ATTAHADI MEDICAL JOURNAL
Journal homepage:
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Hydrogen Peroxide as a Potent Antimicrobial Agent in Contact Lens Care Solutions

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Keywords:
Hydrogen Peroxide, Contact Lens, Cleaning Solution, Acanthamoeba.

Received *02 Feb 25*
Accepted *30 March 25*
Published *11 April 25*

A B S T R A C T

Assessing lens visibility in terms of transmittance is vital for those who wear contact lenses. To improve this visibility, various contact lens solutions are applied to disinfect the lenses effectively. Three bacterial strains sourced from contact lens storage cases were utilized to detect and characterize a biofilm. Three models were utilized, specifically, the biofilm model, the aging well plates model, and Planktonic model. Hydrogen peroxide is still the main contact solution that has an antimicrobial effect against different microbes. The effect of hydrogen peroxide was tested against three either in planktonic or biofilm formation strains, including Stenotrophomonas maltophilia (ALC-01), Elizabethkingia meningoseptica (3AS), and Achromobacter (AH-2A), along with Acanthamoeba castellanii 50370. It shows a higher effective contact lens solution compared with another common multipurpose contact lens solution. The hydrogen peroxide formulation shows significant disinfection potential and is compatible with multiple contact lens types, suggesting it could serve as a valuable option for improving lens hygiene and visibility.

Citation info. Hamed A, Essa E, Alaharash A, Alshourif F. Hydrogen Peroxide as a Potent Antimicrobial Agent in Contact Lens Care Solutions. Attahadi Med J. 2025;2(2):80-86. https://doi.org/10.69667/amj.25203

INTRODUCTION

Contact lens users are identified as a notable risk factor for the development of microbial keratitis (MK) [1,2]. Factors contributing to this risk include poor hygiene practices associated with contact lens maintenance, as well as the use of homemade saline solutions and tap water [3,4]. The storage case for contact lenses may become contaminated with protein residues, promoting the growth of various microorganisms, including bacteria, yeast, and fungi, which can serve as a food source for amoebae. These microorganisms can lead to MK, with potential culprits ranging from bacterial toxins to fungi, and including the free-living amoeba Acanthamoeba, which is commonly found in nature [5,6]. Acanthamoeba is regarded as an opportunistic microorganism, capable of infecting both immunocompetent individuals, resulting in ulcerative keratitis and immunocompromised individuals, potentially causing severe brain infections. The life cycle of Acanthamoeba consists of two distinct stages: the first is the active trophozoite stage [figure 1], where these organisms actively feed on bacteria, yeast, and algae while reproducing. The second stage is the dormant cyst stage, which occurs when environmental conditions such as food supply, oxygen, pH, or temperature become unfavourable, trophozoites encase themselves in a protective cyst wall [figure 2], significantly reducing their metabolic activity. Amoebae are often referred to as the Trojan horses of the microbial realm because of their capacity to harbour and sustain various pathogenic microorganisms [7,8].

The presence of bacteria alongside acanthamoeba as contaminants in contact lenses and their cases may play a crucial role in the proliferation and persistence of these organisms. Nevertheless, there are only a limited number of documented instances of co-infection involving both fungal pathogens and acanthamoeba keratitis in existing literature [9]. Although Acanthamoeba keratitis (AK) is infrequent, it poses a significant risk as a complication of contact lens wear, potentially resulting in loss of vision. The growing concern regarding the pathology of this organism emerged when it began to spread [10], prompting increased scrutiny on the safety of disinfection practices and the formulation of solutions for soft contact lenses [11]. Persistent efforts have been made to enhance the efficacy of contact lens solutions against this resilient microorganism. Hydrogen peroxide (3%) is widely recognized as a standard disinfectant for contact lens cleaning, owing to its extensive effectiveness against bacteria, fungi, and Acanthamoeba species [12]. It is a chemical commonly present in various products, including certain solutions for cleaning contact lenses. It is particularly effective in dislodging and eliminating debris from lenses, such as proteins and fatty residues. When utilized properly, hydrogen peroxide solutions are safe for ocular use. Multipurpose disinfecting solutions (MPDS) are formulated with multiple ingredients, such as biocidal preservatives, buffer solutions, and other compounds that promote lens comfort and cleaning for rinsing, disinfection, and storage purposes [13,14]. The selection of MPDS for contact lens care is attributed to its wide-ranging antimicrobial efficacy against bacteria and fungi, along with its ability to eliminate acanthamoeba [15].

