ORIGINAL ARTICLE

Microbial Contamination and Biofilm Formation in Ophthalmic Solutions and Ophthalmic Instruments at Optometry Practice

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ABSTRACT

Background: Maintaining sterility and preventing microbial contamination are critical in optometry, where multiple surfaces, devices, and solutions contact the patient's eyes, posing an infection risk. Contamination, especially from biofilm-forming pathogens, can occur from airborne droplets, surface contact, and improper disinfection. This study investigates microbial contamination and biofilm formation in solutions and on the surface of ophthalmic instruments commonly used in optometry practices. **Methodology:** Samples were collected from a university-affiliated optometry practice deemed a centre for clinical practice, teaching, and research. Samples were obtained from the dropper tip's ophthalmic bottles and the bulk solution, repeated for both newly opened bottles and after one month of use. One-time samples from various ophthalmic instruments (slit lamps, trial frames, trial lenses, and occluders) were also collected after clinical usage. Contact lens containers were also sampled from the outer edge of the bottles. All samples were placed on Congo Red Agar (CRA) for microbial analysis. **Results:** Microbial contamination was observed from the dropper tips of newly opened bottles but not in the solutions. After one month of use, microbial contamination increased from dropper tips but remained absent in solutions. No biofilm formation was recorded before and after one month of use. Ophthalmic instruments exhibited substantial contamination after use, with some showing biofilm formation. Contact lens containers showed contamination without biofilm formation. **Conclusion:** This study shows bacterial presence on the ophthalmic instruments and solution packaging used in the study location. The most common contamination occurs at the dropper tip while the solution remains pristine. Microbial biofilm observed on ophthalmic tools underscores the importance of diligent sanitation procedures for optometrists.

INTRODUCTION

Maintaining sterility and preventing microbial contamination are critical concerns in optometry, where practitioners routinely handle delicate ophthalmic instruments and administer various solutions to patients. The numerous surfaces, devices, and solutions that come into contact with a patient's eyes and mucous membranes create ample opportunities for transmitting pathogenic microorganisms. Contamination can arise through direct contact between individuals and contact with contaminated objects or surfaces (Lau et al., 2024). Microbial transmission may occur via airborne droplets, surface contact, and improper disinfection practices (Lian et al., 2017). Consequently, microbial contamination that produces biofilms can lead to ocular infections, exacerbating existing conditions and compromising patient well-being (Kyei et al., 2019).

Several studies have investigated the prevalence of microbial contamination in ophthalmic instruments and solutions used in clinical settings (Mohapatra, 2017; Tsegaw et al., 2017; Rutala & Weber, 2016). These findings highlight the need for robust decontamination protocols and adherence to best practices to minimise healthcare-associated infection risks. Contamination in ophthalmic solutions risks infection transmission and diminishes the quality and stability of these solutions, undermining treatment efficacy (Noor et al., 2015). A study assessing the sterility of opened multi-dose ophthalmic medications found that 50% of containers tested positive for bacterial or fungal contamination (Tamer et al., 1994). This underscores the importance of properly labelling and storing opened multi-dose containers to maintain sterility (Tamer et al., 1994).

Decontaminating ophthalmic instruments is crucial, as they can quickly become contaminated when used on patients' eyes or mucous membranes (Mohapatra, 2017; Rutala & Weber, 2016). Contaminated medical devices have been linked to outbreaks and infections within healthcare settings, emphasising the need for rigorous

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sterilisation and disinfection protocols (Infectious Control Unit, 2019). Inadequate cleaning can lead to healthcare-associated infections (Graham et al., 2008). Thus, microbial contamination in ophthalmic instruments and solutions necessitates strict adherence to best practices for patient safety (Hart et al., 2021). Nonetheless, an established disinfection guideline for high-risk ophthalmic instruments remains elusive, leading clinics to create protocols based on limited evidence and manufacturer advice (Dart et al., 1995).

Although studies on microbial contamination are prevalent, biofilm formation is often overlooked. Biofilm-producing microorganisms are particularly concerning due to their heightened resistance to antimicrobial agents, including antibiotics and disinfectants (Navon-Venezia et al., 2017; Khatoon et al., 2018; Lajhar et al., 2018). Biofilms consist of microbial communities that adhere to surfaces and form protective matrices, shielding them from environmental stresses and the immune system (Shree et al., 2023; Muhammad et al., 2020; Gunn et al., 2016). This facilitates chronic infections and complicates treatment, as biofilm-associated bacteria are significantly more resistant than their planktonic counterparts (Sahoo & Meshram, 2024). Addressing biofilm contamination is essential to improve health outcomes in optometry practices, necessitating targeted strategies for monitoring and controlling biofilm formation in instruments and solutions.

