to be more effective compared with OPTI-FREE PureMoist against certain gram-negative bacteria including *Achromobacter xylosoxidans*, *Delftia acidovorans,* and *Stenotrophomonas maltophilia*.[42] However, this finding contrasts with the present study in which Biotrue was the least effective of all the tested MPDS against the *P aeruginosa* clinical strains tested. The findings of the current study are in general agreement with another study[37] on the most to least active MPDS, OPTI-FREE PureMoist > Complete RevitaLens > Biotrue, against fungal and bacterial isolates including *P aeruginosa*.

Individually, disinfectants were not effective against the *P aeruginosa* isolates used in this study. The higher MICs and MBCs of individual disinfectants compared with the concentration of these disinfectants at the MICs and MBCs of MPDS suggested that it was the combination of excipients in MPDS that contributed to the inhibition of growth and killing of bacteria. The current study examined whether this effect was due to the combination of disinfectants within the MPDS, but synergy was only observed for the combination of the three disinfectants in Renu Advance. Indeed, for the combination of Polyquad-1+PAPB there was antagonism between the two disinfectants for all the three strains of *P aeruginosa* tested. All MPDS contain additional excipients to disinfectants. These include surfactants and ethylenediaminetriacetic acid with their known antimicrobial activity.[40] The other components of MPDS including acids and alcohols may also have antimicrobial activity.[43] Together, it might be the combination of excipients with disinfectants in MPDS that contribute to the overall antimicrobial activity. It would be useful in future studies to test the efficacy of other components of MPDS in combination with the disinfectants and to add a possible comparator with common usage in ophthalmic solutions like benzalkonium chloride in MPDS for rigid gas-permeable lenses (chlorhexidine).

Most of the *P aeruginosa* isolates were susceptible to most of the antibiotics except for imipenem, and only one strain showed multiantibiotic resistance (i.e., was resistant to two or more antibiotics from different classes). Few strains were susceptible to the first-generation fluoroquinolone ciprofloxacin (88%) compared with the later generation levofloxacin (100%). This is in contrast to a historical report where more strains were susceptible to ciprofloxacin than levofloxacin[16] and also where susceptibility of both the antimicrobials was equivalent.[44] The lower rate of susceptibility to ciprofloxacin is important because ciprofloxacin is frequently prescribed as monotherapy for the treatment of corneal infections in Australia.[45] Therefore, careful evaluation of changes to the susceptibility of isolates is warranted. Different fluoroquinolones are available in different jurisdictions such as moxifloxacin and besifloxacin in United States, but these are not available in Australia and hence were not included in the present study. The location of the study should determine the panel of antibiotics tested in future studies.

Imipenem was the least effective beta-lactam, with only 29% of strains being susceptible to it. This level of resistance of *P aeruginosa* to imipenem has recently been reported.[46] Therefore, imipenem is not a suitable treatment for *P aeruginosa* keratitis. Of the aminoglycosides, 100% of the *P aeruginosa* isolates were susceptible to gentamicin and 94% to tobramycin. These data are consistent with a surveillance study of keratitis isolates of *P aeruginosa* from Sydney, Australia, that showed 100% of isolates were susceptible to these two aminoglycosides.[47] Similar susceptibility (>99%)

to aminoglycoside has been reported from the United States[48], whereas susceptibility to aminoglycosides was lower in Pakistan (36% gentamicin)[49] and India (~77% to gentamicin).[50]

The resistance to polymyxin B (which potentially shares the same mechanism of action to disinfectants through targeting the cell membrane of bacteria)[51] found in strain 123 might be due to the role of two component systems present in *P aeruginosa* including PhoPQ, PmrAB, ParRS, CprRS, and ColRS that, when mutated, result in the modification of lipopolysaccharide and efflux pumps[52] or acquisition of external *mcr-1* gene.[53] This strain showed high MIC and MBC values for MPDS and disinfectants that may be due to the use of the same resistance mechanisms. This requires further investigation. Overall, there was no relationship between resistance to antibiotics and relative resistance to MPDS or disinfectants, even though resistance to disinfectants can be mediated by *qac* genes[54] and these genes can be carried on mobile genetic elements that can also carry antibiotic resistance genes.[55] These findings may indicate that exposure to disinfectants does not contribute to the acquisition of antibiotic resistance genes in *P aeruginosa*.