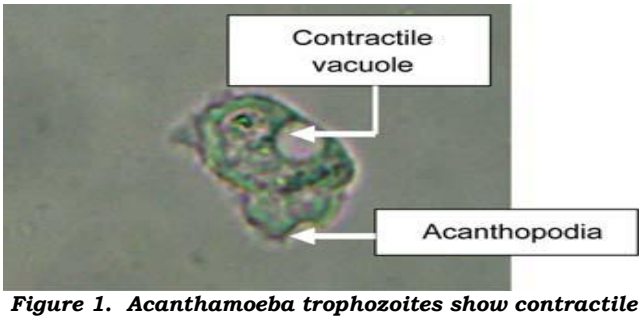

Figure 1. Acanthamoeba trophozoites show contractile vacuole (pale area) and acanthopodia.

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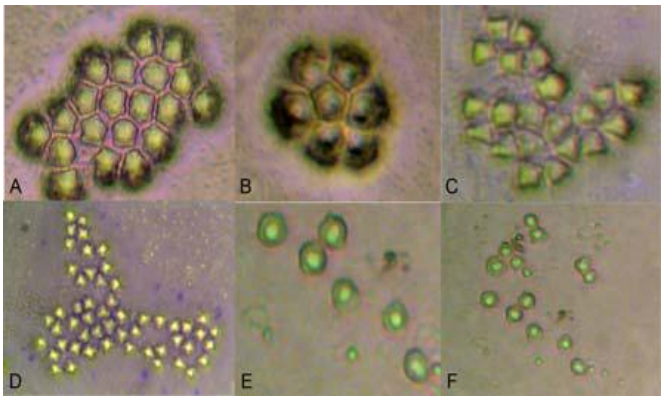

Figure 2. Cysts of various Acanthamoeba spp. growing on non-nutrient agar.

Hydrogen peroxide solutions typically lack preservatives, making them suitable for individuals who are allergic or sensitive to certain components found in other multipurpose contact lens solutions. As a potent oxidizing agent, hydrogen peroxide has a chemical structure with unpaired electrons, rendering it highly reactive and capable of damaging cellular macromolecules, including proteins, lipids, and nucleic acids. This solution will be evaluated for its antimicrobial agents intended for use in disinfecting contact lenses. Assays will be established to determine the antimicrobial efficacy of hydrogen peroxide in the context of contact lens care, alongside comparisons to other formulations. The efficacy will be assessed against a variety of bacteria and Acanthamoeba in both planktonic (free-floating) and biofilm growth environments.

MATERIAL AND METHOD

Two types of culture media are used: tryptone soya agar (TSA) and Tryptone soya broth (1% TSB). The media are prepared as shown in Table 1, autoclaved, and kept in the refrigerator until use. Various commercial contact lens disinfectant solutions are also used and shown in Table 2.

Table 1. Media Composition

Tryptone soya agar (TSA) 20g in 500 ml. water
Tryptone soya broth (1% TSB). 1g in 100 ml. water

Table 2. Different types of commercial disinfecting solutions

Contact lens solution	Preservatives	Uses
Revitalense	Alexidine 0.00016% Poly quaternium -1 0.0003 (PQ-1)	For silicon hydrogel and soft contact lenses
Menicare plus (MP)	Polyhexamethylene biguanide 0.0005%	For all rigid gas permeable lenses
All in ON light (AIOL)	Polyhexanide 0.0001%	For all contact lenses
Pure moist (PM)	Poly quaternium -1 0.001% (PQ-1). Myristamidopropyl dimethylamine 0.0006%	For silicone hydrogel and soft contact lens
Biotrue	Polyaminopropyl biguanide 0.00013%, PQ-1 0.0001%	For soft contact lenses including silicone hydrogel lenses
Boston	Chlorhexidine gluconate 0.003% Polyaminopropyl biguanide 0.0005%	For gas permeable contact lens
Hydrogen peroxide H2O2		Used as a positive control
Dulbecco's phosphate buffer saline (DPBS)		Used as a negative control

The initial phase of this research involved several key actions. Three bacterial strains sourced from contact lens storage cases provided by the Department of Microbiology and Immunology, Medical Sciences, University of Leicester, UK. were utilized to detect and characterize a biofilm. These strains include Stenotrophomonas maltophilia (ALC-01), Elizabethkingia meningoseptica (3AS), and Achromobacter (AH-2A), along with Acanthamoeba castellanii 50370. During the experiment, three models were utilized, specifically:
1. Bio-film model. 2. Aging well plates model.3. Planktonic model.

Bio-film model

Following an overnight culture on Tryptone soya agar (TSA) plates at 35°C in an aerobic environment, bacteria were collected using sterile cotton-tipped swabs and re-suspended in 1% Tryptone soya broth (TSB) to an optical density (OD600) of 0.18-0.2. Two milliliters of this bacterial suspension were inoculated into the wells of a 12-well tissue culture plate and incubated at 32°C for 18-20 hours. After incubation, the bacterial solution was removed, and the wells were washed with 2 mL of DPBS (Dulbecco's Phosphate-Buffered Saline). Each well was subsequently filled with 3 mL of contact lens solutions, with 3.5% H2O2 and DPBS included as controls. The plates were then incubated at 25°C for either 6 or 24 hours. The walls of the wells were scrubbed with sterile swabs, and 100 µL of the solution was pipetted onto a TSA plate and spread using a spreader. The TSA plates were incubated at 32°C for 24 hours, after which they were photographed according to the established protocol.

