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Efficacy of two contact lens disinfecting solutions in reducing growth rate of bacteria and eradication of biofilms

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Abstract

Efficacy of two types of contact lens disinfecting (CLD) solutions, most frequently purchased from Jordanian pharmacies by contact lens wearers, was investigated for reducing growth rate of methicillin resistant and methicillin sensitive *Staphylococcus aureus*, *Serratia marcesence*, *S. liquefacience* and *Acinetobacter spp*., previously isolated from In-use disinfecting solutions in CL cases. Each species was cultured in a clean and dirty conditions (to mimic real situation) of two fresh CLD solutions separately: A and B whose active agents are: Polyhexamethylene biguanide hydrochloride and Polyaminopropyl biguanide respectively. Log reduction of these bacteria have exceeded ISO 14729 acceptable criteria (3 log) and reached up to 5 log reduction. Dirty conditions have marked effect in reducing efficacy of CLD solution (A) to kill bacterial species under test. Biofilms produced by *S. marcescens* and *S. liquefaciens* were reduced by more than 50% after 24 hrs. using either CLD solutions, though it was less than that reduced after 4 hrs. Biofilm of methicillin resistant *Staphylococcus aureus* (MRSA) was the most affected by either CLD solutions after 4 hours. This study concluded that CL wearers should pay great consideration to cleaning and disinfection practices to decrease bacterial growth, reduce chances of biofilm formation.

Keywords: Contact lenses (CLs); Disinfecting solutions; Biofilm eradication; Growth reduction; MRSA

1. Introduction

For more than a century, CLs are one of the most biomedical appliances achieved clinical spread; they are used for refractive errors correction and for beautifying reasons. Commensal microflora normally found on eyelid margins, conjunctivae and microflora from wearer's fingers, while inserting lenses, may contaminate In-use CLD solutions found in CL storage cases. Potential pathogens that may exist temporarily on the ocular surface, may also be inoculated into contact lenses and ultimately to CLD solutions resulting in decreased preservative efficacy [1, 2]. Thereby, CLD solutions will act as a good substrate for these microbes, support their growth and act as a vector for potential pathogens through which eye infections could result [3]. Furthermore, grown bacteria may adhere to and transfer to contact lenses, may build up on the lenses developing into biofilm and eventually may transfer to the ocular surface. Owing to the very poor blood supply to the anterior chamber of eyes, defense against microbial invasion is at minimum, hence, bacteria can invade and colonize the cornea or conjunctiva producing infections and inflammatory complications including keratitis as infiltrative keratitis [4, 5, 6].

Polyhexamethylene biguanide (PHMB) is a chemical biocide used as an active ingredient in many products including disinfectant solutions, wet wipes, wound irrigation solutions and sterile dressings. Polyaminopropyl biguanide (PAPB) is a polymer or oligomer where biguanide functional groups are connected by propyl hydrocarbon chains, is an active material of some disinfectants and a preservative used for disinfection on skin and in cleaning solutions for contact lenses. It is also an ingredient in much deodorant body sprays [7].

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By using different types of CLD solutions, Nzeako and Al-Sumri [8] have reported that 65% and 5% of user solutions containing polyhexanide and PAPB revealed microbial growth respectively. Despite these solutions are containing biocides, bacterial biofilm if formed is undoubtedly show increased resistance to their antimicrobial activity [4, 9].

Recent studies [2, 8,10] have identified the most common bacterial species isolated from CLs cases and CLD solutions including: Staphylococci, *Pseudomonas aeruginosa*, *Streptococcus spp*., *Escherichia coli, Acinetobacter spp* and*Serratia spp.*

The aim of the present study is to investigate the effect of two most commonly bought CLD solutions from Jordan pharmacies, to reduce growth rate of methicillin resistant and methicillin sensitive *Staphylococcus* (MRSA and MSSA respectively)*, Serratia marcesence, S. liquefacience* and *Acinetobacter spp*., previously isolated from CLD solutions found in CL cases. Also, assessing disinfecting potentials of these two solutions in eradication biofilms formed experimentally by each of the above bacterial species.