Acknowledgments

Supported by the UNSW Sydney Australia.

Authors' contributions: MW: Conceptualization of the study and corrections. MK: Experimental procedures and writing of the manuscript. FS: Conceptualization of the study and editing. All authors have approved the final article.

Disclosure: **M. Khan,** None; **F. Stapleton,** None; **M.D.P. Willcox,** None

References

1. Stapleton F, Keay L, Edwards K, Naduvilath T, Dart JKG, Brian G, et al. The incidence of contact lens–related microbial keratitis in Australia. *Ophthalmol*. 2008;115:1655–1662.
2. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea*. 2008;27:22–27.
3. Henriques M, Sousa C, Lira M, Elisabete M, Oliveira R, Oliveira R, et al. Adhesion of Pseudomonas aeruginosa and Staphylococcus epidermidis to silicone–hydrogel contact lenses. *Optom. Vis. Sci.* 2005;82:446–450.
4. Szczotka-Flynn LB, Imamura Y, Chandra J, Yu C, Mukherjee PK, Pearlman E, et al. Increased resistance of contact lens related bacterial biofilms to antimicrobial activity of soft contact lens care solutions. *Cornea*. 2009;28:918–926.
5. Vijay AK, Willcox M, Zhu H, Stapleton F. Contact lens storage case hygiene practice and storage case contamination. *Eye Cont Lens*. 2015;41:91–97.
6. Efron N, Morgan PB, Woods CA, Consortium TICLPS. An international survey of daily disposable contact lens prescribing. *Clin Exp Optom*. 2013;96:58–64.
7. Russell AD. Bacterial resistance to disinfectants: present knowledge and future problems. *J Hos Infec*. 1999;43:S57–S68.
8. Lakkis C, Fleiszig SMJ. Resistance of Pseudomonas aeruginosa isolates to hydrogel contact lens disinfection correlates with cytotoxic activity. *J Clin Microb*. 2001;39:1477–1486.
9. Bruinsma GM, Rustema-Abbing M, van der Mei HC, Lakkis C, Busscher HJ. Resistance to a polyquaternium-1 lens care solution and isoelectric points of Pseudomonas aeruginosa strains. *J Antimicrob Chemother*. 2006;57:764–766.
10. Tabata A, Nagamune H, Maeda T, Murakami K, Miyake Y, Kourai H. Correlation between resistance of Pseudomonas aeruginosa to quaternary ammonium compounds and expression of outer membrane protein OprR. *Antimicrob Agents Chemother*. 2003;47:2093–2099.
11. Subedi D, Vijay AK, Willcox M. Study of disinfectant resistance genes in ocular isolates of Pseudomonas aeruginosa. *Antibiotics (Basel, Switzerland)*. 2018;88:2–7.
12. Sauer A, Meyer N, Bourcier T. Risk factors for contact lens-related microbial keratitis: a case-control multicenter study. *Eye Cont Lens*. 2016;42:158–162.
13. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *Clin Infect Dis*. 2002;34:634–640.
14. Moshirfar M, Mirzaian G, Feiz V, Kang PC. Fourth-generation fluoroquinolone-resistant bacterial keratitis after refractive surgery. *J Cataract Refract Surg*. 2006;32:515–518.
15. Subedi D, Vijay AK, Willcox M. Overview of mechanisms of antibiotic resistance in Pseudomonas aeruginosa: an ocular perspective. *Clin Exp Optom*. 2018;101:162–171.
16. Kowalski RP, Pandya AN, Karenchak LM, Romanowski EG, Husted RC, Ritterband DC,