Aging Well Plates Model

In this aging model, 3 mL of a contact lens disinfection solution is introduced into a 12-well plate. The plate is subsequently incubated at 25°C for either 6 or 24 hours to facilitate aging. Hydrogen peroxide and DPBS serve as control agents. After the aging process, the plate is emptied, and 2 mL of a bacterial suspension, prepared to an optical density (OD) of 0.18-2.0, is added to each well. The plates are then incubated for 24 hours at 32°C. Following incubation, the walls of the wells are gently swabbed with sterile swabs. A volume of 100 µL from each well is pipetted onto a TSA plate, spread evenly, and then incubated at 32°C for 24 hours. Finally, the plates are photographed according to the specified protocol.

Planktonic model

A bacterial suspension was prepared as previously described, achieving an optical density (OD600) of 0.18-0.20. To a 12-well plate, 3 mL of contact lens solution was added. H2O2 and DPBS were also utilized to fill the wells for control purposes. Following this, 30 µL of the bacterial suspension was introduced into each well. The plates were incubated at 32°C for either 6 or 24 hours. After incubation, the walls of the wells were gently rubbed with sterile swabs. A volume of 100 µL from each well was then pipetted onto a TSA plate and spread evenly. The plates were incubated at 32°C for 24 hours, after which they were photographed in accordance with the protocol.

Biofilm formation and quantification

The bacteria Stenotrophomonas maltophilia (ALC-01), Elizabethkingia meningoseptica (3AS), and Achromobacter (AH-2A) are cultured on TSA for a duration of 18 to 24 hours at a temperature of 35°C. The colonies are then collected into a 0.5% TSB solution and adjusted to an optical density (OD600) of 0.18 to 0.20, ensuring the absence of visible clumps through vortexing. A volume of 2 ml of the bacterial suspension is added to the wells of a 12-place microtiter plate, followed by incubation for 18 to 24 hours at 35°C. After incubation, the solution is discarded from the wells, and 2 ml of DPBS is introduced. Decantation is performed, and the wells are subsequently filled with another 2 ml of DPBS. Next, 200 µl of 0.2% crystal violet stain is added and allowed to sit at room temperature for 15 minutes. Following this, decantation is conducted, and the wells are gently washed three times with DPBS. The plates are then left to dry, after which 2 ml of ethanol is added, mixed, and allowed to stand at room temperature for 10 minutes. Finally, the eluted stain is measured and recorded at OD 600.

Results

The findings of this study suggest that the biofilm layer plays a significant role in the protective function of microorganisms associated with biofilms from the effects of contact lens solutions. The subsequent figures illustrate the distinctions between bacterial behavior in planktonic and biofilm growth states. The development of bacterial communities, or biofilms, can be

affected by the presence of eye contact lens solutions. In natural environments, bacteria often form biofilms, which serve as a strategy to enhance their defensive responses to stress. Additionally, the formation of biofilms in bacteria provides protection against phagocytosis by protozoan predators [16]. Adherent bacteria may also exist on contact lenses, making the use of disinfecting solutions advisable to mitigate this risk. However, it is important to note that some of these solutions could act as catalysts for biofilm development, thereby exacerbating the problem [17].

Different strains of microorganisms have been found to possess varying susceptibilities to biocides, with adhered microbes exhibiting higher resistance than those in a planktonic state [18]. Furthermore, a specific formulation of a multipurpose disinfecting solution for contact lenses may facilitate the encystment of Acanthamoeba [22]. In the context of the planktonic model, the strains 3AS, AH-2A, and ALC-01 were exposed to various eye lens solutions, which were shown to effectively inhibit bacterial growth. This was depicted in all the figures provided (fig. 3 & 4), (fig. 7 &8), (fig 9 & 10).

Conversely, during the biofilm model investigation, the same bacterial strains displayed distinct actions, leading to the development of biofilm that was resistant to disinfectant activity and fostered an environment favourable for growth (Fig. 5 & 6), (fig. 11 & 12). On the other hand, some solutions display strong antimicrobial activity against this biofilm, including Boston and hydrogen peroxide (Fig. 5). It is evident that Menicare plus (MP) is effective against the biofilm formed by 3AS (Fig. 6), while it does not have a similar effect on the planktonic growth of AH-2A and ALC-01 (Fig. 7), (Fig. 9). This may be explained by the solution's capacity to penetrate the 3AS biofilm more effectively and the phenotypic differences present in the biofilms of each strain. Moreover, in terms of preventing biofilm development, Revitalens demonstrates the capability to inhibit the biofilm growth of the ALC-01 strain (Fig,12). Ultimately, the study found that hydrogen peroxide was the most potent contact lens solution, capable of inhibiting both planktonic and biofilm models across all strains tested.