2. Material and methods

2.1. Bacterial Isolates

MRSA, MSSA*, Serratia marcesence, S. liquefacience* and *Acinetobacter spp*., were provided from the study of AL-Khani [10]. Isolates were grown for 18-24 hr. on Tryptic Soy Agar (TSA) at 35°C. Cultures were suspended in sterile 0.9% NaCl to obtain a concentration of 1.0×10^7-1.0×10^8 CFU/ml [10,11].

2.2. Contact lens disinfecting (CLD) solutions:

Two CLD solutions coded A and B, most commonly used were bought from Jordanian pharmacies. Both solutions were within expiration date and were tested according to manufacturer's labeled recommendation for disinfection time. Ingredients and recommended disinfection time for each solution are shown in (Table1).

Table 1 Ingredients and recommended disinfection times of the two tested contact lens solutions

Solution code	Disinfectant agent	Other ingredients	Recommended time
A	Polyhexamethylene biguanide (Polyhexanide)	Potassium Chloride, Disodium Edetate Poloxamer, Sodium Hyaluronate, HPMC, Sodium Phosphate Buffer.	at least 4 hr.
B	Polyaminopropyl biguanide	Hydroxyalkylphosphonate, boric acid, Ethylenediaminetetraacetic acid disodium, poloxamine, sodium borate and sodium chloride.	at least 4 hr.

2.3. Reduction of Growth Rate

The experiment was conducted under clean and dirty conditions to mimic real situation. For clean conditions, aliquots of 0.5 ml bacterial suspensions were added to 4.5 ml of either CLD solutions. To provide dirty conditions, the above steps were repeated with the addition of 0.3% yeast [12,13] and the tubes were vortexed thoroughly. Positive control was prepared by the addition of 0.5 ml of each bacterial suspension to tubes containing 4.5 ml of 0.9% NaCl and negative control was prepared by addition 0.5 ml of either CLD solutions to tubes containing 4.5 ml of 0.9% NaCl [11, 14]. All tubes were allowed to stand at room temperature for 4 hrs. From each test tube, 0.5 ml was withdrawn and diluted with 0.4 ml tryptic soy broth (TSB) containing Tween 80 (1%) and left at least for 10-15 minute at room temperature to neutralize residual solution [11, 15]. Aliquots of 10µl and 100µl were plated on TSA plates and incubated at 35°C for 24-48hr. According to Laxmi Narayana, et al. [16], the number of surviving bacteria, colony forming unit (CFU/ml) was counted and the logarithmic reduction in growth by each CLD solution and the control was calculated as following:

$$\text{Log reduction} = \text{Log}\,10\ (\text{Control CFU/ml}) - \text{Log}\,10\ (\text{Final CFU/ml})$$

2.4. Biofilm Formation and Inhibition Assay

To test the ability of CLD solution to eradicate biofilm; one colony of each isolate was transferred to TSB and incubated at 35^0 C for 24 hrs. Aliquots 100 µl of TSB inoculum were placed in wells of 96-microtittre plates filled with 100 µl of

either CLD solutions. This procedure was repeated but with the addition of one drop of 0.3% yeast to provide dirty conditions. Control samples contained 100µl of TSB inoculum with 100µl of pure TSB. Two sets of plates were prepared to be incubated for two periods: 4 and 24 hrs. at 35°C. After each period, the contents of plates were poured off and the wells were washed with running tap water. Wells were stained with crystal violet for 15 min then excess crystal violet was washed away with running tap water and the stain was dissolved by ethanol then allowed to air dry [17, 18]. Optical density (OD) of each well was measured at 600 nm by AccuReader M965 Microplate Reader (Metertech Inc.). Inhibition of biofilms was calculated as following

$$\text{Inhibition Biofilm \%} = \frac{\text{OD of Control} - \text{OD of Solution}}{\text{OD of Control}} \times 100$$

2.5. Data Calculation

Microsoft Excel was used for data storage and graphs generation.

