Risks, Identification, and Antibiotic Susceptibility Against High Prevalence of Bacterial Infection in Contact Lens Solutions

Rana S. Hasan [1], Sawsan M. Alomari [1], Roaa A. Thamer [1], Mohammed Fadhil Haddad [1*]

Abstract

Background: Contact lens wear has become increasingly common for vision correction and cosmetic purposes. However, it also causes risks of microbial contamination leading to ocular infections. The aim of our study was to investigate the bacterial contamination in commercial contact lens solutions. Method: A total of 33 samples were collected and analyzed for bacterial presence using both phenotypic and molecular methods. Phenotypic diagnosis involved culturing samples on specific media and utilizing the Vitik system. Results: Our results showed the presence of various bacterial groups in contact lens preservation solutions, with Pseudomonas aeruginosa and Staphylococcus epidermidis being the most prevalent at 27.2% and 21.2%, respectively. The highest percentage of infections occurred in the age range of 12-20 years (41%), followed by 21-30 years (22%), 31-40 years (19%), 41-50 years (13%), and 51-60 years (5%). Regarding education level, individuals with primary education showed a higher percentage of eye infections (63%) compared to those with secondary education (27%) or higher education (10%). A higher percentage of infections was observed among individuals wearing lenses for 24 hours (57%) compared to those wearing them for 12, 6, or 1 hour, at 22%, 14%, and 7% respectively. Conclusion: In conclusion, the study demonstrated the importance of adherence to proper lens care practices to improve lens safety and reduce microbial contamination. Effective measures are essential to mitigate the risks associated with contact lens wear and maintain optimal eye health.

Keywords: Pseudomonas, Staphylococcus, Contact lenses, microbial contamination, antibiotic susceptibility, eye infections, disinfection solutions.

Significance | This study determiend the microbial contamination in contact lens solutions for preventing eye infections and improving lens safety practices.

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Introduction

The contact lens is an artificial device used to correct refractive errors, with its front surface serving as a substitute, particularly for the cornea. Nowadays, wearing lenses is increasingly common, catering to various needs such as correcting nearsightedness, cosmetic enhancements, and repairing refractive errors. Initially developed for patients with corneal and iris distortions, contact lenses, also known as circular decorative lenses, have evolved to enhance a person's appearance (Abadi et al., 2021). Contact lenses come in different types, including rigid gas-permeable lenses, soft lenses, and hard lenses, distinguished by the materials used in their production (Abdelkader A et al., 2014). Although invented in 1887, contact lenses weren't widely adopted until 1938 (Abid et al., 2014). Prolonged lens wear can induce changes in the cornea, exacerbating pre-existing conditions and giving rise to various issues. These issues stem from factors such as the type of lenses used, their frequency of replacement, the effectiveness of lens cleaning, and

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individual wearer characteristics (Bourne, 2001). Ocular infections associated with contact lens use encompass a range of conditions, including orbital cellulitis, conjunctivitis, keratitis, endophthalmitis, blepharitis, stye, and dacryocystitis, manifesting in symptoms like redness, pain, discharge, watery eyes, and dryness (Alasadi et al., 2022). Contact lens wear poses a risk for eye diseases by introducing pathogens, leading to microbial adherence and multiplication, potentially resulting in infectious keratitis (Janabi et al., 2013). Corneal hypoxia, a consequence of wearing contacts, compromises epithelial integrity, providing an entry point for microorganisms (Al-Mujain, 2020). Improper lens usage, including handling lenses with unwashed hands, is among the significant risk factors for eye diseases (Al-Shimmary, 2021). Microbial contamination of lenses, including fungi, bacteria, and viruses, exposes the cornea and conjunctiva to infections (Altaa et al., 2014). Microorganisms can colonize the eye, potentially leading to viral, fungal, and bacterial diseases and infections. The manifestation of symptoms and the development of infection depend on the specific pathogen and its ability to cause disease, as the pathogenicity varies among microorganisms (Ashurst et al., 2023). Bacteria are considered a significant risk factor for ocular infections worldwide. Infections can be mono- or polymicrobial and are associated with various factors such as contact trauma, surgery, lens usage, dry eye conditions, age, and previous ocular infections (Aso et al., 2017).