This study investigated the direct contamination of solutions and containers usually used in optometry practices and the indirect contamination of ophthalmic instruments commonly used in optometry practices, particularly the presence of biofilm producers.

MATERIALS AND METHODS

Ophthalmic solution sampling

Samples were obtained from seven bottles of ophthalmic solutions: normal saline solutions (sodium chloride 0.9%, 500ml bottle; Bottle-1, Bottle-2, Bottle-3), cycloplegic agent (cyclopentolate hydrochloride 1% with benzalkonium chloride 0.01% preservative, 15ml bottle; Bottle-4) and anesthetic agent (proparacaine hydrochloride 0.5% with benzalkonium chloride 0.01% preservative, 15ml bottle; Bottle-5, Bottle-6, Bottle-7). Due to a manufacturing issue that led to supply shortages, samples were taken from only one bottle of cycloplegic agent.

Samples were taken from the dropper tip (Figure 1) and the solution inside the bottles. Samples from the dropper tips were taken using damp sterile swab sticks (sticks were dipped into sterile distilled water). A damp swab stick enhances the attachment of bacteria compared to a dry swab stick (Pichon et al., 2019). Samples from the dropper tips were placed in sterile plastic containers before laboratory analysis.

Ophthalmic solutions were sampled directly by placing a drop on Congo Red Agar (CRA). To avoid contamination from tips to solutions, each dropper tip was decontaminated using an alcohol swab (70% isopropyl alcohol) before sampling the solution. Care was taken to ensure the alcohol did not enter bottles. The decontaminated tip was left to dry for one minute before sampling.

Sampling was repeated twice: on a newly opened bottle and again one month after the opening date. The usage of the ophthalmic solutions was recorded. The sampling of each ophthalmic solution was triplicated to enhance the validity of the test.

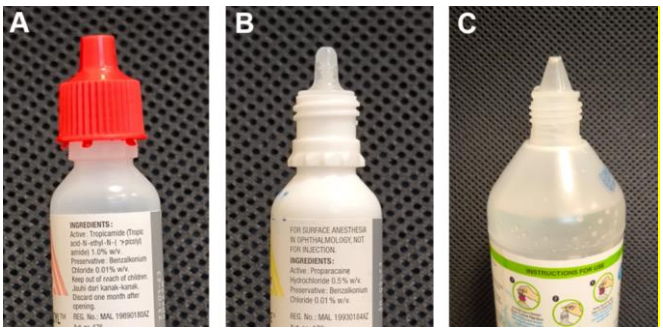

Figure 1: The bottle's tips: (A) shows a 15ml capped bottle of a cycloplegic agent, (B) shows a 15ml uncapped bottle of an anesthetic agent, and (C) shows a 500ml uncapped normal saline bottle. Samples were taken from uncapped bottle tips

Ophthalmic instruments sampling

Ophthalmic instruments from three optometry cubicles (Cubicle-1, Cubicle-2, Cubicle-3) in a university-affiliated optometry practice were chosen for sampling. Samples were collected immediately after a clinic session using the same procedure in sampling ophthalmic solution dropper tips elaborated previously. Samples were taken on slit lamps (SL) in three areas, including the joystick, headrest, and chin rest. Samples were also taken on trial frames, trial lenses, and occluders.

Contact lens (CL) container sampling

Six containers of CL were sampled. The CLs were randomly selected from the available CLs in the optometry practice. Samples were taken from the outer edge of the contact lens bottles. The technique used for collecting the samples

was the same as that used for collecting samples from the dropper tips.