et al. An in vitro resistance study of levofloxacin, ciprofloxacin, and ofloxacin using keratitis isolates of Staphylococcus aureus and Pseudomonas aeruginosa. *Ophthalmol*. 2001;108:1826–1829.
17. Choy MH, Stapleton F, Willcox MD, Zhu H. Comparison of virulence factors in Pseudomonas aeruginosa strains isolated from contact lens-and non-contact lens-related keratitis. *J Med Microbiol*. 2008;57:539–546.
18. Borkar DS, Acharya NR, Leong C, Lalitha P, Srinivasan M, Oldenburg CE, et al. Cytotoxic clinical isolates of Pseudomonas aeruginosa identified during the Steroids for Corneal Ulcers Trial show elevated resistance to fluoroquinolones. *BMC Ophthalmol*. 2014;54:1–6.
19. Hancock RE, Speert DP. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment. *Drug Resist Updat*: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy, 2000;3:247–255.
20. Subedi D, Vijay AK, Kohli GS, Rice SA, Willcox M. Nucleotide sequence analysis of NPS-1 β-lactamase and a novel integron (In 1427)-carrying transposon in an MDR Pseudomonas aeruginosa keratitis strain. *J Antimicrob Chemother*. 2018;73:1724–1726.
21. Green MD, Apel AJ, Naduvilath T, Stapleton FJ. Clinical outcomes of keratitis. *Clin Exp Ophthalmol*. 2007;35:421–426.
22. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. *Antimicrob Agents Chemother*. 2006;50: 43–48.
23. Mohammadinia M, Rahmani S, Eslami G, Ghassemi-Broumand M, Aghazadh Amiri M, Aghaie G, et al. Contact lens disinfecting solutions antibacterial efficacy: comparison between clinical isolates and the standard ISO ATCC strains of Pseudomonas aeruginosa and Staphylococcus aureus. *Eye (London, England)*, 2012;26:327–330.
24. Watanabe K, Zhu H, Willcox M. Susceptibility of Stenotrophomonas maltophilia clinical isolates to antibiotics and contact lens multipurpose disinfecting solutions. *Invest Ophthalmol Vis Sci*. 2014;55:8475–8479.
25. Kirven LA, Thornsberry C. Minimum bactericidal concentration of sulfamethoxazole-trimethoprim for Haemophilus influenzae: correlation with prophylaxis. Antimicrob Agents Chemother. 1978;14:731–736.
26. Taylor P, Schoenknecht F, Sherris J, Linner E. Determination of minimum bactericidal concentrations of oxacillin for Staphylococcus aureus: influence and significance of technical factors. Antimicrob Agents Chemother. 1983;23:142–150.
27. White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. *Antimicrob Agents Chemother*. 1996;40:1914–1918.
28. Stein C, Makarewicz O, Bohnert JA, Pfeifer Y, Kesselmeier M, Hagel S, et al. Three dimensional checkerboard synergy analysis of colistin, meropenem, tigecycline against multidrug-resistant clinical Klebsiella pneumonia isolates. PloS One, 2015;10:0126479.
29. Oo TZ, Cole N, Garthwaite L, Willcox MDP, Zhu H. Evaluation of synergistic activity of bovine lactoferricin with antibiotics in corneal infection. *J Antimicrob Chemother*. 201; 65:1243–1251.
30. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother*. 2003;52;1.
31. Clinical and laboratory standard institute CLSI, 2012.
32. Kowalski RP, Yates KA, Romanowski EG, Karenchak LM, Mah FS, Gordon YJ. An ophthalmologist's guide to understanding antibiotic susceptibility and minimum inhibitory concentration data. *Ophthalmology*. 2005;112:1987.e1–.e6.
33. Levy B, Heiler D, Norton S. Report on testing from an investigation of fusarium keratitis in contact lens wearers. *Eye Cont Lens*. 2006;32:256–261.
34. McDonnell G, Russell AD. Antiseptics and disinfectants: Activity, action, and resistance. *Clin Microbiol Rev*. 1999;12:147–179.
35. Ruiz-Linares M, Ferrer-Luque CM, Arias-Moliz T, de Castro P, Aguado B, Baca P. Antimicrobial activity of alexidine, chlorhexidine and cetrimide against Streptococcus mutans biofilm. *Ann Clinical Microbiol Antimicrob*. 2014;13:41.
36. Cazzaniga A, Serralta V, Davis S, Orr R, Eaglstein W, Mertz PM. The effect of an antimicrobial gauze dressing impregnated with 0.2-percent polyhexamethylene biguanide as a barrier to prevent Pseudomonas aeruginosa wound invasion. Wounds. 2002;12:69–76.
37. Gabriel MM, McAnally C, Bartell J. Antimicrobial efficacy of multipurpose disinfecting solutions in the presence of contact lenses and lens cases. *Eye & Cont Lens*. 2018;44:125–131.
38. Codling CE, Maillard JY, Russell AD. Aspects of the antimicrobial mechanisms of action of a polyquaternium and an amidoamine. *J Antimicrob Chemother*. 2003;51:1153–1158.
39. Artini M, Cellini A, Scoarughi GL, Papa R, Tilotta M, Palma S, et al. Evaluation of contact lens