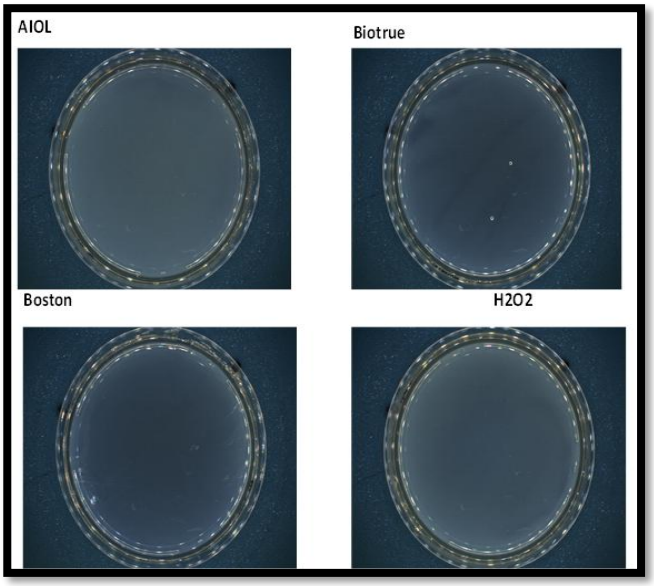

Figure 3. Contact lens solutions inhibit planktonic Elizabethkingi meningoseptica (3AS) strain from growth after 24 hours.

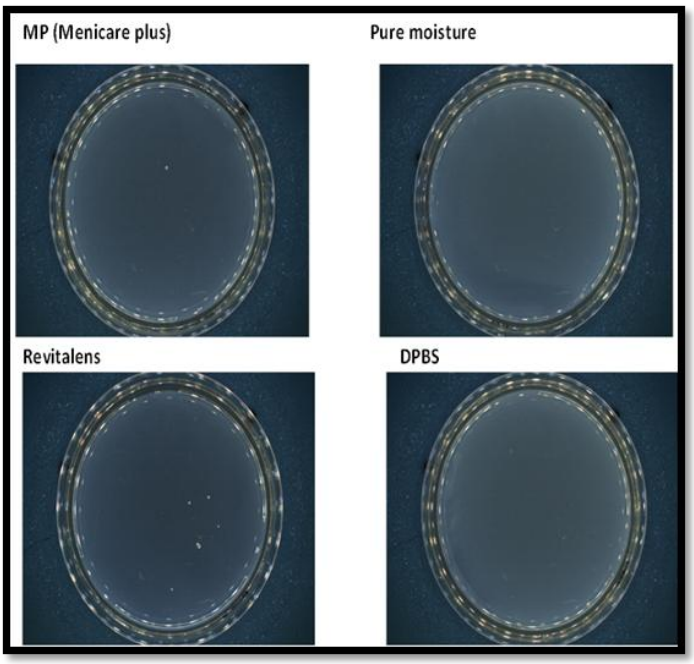

Fig. 4: Contact lens solutions inhibit planktonic Elizabethkingia meningoseptica(3AS) from growth after 24hrs.

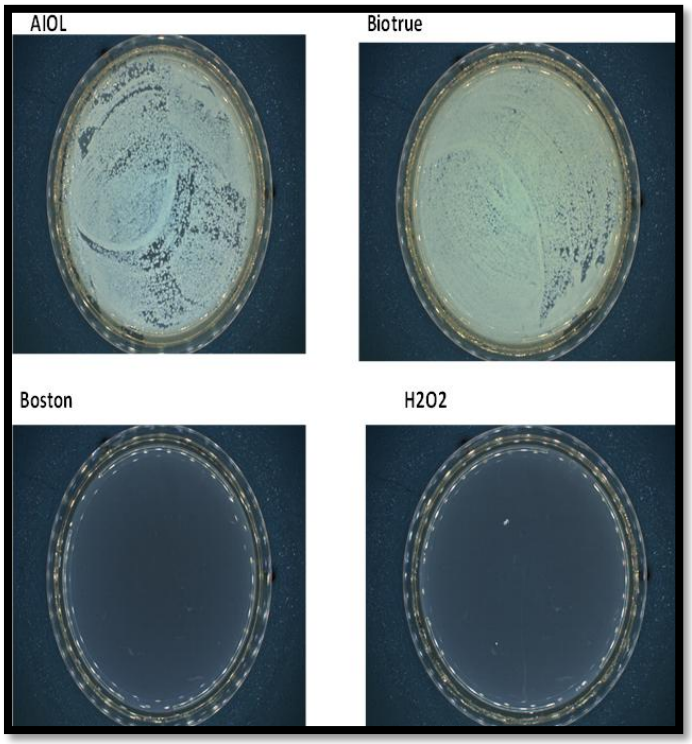

Fig. 5: Biofilm formation protect Elizabethkingia meningoseptica (3AS) from theaction of some solution.