3. Results

Log reduction at the minimum recommended disinfection time for the two CLD solutions: A and B against *S. marcescens, S. liquefaciens, Acinetobacter spp*., MRSA and MSSA in clean and dirty conditions, are illustrated in Tables (2) and (3) respectively.

Table 2 Efficacy of (A) disinfecting solution in log reduction of tested bacteria under clean and dirty conditions, after 4hr

Test bacteria	Clean	Dirty
Acinetobacter spp.	5.6148972	5.342423
S. marcescens	5.5888317	5.519828
S. liquefaciens	5.5599066	5.394452
MRSA*	5.6148972	5.541579
MSSA**	5.553883	5.318063

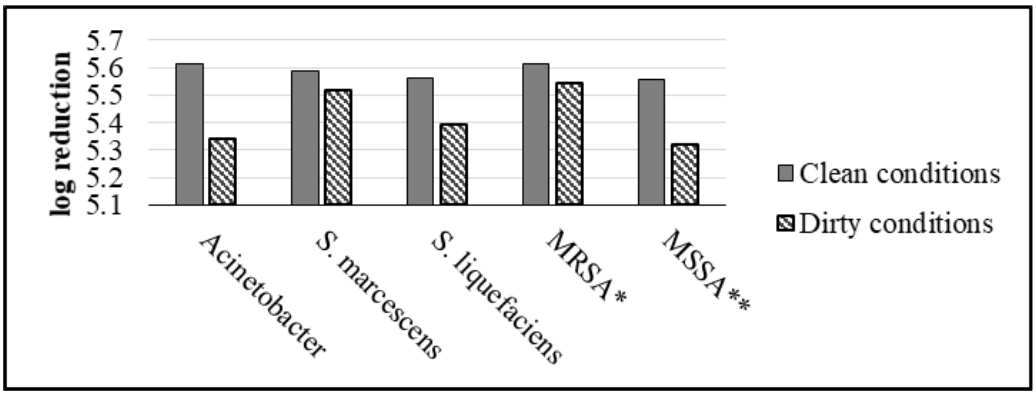
*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureus*

Table 3 Efficacy of (B) disinfecting solution in log reduction of tested bacteria under clean and dirty conditions, after 4hr

Test organisms	Clean	Dirty
Acinetobacter spp.	5.625312	5.535294
S. marcescens	5.585461	5.553883
S. liquefaciens	5.609594	5.574031
MRSA*	5.580925	5.536558
MSSA**	5.556303	5.444045

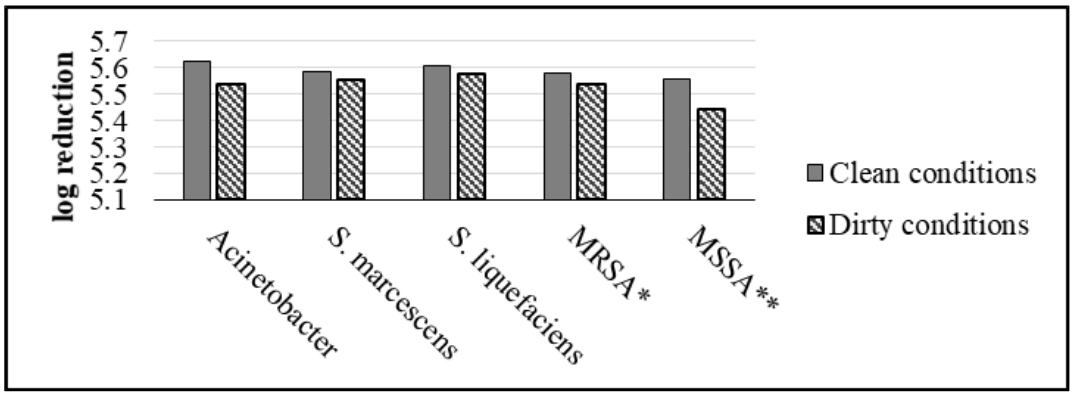
*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureus*

Generally, CLD solutions (A) was more efficient in reducing bacterial growth and is clearly evident in clean as compared to dirty condition. CLD solution (B) was more effective in reducing bacterial growth in dirty conditions than CLD solutions (A) as illustrated in figures (1) and (2). CLD solution (B) exerted almost similar and high efficacy in reducing growth of MRSA, *S. marcescens, S. liquefaciens* and *Acinetobacter spp*. in both clean and dirty conditions.