multipurpose solutions on bacterial biofilm development. *Eye & Cont Lens*. 2015;41:177–182.
40. Guan A, Li Z, Phillips KS. The effects of non-ionic polymeric surfactants on the cleaning of biofouled hydrogel materials. Biofouling. 2015;31:689–697.
41. Abjani F, Khan NA, Jung SY, Siddiqui R. Status of the effectiveness of contact lens disinfectants in Malaysia against keratitis-causing pathogens. *Exp Parasitol*. 2017;183:187–193.
42. Callahan D, Kovacs C, Lynch S, Rah M. Biocidal efficacy of multipurpose solutions against Gram-negative organisms associated with corneal infiltrative events. *Clinical Exp. Optom*. 2017;100:357–364.
43. Jeffrey D. Chemicals used as disinfectants: active ingredients and enhancing additives. *Revue Scientifique et Technique-Office International des Epizooties*. 1995;14:57–57.
44. Thibodeaux BA, Dajcs JJ, Caballero AR, Marquart ME, Girgis DO, O'Callaghan RJ. Quantitative comparison of fluoroquinolone therapies of experimental gram-negative bacterial keratitis. *Curr Eye Res*. 2004;28:337–342.
45. Keay L, Edwards K, Naduvilath T, Taylor HR, Snibson GR, Forde K, et al. Microbial keratitis: predisposing factors and morbidity. *Ophthalmol*. 2006;13:109–116.
46. Spierer O, Miller D, O'Brien TP. Comparative activity of antimicrobials against Pseudomonas aeruginosa, Achromobacter xylosoxidans and Stenotrophomonas maltophilia keratitis isolates. *Br J Ophthalmol*. 2018;102:708–712.
47. Watson S, Cabrera-Aguas M, Khoo P, Pratama R, Gatus BJ, Gulholm T, et al. Keratitis antimicrobial resistance surveillance program, Sydney, Australia: 2016 Annual Report. *Clin Exp Ophthalmol*. 2018;47:20–25.
48. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in South Florida and emerging resistance to fluoroquinolones. *Ophthalmol*. 2000;107:1497–1502.
49. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug resistance profile and biofilm forming potential of Pseudomonas aeruginosa isolated from contact lenses in Karachi-Pakistan. *BMC Ophthalmol*. 2013;13:1–8.
50. Lalitha P, Manoharan G, Karpagam R, Prajna NV, Srinivasan M, Mascarenhas J, et al. Trends in antibiotic resistance in bacterial keratitis isolates from South India. *Br J Ophthalmol*. 2017;101:108–113.
51. Ikeda T, Ledwith A, Bamford CH, Hann RA. Interaction of a polymeric biguanide biocide with phospholipid membranes. *Biochimica et Biophysica Acta - Biomembranes*. 1984;769:57–66.
52. Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol*. 2014;643:1–18.
53. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16:161–168.
54. Boost MV, Chan J, Shi GS, Cho P. Effect of multipurpose solutions against Acinetobacter carrying QAC genes. *Optom Vis Sci. Official Publication of the American Academy of Optometry*. 2014;91:272–277.
55. Chapman JS. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeterior Biodegrad*. 2003;51:271–276