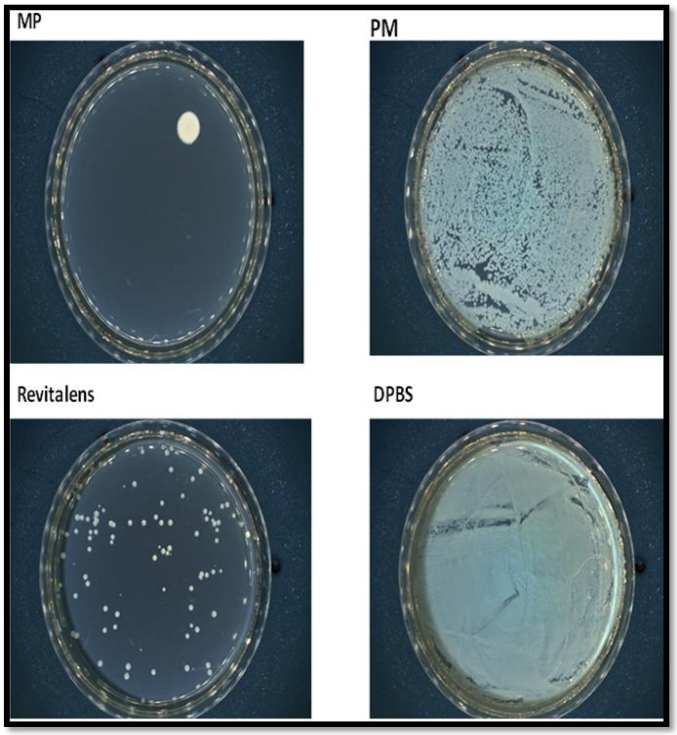

Fig. 6: Biofilm formation protects Elizabethkingia meningoseptica (3AS) from the action of the contact lens solution.

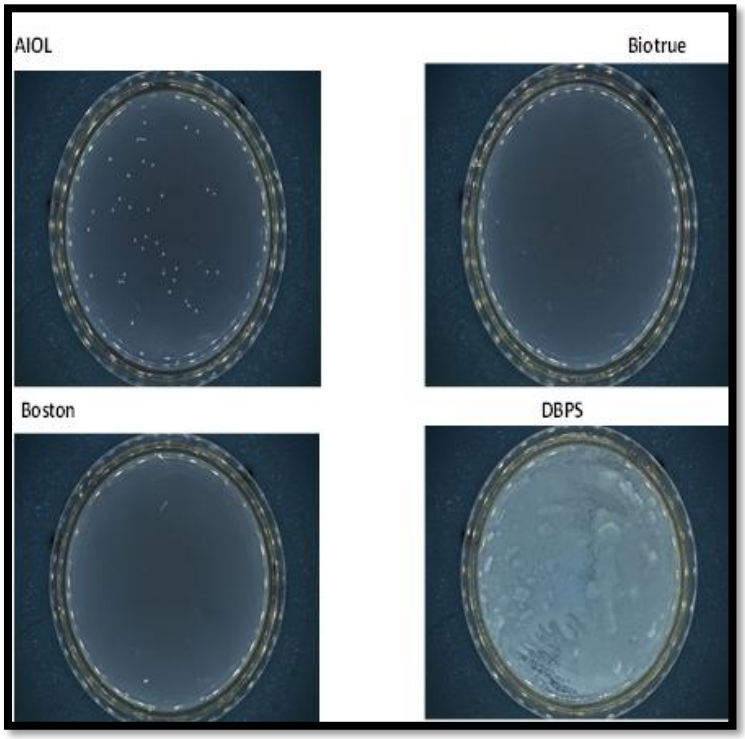

Fig. 8: Contact lens solutions used inhibit planktonic Achromobacter (AH-2A) from growth after 24hrs.