*MRSA; methicillin resistant S. aureus **MSSA; methicillin sensitive S. aureus

Figure 1 Log reduction of tested bacteria in (A) disinfecting solution under clean and dirty conditions, after 4 hrs



*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureus*

Figure 2 Log reduction of tested bacteria (B) disinfecting solution under clean and dirty conditions, after 4 hrs

3.1. Biofilm formation and eradication

Optical density of the remaining biofilm in microtiter plates was measured twice, after 4 and 24 hr. of application of CLD solutions (Tables 4 and 5).

Table 4 Optical density (OD) and percentage inhibition of biofilm using solution A against isolates for two time periods under clean and dirty conditions

Test bacteria	OD of Control	OD of Clean conditions	Inhibition biofilms (%)	OD of Dirty conditions	Inhibition biofilms (%)	Period
Acinetobacter spp.	0.177	0.052	70.6%	0.053	70.1%	After 4hr
S. marcescens	0.275	0.055	80%	0.054	80.4%	
S. liquefaciens	0.37	0.051	86.2%	0.055	85.1%	
MRSA*	0.645	0.061	90.5%	0.055	91.5%	
MSSA**	0.192	0.065	66.1%	0.056	70.8%	
Acinetobacter spp.	0.1	0.055	45%	0.058	42%	After 24hr
S. marcescens	0.38	0.057	85%	0.059	84.5%	
S. liquefaciens	0.38	0.061	83.9%	0.058	84.7%	
MRSA	0.95	0.364	61.7%	0.37	61.%	
MSSA	0.1	0.052	48%	0.052	48%	

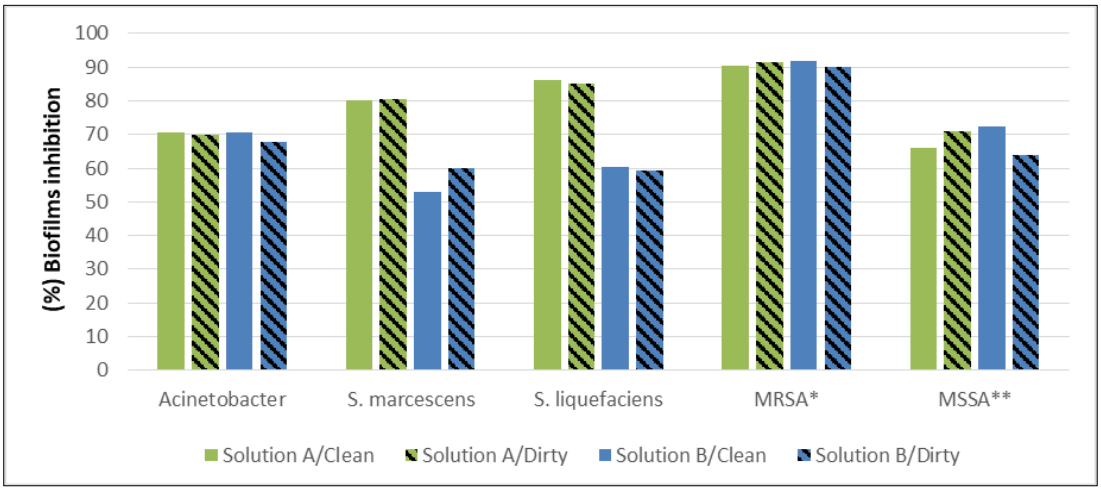
*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureus*

Table 5 Optical density (OD) and percentage inhibition of biofilm using solution B aganist isolates for two time periods under clean and dirty conditions