Microbial analysis

Preparation of Congo Red Agar (CRA) plate

The CRA was utilised to differentiate biofilm-producing bacteria while also indicating the presence of contamination by non-biofilm producers. Biofilm is observed as black colonies on CRA, whereas non-biofilm is observed as red colonies (Melo et al., 2013). In preparing the CRA, a mixture of Brain Heart Infusion Agar (23.5g), Agar Technical No.2 (5.0g), glucose (2.5g), and Congo red dye (0.4g) were infused into 500ml of distilled water. The mixture was then shaken thoroughly and sterilised in the autoclave machine at 121°C for 15 minutes. The mixture was then poured into Petri dishes and solidified at room temperature. Two plates from each manufacturing batch were placed in the incubator overnight as control plates to ensure no contamination, while the remaining plates were stored at 2°C to 8°C.

Bacterial isolates on CRA plate

Samples from swabs were streaked on the CRA plates. Ophthalmic solution samples directly placed on the CRA plates were streaked using an inoculating loop strictly under aseptic conditions, using the quadratic streaking technique (Tantray et al., 2023). The cultures were then incubated in 5% carbon dioxide (CO2) at 37ºC for at least three days. They were monitored daily until day five of incubation for bacterial growth and the presence of black colonies. Streptococcus mutans, a biofilm producer, was used as the positive control strain.

RESULTS

Samples from unused (newly opened) ophthalmic solutions showed that two dropper tips were contaminated. The contamination was found at the dropper tip of the normal saline Bottle-3 and anesthetic agent Bottle-6. No biofilm was observed from the contaminated dropper tip samples. No contamination was recorded in all ophthalmic solutions (Table 1). Taking the negative contamination in all solutions, they were deemed safe for study after ensuring proper disinfection of the dropper tip.

Table 2 lists the usage of various ophthalmic solutions in one month, with an average of 10.4 usage per month. Microbial contaminations were observed after one month of usage on the dropper tip of normal saline Bottle-2 and -3 (66% of samples), cycloplegic agent Bottle-4 (100%), and anesthetic agent Bottle-6 and -7 (66%) (Table 3). No biofilm was observed from the contaminated dropper tip samples. None of the solutions, however, were observed to have positive contamination.

Table 1: Baseline data of newly opened bottles of various ophthalmic solutions. Samples were taken and analysed from the dropper tips and ophthalmic solution contents

Ophthalmic solution	DTC	BPT	SC	BPS
Normal saline (3 samples)	Bottle-3	-ve	ND	-ve
Cycloplegia (1 sample)	ND	-ve	ND	-ve
Anesthesia **(3 samples)**	Bottle-6	-ve	ND	-ve

DTC: Dropper Tip Contamination
BPT: Biofilm Presence from Tip
SC: Solution Contamination
BPS: Biofilm Presence from Solution
-ve: Negative
ND: Not Detected

Table 2: The usage record of various ophthalmic solutions in one month

Bottle Number	Number of usage
Normal Saline Bottle-1	11
Normal Saline Bottle-2	12
Normal Saline Bottle-3	12
Cycloplegic Agent Bottle-4	7
Anesthetic Agent Bottle-5	10
Anesthetic Agent Bottle-6	10
Anesthetic Agent Bottle-7	11

Table 3: Bacterial contamination of various ophthalmic solutions after one month of open bottle. Samples were taken and analysed from the dropper tips and ophthalmic solution contents

Ophthalmic solution	DTC	BPT	SC	BPS
Normal saline (3 samples)	Bottle-2, Bottle-3	-ve	ND	-ve
Cycloplegia (1 sample)	Bottle-4	-ve	ND	-ve
Anesthesia **(3 samples)**	2 (67%) Bottle-6, Bottle-7	-ve	ND	-ve

DTC: Dropper Tip Contamination
BPT: Biofilm Presence from Tip
SC: Solution Contamination
BPS: Biofilm Presence from Solution
-ve: Negative
ND: Not Detected

The ophthalmic instruments observed various contaminations after usage (Table 4). The trial frames in all optometry cubicles were contaminated, but there were no observable biofilms. The trial lens in Cubicle-2 was deemed contaminated, but no biofilm was presented. The occluders in Cubicle-1 and -2 were observed to have

bacterial contamination, with Cubicle-2 showing the formation of biofilms. Contaminations were positive from various parts of SL: Cubicle-1 and -3 on SL's joystick, Cubicle-2 and -3 on SL's headrest, and Cubicle-1 and -2 on SL's chinrest. Biofilm was found on the SL's headrest of Cubicle-2.

Two CL containers were deemed contaminated (Table 5). No biofilm was presented from the contaminated CL containers.