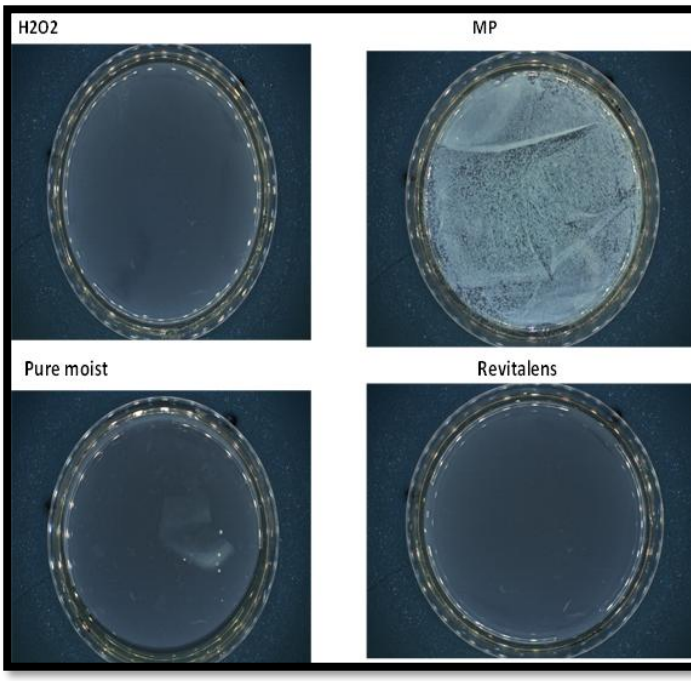

Fig 7: Contact lens solutions inhibit planktonic Achromobacter (AH-2A) from growth after 24 hours.

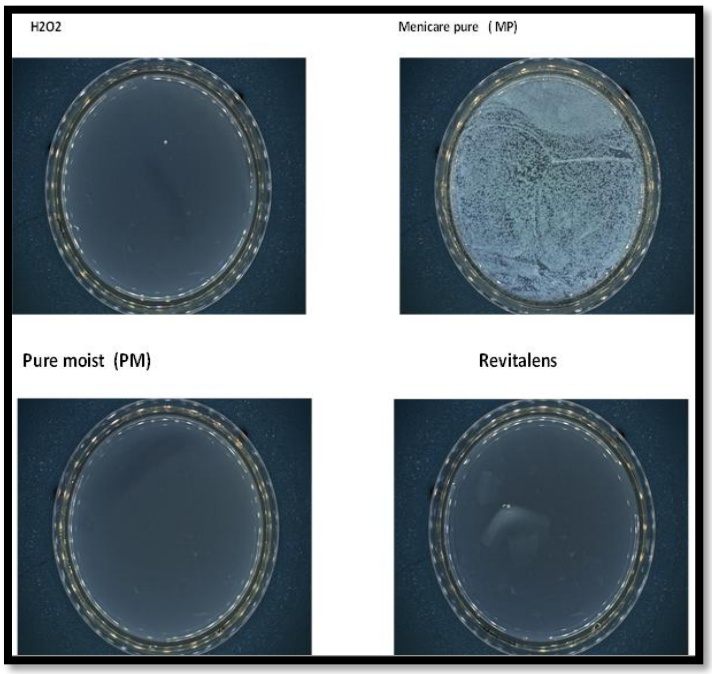

Fig 9: Contact lens solutions used inhibit planktonic state in ALC-01 from growth after 24hrs.

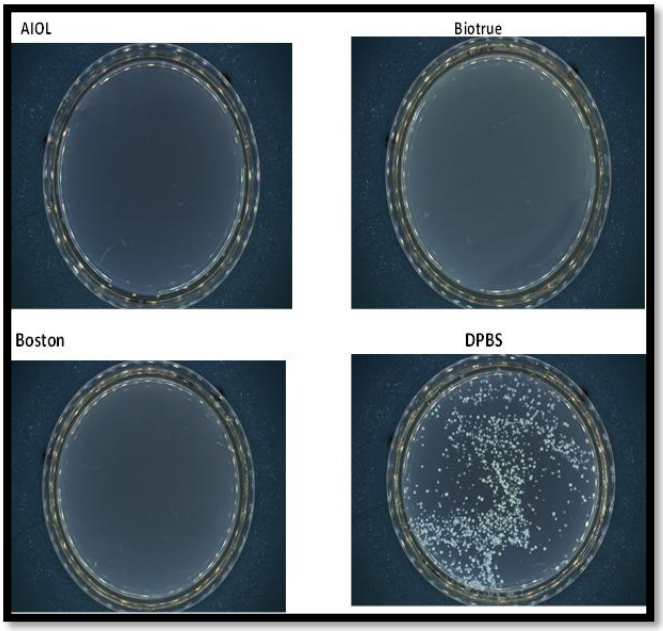

Fig.10: Contact lens solutions used inhibit planktonic Stenotrophomonas maltophilia (ALC-01) state in from growth after 24hrs.