Test bacteria	OD of Control	OD of Clean conditions	Inhibition biofilms (%)	OD of Dirty conditions	Inhibition biofilms (%)	Period
Acinetobacter spp.	0.177	0.052	70.6%	0.057	67.8%	After 4hr
S. marcescens	0.275	0.129	53.1%	0.11	60%	
S. liquefaciens	0.37	0.147	60.3%	0.151	59.2%	
MRSA*	0.645	0.053	91.8%	0.064	90.1%	
MSSA**	0.192	0.053	72.4%	0.069	64.1%	
Acinetobacter spp.	0.1	0.05	50%	0.067	33%	After 24hr
S. marcescens	0.38	0.06	84.2%	0.098	74.2%	
S. liquefaciens	0.38	0.06	84.2%	0.101	73.4%	
MRSA	0.95	0.35	63.2%	0.367	61.4%	
MSSA	0.1	0.06	40%	0.068	32%	

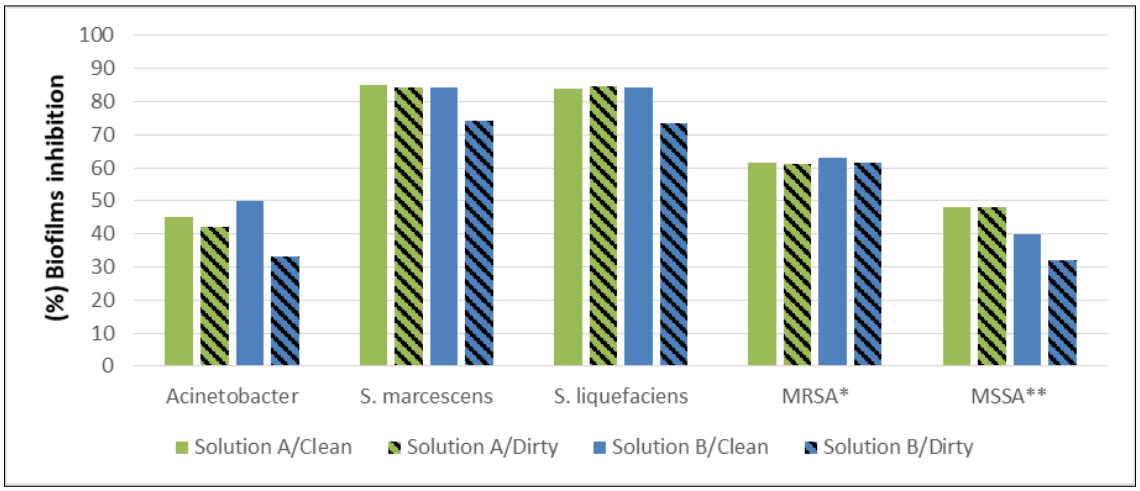
*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureu*

Both CLD solutions have the ability to reduce biofilms. Figures (3) and (4) clarify percentage reduction of biofilms as compared to control for each bacterium. After 4hr, solutions A and B were able to reduce biofilms by more than 50% of all tested bacterial biofilms, regardless of the cleanness/dirtiness status. Solution (A) achieved significantly higher inhibition rate on biofilms produced by *S. marcescens* and *S. liquefaciens* as compared to solution B (Fig. 3). MRSA biofilm was the most affected by either CLD solutions after 4 hours (Fig. 3). Compared to results achieved after 4hr (Fig 3), percentages of biofilm inhibition of *Acinetobacter*, MRSA and MSSA were comparably lower after 24hr (Fig 4).



*MRSA; methicillin resistant S. aureus **MSSA; methicillin sensitive S. aureus

Figure 3 Efficacy of disinfecting solutions (A)and (B) for inhibition biofilm after 4 hrs



*MRSA; methicillin resistant *S. aureus* **MSSA; methicillin sensitive *S. aureus*

Figure 4 Efficacy of disinfecting solutions (A) and (B) for inibition biofilm after 24 hrs

4. Discussion

The active agents of the two disinfecting solutions evaluated in the present study

PAPB (A) and PHMB (B) are molecularly closely related [7].