Table 4: Bacterial contamination of various ophthalmic instruments from three optometry cubicles after a clinical session

Sampling area	Location of contamination	Biofilm presence
Slit lamp's joystick	Cubicle-1, -3	-ve
Slit lamp's headrest	Cubicle-2, -3	Cubicle-2
Slit lamp's chin rest	Cubicle-1, -2	-ve
Trial frame	Cubicle-1, -2, -3	-ve
Trial lenses	Cubicle-2	-ve
Occluders	Cubicle-1, -2	Cubicle-2

-ve: Negative

Table 5: Bacterial contamination of contact lens containers

No. of contact lens containers tested	Contaminated	Biofilm
6	2	-ve

-ve: Negative

DISCUSSION

The issue of microbial contamination in optometry practices is a significant concern that warrants careful consideration. Ophthalmic solutions, such as diagnostic agents, eye drops, and other topical medications, can serve as potential vectors for the transmission of harmful microorganisms, posing a risk to patients' ocular health and overall well-being (Zilliox et al., 2020; Chua et al., 2021; Kyei et al., 2019). This study investigated three types of ophthalmic solutions, including normal saline, cycloplegic, and anesthetic agents, considering their frequent usage in a typical optometry practice. Both cycloplegic and anesthetic agents contain benzalkonium chloride 0.01% as a preservative, while the normal saline was deemed preservative-free. Preservatives in ophthalmic solutions help maintain sterility and prevent microbial contamination, especially in multi-dose solutions, which are susceptible to repeated use. Preservatives help to inhibit bacteria and other microorganisms, ensuring the product's safety and efficacy (Chua et al., 2021; Bachewar et al., 2018). The current study found no contamination at baseline or after one month of use. This was also true for preservative-free normal saline. However, some studies have questioned the efficacy of certain preservatives in ophthalmic solutions, suggesting the need for better methods to eliminate microbial contamination (Jayant & Halami, 2020).

Microbial contaminations in this current study were spawned from the dropper tip without the formation of biofilm. Contamination was also detected on dropper tips in an unused normal saline and anesthetic agent without compromising the solution. A similar observation was reported by Tsegaw et al. (2017), who observed that 11% of their samples were contaminated at the dropper tip without compromising the residual content. They even found contaminations in samples used for less than seven days. Nevertheless, solutions of more than seven days dominated the reported incidence. In contrast, a study by Chua et al. (2021) reported an average contamination rate of 25% from the dropper tip, 17% in residual content, and 8% of both dropper tip and residual content over 14 and 30 days of preserved ophthalmic drugs (POD) usage. Interestingly, Chua et al. (2021) also reported contamination in nine unused PODs they tested, similar to the current findings of bottle tip contamination on one unused normal saline and anesthetic agent. To reverberate, the current study also observed contamination of CL storage containers. Unfortunately, the solution residue in the containers was not sampled to provide a more conclusive finding.

Existing research has identified several key risk factors for microbial contamination of ophthalmic solutions. Certain therapeutic classes, such as steroid-containing anti-inflammatory solutions, appear more susceptible to contamination than others (Zilliox et al., 2020; Chua et al., 2021). The duration of product use is also a critical factor, with more extended periods of use increasing the likelihood of contamination (Chua et al., 2021). Additionally, the physical appearance of the bottle, such as signs of tampering or cloudiness, can serve as visual cues for potential contamination.

The sources of contamination can arise from various routes, including improper handling by optometrists, inadequate disinfection of equipment and surfaces, and even the intrinsic formulation of the solutions themselves (Chua et al., 2021). The impact of such contamination on patient health can be severe, leading to the development of ocular infections, corneal ulcers, and other sight-threatening complications (Zilliox et al., 2020; Kyei et al.,

2019). Certain microorganisms, such as Gram-negative rod bacteria and Micrococcus species, have been frequently isolated from contaminated ophthalmic solutions, underscoring the potential for serious clinical consequences (Chua et al., 2021).