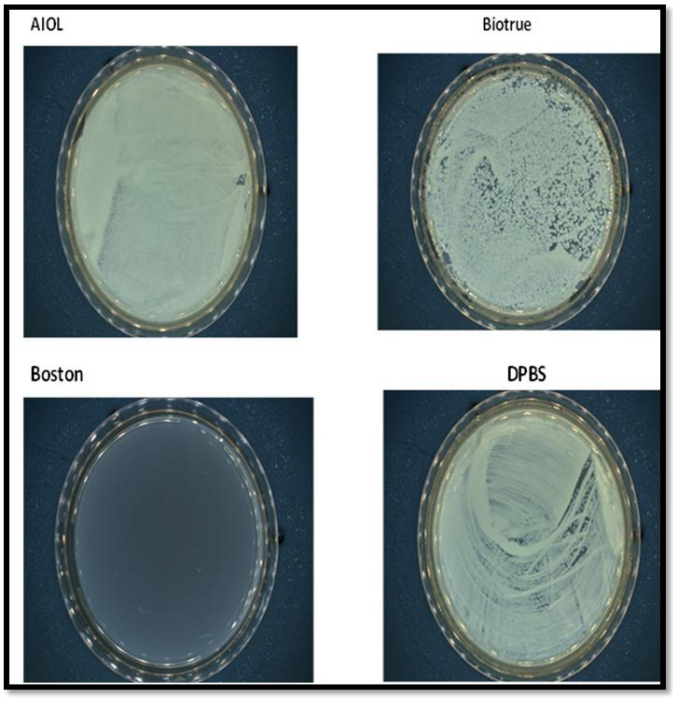

Fig. 11: Biofilm model in Stenotrophomonas maltophilia (ALC-01) resistant action of contact lens solution and grow after 24 hours.

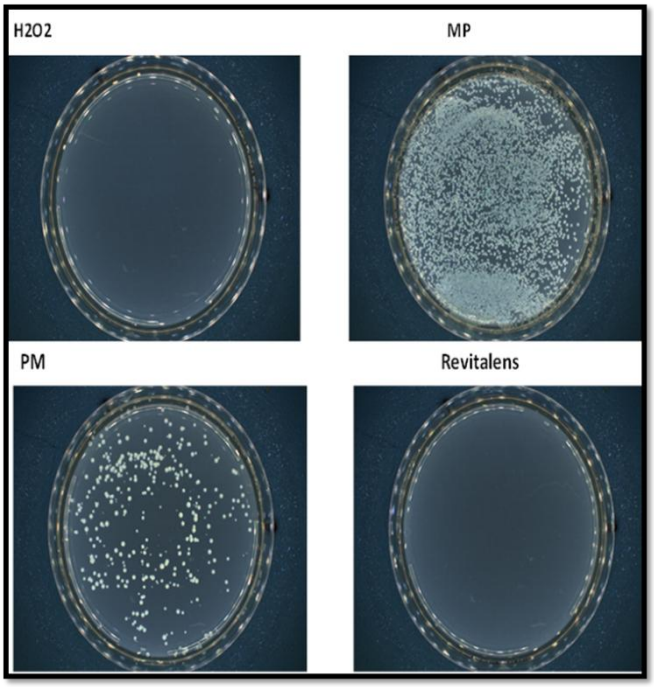

Fig. 12: Biofilm model in Stenotrophomonas maltophilia (ALC-01) resistant action of contact lens solution and grow after 24 hours.

The establishment and assessment of biofilm formation

The method for assessing biofilm formation and quantification involved the use of various disinfectant solutions, with crystal violet serving as an indicator. The absorbance measured correlates with the concentration of eluted crystal violet, which indicates biofilm formation. In the case of the 3AS strain, Menicare Plus and hydrogen peroxide emerged as the primary solutions for evaluating biofilm development (fig.13). A similar outcome was observed for the ALC-01 strain, except phosphate buffer, which functioned as a negative control (fig. 15). Conversely, the Revitalens and hydrogen peroxide solution exhibited the highest absorbance in the AH-2A strain (fig. 14).

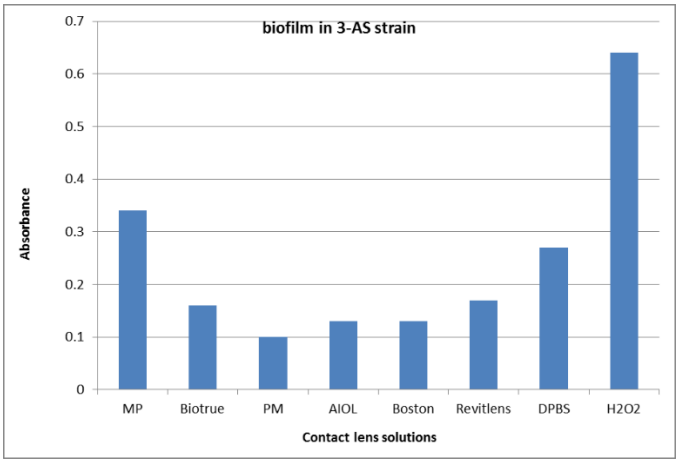

Fig. 13: Biofilm testing of Elizabethkingia meningoseptica (3AS), in the presence of different contact lens solutions using biofilm formation and quantification method.