Over the past years, CLD solutions have been continually improved to contain combinations of cleaning, disinfecting, moisturizing, preventing of tear agents and become more efficient for surface sterilization of CLs. Nevertheless, CLD solution with the same formulations, but manufactured by different companies, may possess different disinfecting potentials [8]. Thereby, extent of microbial contamination of storage cases varies with the use of different formulations of CLD solutions [19].

Despite using disinfecting agent, CLs cases are the most allegeable item to be contaminated which in turn introduced to disinfectant solution resulting in decreased preservative efficacy [3].

According to International Standards Organization (ISO) 14729 guidelines that determined standard in industry of active CLD solutions against microorganisms. CLD solution is considered effective, if it reduces viability of initial concentration of bacteria species by at least 3 log which is (99.9%) of bacteria concentration at recommended exposure time [20].

Although there were differences between the efficacies of the two CLD solutions A and B , both exceeded the required 3.0 log reduction in growth of isolates recovered from CL storage cases (Figures 1 and 2). Mohammadinia et al., (11) reported that clinical isolates are more resistant to solutions than standard strains therefore; they concluded that acceptance test criteria of CLD solutions cannot be ensured for effectiveness with standard strains only. Lever and Borazjani [21] tested efficacy of CLD solution whose active agent is PHMB, they found it was exceeding the minimum acceptable criteria after one hour which is one quarter of labeled minimum disinfection time with reference strain.

The two CLD solutions were shown to be more effective in clean conditions than in dirty conditions. Polyhexanide works by electrostatic interaction, it has positive charge that binds to phosphate negative charge of phospholipids at bacteria cell wall, protective out layer and cell membrane were splintered, then cytoplasm leak causing cell death [22]. This means that the disinfectant is reacting with the organic matter instead of bacteria or react with impurities adhered to bacterial cell surface then prevent PHMB or/and PAPB from binding to bacterial cell wall. This explains importance of good cleaning practices where impurities reduce disinfectant efficacy; thus, providing more suitable environment for microbes.

CLD solution (B) efficacy was the highest in log reduction of growth of all tested bacteria. According to Laxmi Narayana et al. [16] study, the two solutions tested containing PAPB and PHMB reached the 3 log and 5 log reduction criteria, respectively. Also, they observed that effectiveness of CLD solutions varies against different bacterial species such as *S. aureus* and *S. marcescens*.

After 4hr, biofilms produced by *S. marcescens* and *S. liquefaciens* using solution (A) achieved significantly higher inhibition rates when compared to solution B(Fig 3). Artini et al. [17] concluded that CLD solutions are able to inhibit biofilm formed by *S. marcescens* and *S. aureus* after 4 hrs.

Noteworthy, after 4 hours, MRSA biofilm reduction was the most affected by both CLD solutions used in present study (Fig. 3). Kamaruzzaman et al. [23] suggested that PHMB is effective against *S. aureus* and can damage biofilm structures between 28 to 37% of biofilm produced by *S. aureus*

CLD solution (B) whose active agent is PAPB was more effective in reducing bacterial growth than CLD solution (A), active agent PHMB, with the maximum efficacy against S*. liquefaciens* and *Acinetobacter spp.* in both clean and dirty conditions. Both CLD solutions displayed the ability to reduce biofilm

These results agree with Rembe, et al. [7] finding who determined antimicrobial efficacy of dressing containing either PAPB or PHMB against *Staphyloccoccus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, according to international standards. They reported that PHMB exhibited high antimicrobial efficacy against *S. aureus* and *E. coli,* in addition to high efficacy in bacterial eradication. Whereas, PAPB displayed no appropriate antimicrobial effects and was not efficient to eradicate bacteria.

5. Conclusion

Significance of CL wearer's commitment to cleaning and disinfection practices will decrease bacterial growth, reduce chances of resistance and/or biofilm formation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors of this study have no financial interest nor received any financial support from the companies that manufacture multipurpose contact lens care solutions.

Statement of ethical approval

Approval to conduct the study was obtained from the Ethical Committee at Isra University (Ph/ 9/2019).

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