The current study observed substantial contaminations of the ophthalmic tools in all tested cubicles. All trial frames were deemed contaminated (100% contamination rate) where inoculation occurred after a clinical session, emphasising the need for thorough disinfecting procedures in optometry practices. Viegas et al. (2017) reported that the trial lens was the most contaminated item in their study location. In the current study, a similar observation was made for occluders, with one occluder contamination developing biofilm. A typical disinfection norm after a clinical session focuses on surfaces in contact with patients, and smaller optometry paraphernalia such as trial frames, trial lenses, and occluders may have been neglected. Sivaraj et al. (2004) tested contamination on non-contact handheld lenses and reported an 81% contamination rate, mostly from skin flora. They tested the same lenses after cleaning with detergent, which saw a reduction of contamination rate to 15%. They recommended that regular lens cleaning should be conducted to reduce the risk of cross-infection.

Moosavi et al. (2005) conducted an analysis of various SL components in an emergency room and outpatient clinic of a hospital. They reported that microbial contamination on SLs increases with usage during clinical sessions. They recommended disinfection of SLs prior to use to eliminate potential machine-patient cross-infection. The same study showed a contamination rate of 52.9% for the headrest, 70.5% for the chinrest, and 17.6% for the transformer switches. In another study, where samples were collected from a SL's headrest and joystick, Sobolewska et al. (2018) reported a contamination rate of 65% on their samples. These findings suggest that SLs are a potential source for the transmission of microorganisms. The same observation was found in the current study, where microbial biofilm was identified from the headrest. This underscores the importance of sanitisation procedures, in which vigorous cleaning using alcohol swabs eliminates bacterial contamination (Graham et al., 2008).

Biofilm is a group of bacteria adhering to surfaces and bound together by a matrix called extracellular polymeric substances (EPS), protecting the bacteria against external factors (Muhammad et al., 2020; Gunn et al., 2016). Over time, the EPS matrix strengthens cell adhesion and cohesion, resulting in a densely packed and firmly attached biofilm. Once formed, biofilms become highly resistant to removal or eradication (Zheng et al., 2021). In its early stages, a biofilm is typically invisible to the naked eye because it consists of a thin layer with minimal microorganisms embedded in the EPS matrix. As the biofilm matures, it becomes more noticeable, often appearing as a slimy film on the surface (Ben-Ari, 1999; Sauer, 2017). The development of biofilms plays a crucial role in the survival of microorganisms by facilitating bacterial growth and serving as a protective barrier, shielding the implanted microorganisms from environmental hazards and antimicrobial treatments (Lebeaux et al., 2014). The ability of certain bacterial species to adhere to various fomite surfaces, including ophthalmic equipment, plays a critical role in contamination. For example, the hydrophobic surface properties of Pseudomonas aeruginosa enhance its tendency to adhere to contact lenses. As a well-known biofilm producer, *Pseudomonas aeruginosa* contamination on contact lenses can significantly increase users' risk of biofilm infections (Bruinsma et al., 2001).

Although the current study observed minimal biofilm formation on contaminated apparatus and solutions in ophthalmic settings, the presence of microbial contaminants, especially species with a high biofilm-forming capacity, can still pose a risk of biofilm-related infections with prolonged exposure. To mitigate the risks associated with microbial contamination, particularly biofilm-producers, optometry practices must implement robust infection control measures, adhere to best practices in handling and administering ophthalmic solutions (Chua et al., 2021), and establish rigorous disinfection protocols for all reusable equipment. This includes frequently cleaning and sterilising instruments between patients using appropriate disinfectants and following manufacturer guidelines. Proper tool handling and storage also help prevent the spread of pathogens and reduce cross-contamination risks. Comprehensive disinfection practices ensure patient safety, especially for those with compromised immune systems.

Limitation

Sample contamination can occur when external substances, such as microorganisms, chemicals, or particles, accidentally enter the sample (Group et al., 2023). These particles can be transmitted through the air or by cross-contamination. To reduce the possibility of airborne cross-contamination, the sample swabbing method onto the CRA was conducted near the Bunsen burner (Bykowski & Stevenson, 2020). However, cross-contamination may occur when handling equipment and samples or when using gloves on the samples.

CONCLUSION

This study shows bacterial presence on the ophthalmic instruments and solution packaging used in the study location. The most common contamination occurs at the dropper tip while the solution remains pristine. Microbial biofilm observed on ophthalmic tools underscores the importance of diligent sanitation procedures for optometrists. The finding highlights the potential of microbial contamination on various ophthalmic solutions and instruments, particularly after extended use. The findings implicate the importance of regular cleaning and sterilisation, adherence to best cleaning practices, and appropriate storage of solutions, which are essential to ensure patient safety and mitigate the risk of infections.

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