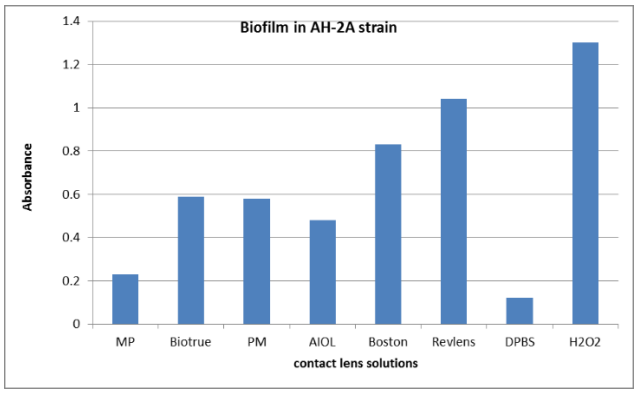

Fig. 14: Biofilm testing of Achromobacter (AH-2A), in presence of different contact lens solution using biofilm formation and quantification method.

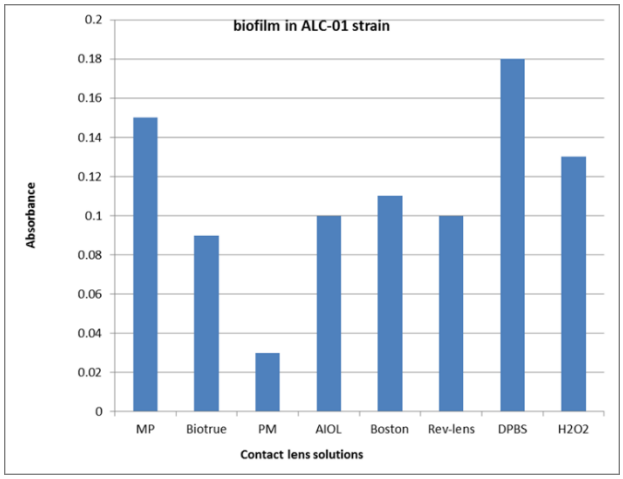

Fig 15: Biofilm testing of Stenotrophomonas maltophilia (ALC-01), in the presence of different contact lens solutions using biofilm formation and quantification method.

DISCUSSION

Contact lenses, along with their corresponding solutions and storage cases, can serve as a medium for significant amounts of both commensal and pathogenic microorganisms to reach the corneal surfaces. Nevertheless, the ocular surface is capable of accommodating and managing these microorganisms under optimal conditions. Conversely, in adverse situations such as injury (trauma) or illness, the use of contact lenses can facilitate the adherence of microorganisms, leading to their colonization of the cornea or conjunctiva, which may result in inflammation and infection [21].

The vast majority of microorganisms inhabiting Earth can be found in communities called biofilms [20]. In this preliminary investigation established and conducted two models to explore the effects of contact lens solutions on bacterial strains that could form biofilms when exposed to disinfectants, which are often present as contaminants in contact lens cases. Bacteria exhibit various properties related to biofilm formation, including their ability to withstand antibiotics, disinfectants, and challenging environmental conditions.

In this study, three different models are utilized: the biofilm model, planktonic model, and aging model. The species under investigation include Stenotrophomonas maltophilia ALC-01, Elizabethkingia meningoseptica 3AS, and Achromobacter AH-2A. Various commercial contact lens solutions are tested as disinfectants, namely All in One Light (AIOL), Biotrue, Boston, Menicare Plus (MP), Pure Moist (PM), Revitalens, and H2O2. Phosphate buffer saline (DPBS) is employed as a control. The findings of this study indicate that ALC-01, 3AS, and AH-2A have a pronounced capability for biofilm formation in the presence of contact lens solution, although with varying levels of adhesion.

Understanding the dynamics of bacterial biofilm formation may contribute to the development of improved antimicrobial approaches for the control and treatment of ocular infections. In the same context, the hydrogen peroxide solution presents strong disinfection abilities and is compatible with multiple contact lens types, suggesting its potential as a beneficial disinfectant for enhancing lens care and visual clarity. This investigation is in agreement with a more recent study by Akram et al. (2025), which focused on the possible actions of hydrogen peroxide solution [23]. Moreover, Studies conducted recently have demonstrated the benefits of hydrogen peroxide in the care of contact lenses, particularly in terms of comfort, user compliance, and disinfection performance. As practitioners update their recommendations, hydrogen peroxide is gaining popularity among contact lens users, especially those with particular concerns such as dry eye or sensitivity to preservatives [23-25]. Although multipurpose solution systems offer convenience, they also come with several potential drawbacks that could influence patient outcomes. These drawbacks include the use of chemical preservatives for disinfection, concerns with biocompatibility, and challenges in maintaining lens care compliance. On the other hand, one-step H2O2 systems, with their specific composition and action mechanism, present an opportunity to mitigate many of the problems associated with multipurpose solutions [26].

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