Therefore, according to *Wu et al.* (2010) and *Dantam et al.* (2016), CLs serve as a vector for commensal (Resident) and transient potential microorganisms to adhere to and transfer to the ocular surface, resulting in inflammation or infection. Several studies were conducted on CL cases, solutions and lenses to identify contamination. *M.O* which was traceable to users' dirty hands, or the tap water used to rinse the lens storage cases, and/or air contamination during drying of the cases. *Coagulase-positive Staphylococci (CoPS)*, *coagulase-negative staphylococci (CoNs)*, *Pseudomonas aeruginosa*, *Streptococcus spp.*, *Escherichia coli* and *Klebsiella spp* and *Serratia spp*. These were the most common species identified (*Wu et al.*, 2011; *Mohamed et al.*, 2017). Aerobic Gram-positive (*Gm+ve*) commensal bacteria, *Staphylococcus*, is commonly found on the hands, face, nose, and skin. It can easily enter the eye and is carried by 50–60% of the general population. Thus, hand-to-eye transmission is most likely the cause of Staphylococcal ocular infection (*Jalbert et al.*, 2000). *Pseudomonas* is an opportunistic pathogen that can thrive in diluted disinfectant solutions and is frequently found in various habitats, including water (*Willcox*, 2007). It has very little nutritional requirements. *P. aeruginosa* keratitis is linked to contact lenses and is challenging to treat due to the possibility of multiple antibiotic resistance (*Chalita et al.*, 2014). Many research have asserted the significance of methicillin-resistant *CoPS* or *CoNS* in ocular infections (*Melton et al.*, 2010).

Research conducted by Nzeako and Al-Sumri (2011) revealed that contact lens disinfecting solutions produced by different companies, despite having identical compositions, exhibited varying disinfection potentials. Furthermore, findings from Lakkis and Fleiszig (2001) indicated that disinfection solutions exhibited selectivity against *Pseudomonas aeruginosa* contamination caused by cytotoxic strains. However, research by Dantam et al. (2014) demonstrated that the use of different formulas of contact lens care solutions influenced the level of microbiological contamination in storage cases.

An effective approach for surface cleaning and sterilization is necessary for contact lenses. Disinfecting care products, including cleansing, disinfecting, moisturizing, and reducing tear agents, have been enhanced to become more effective (Szczotka-Flynn et al., 2010). When bacterial biofilm forms, it becomes immune to the antimicrobial action of disinfectants, even when they are included in solutions (Wu et al., 2010). The importance of CL wearers' adherence to washing and disinfection procedures is indicated by bacterial resistance to preservatives (Mayo et al., 1987). The research by Dantam et al. (2014) mandated that CL wearers use their lenses daily and replace them regularly. Each group employed one of the four types of CL care solutions for two weeks. Regardless of the treatment used, contamination was identified in 80% of CL cases. CL cases kept in 0.00013% polyaminopropyl biguanide (PAPB) and 0.0001% polyquaternium solution were compared to those maintained in disinfection solution containing 0.001% polyquaternium-1 and 0.0006% myristamidopropyl dimethylamine. Solutions containing 3% hydrogen peroxide and 0.79% NaCl showed significantly higher contamination levels in CL patients compared to solutions containing 0.0003% Polyquaternium-1 and 0.00016% Alexidine.

A biofilm is a community of bacterial cells that adhere to one another, to solid surfaces, or to tissues in an irreversible manner. It comprises a matrix of polymeric substances (Donlan et al., 2002). Biofilms were not linked to infection until the 1970s, when mucoid strains of *Pseudomonas aeruginosa* were found in individuals with chronic cystic fibrosis, according to Høiby et al. (1973). Following that discovery, bacterial biofilms were associated with several infectious disorders. Biofilm-forming microorganisms are incredibly resistant to antibiotics and may grow on a variety of medical equipment types (Costerton et al., 1999).

The capacity of three distinct kinds of contact lens (CL) solutions to suppress bacterial biofilms was examined by Artini et al. (2015). Oxychlorite, polyaminopropyl biguanide (PAPB), polyquad, and aldox are present in these three distinct CL solutions as disinfection agents. After four hours, all CL treatments may prevent *Serratia marcescens and Staphylococcus species* from forming biofilms and decrease the production of Pseudomonas biofilm.

The current study aimed to screen for contamination of microbes found in commercial lens solution and factors that affect the increase in eye infections in Mosul city. Recently, many techniques for identifying bacteria have been tested, with molecular methods such as polymerase chain reaction (PCR) being broadly applied to detect and characterize microorganisms. Sequence analysis of the 16S rRNA accurately identifies unknown bacteria to the genus level, especially in the classification of bacterial species (Al-Shimmary et al., 2021).

Materials and methods

Samples

Samples were collected through the period from January until March 2023. Samples were taken with sterile cotton swabs from users that appeared of them eye inflammation whose used contact lenses with ages ranged from 12 to 60 years old. All practical experiments were processed and completed in microbiology laboratory which is equipped with devices, instruments, culture media, chemicals, glassware and materials necessary for researchers.

All participants signed the informed consent without any obligation. The informed consent explains study objective, procedures applied to the samples and enough information to make an informed decision. Participant's inquiries were answered and clarified. Contact lens wearers included in the study aged >12 years and currently wearing CLs. All of them use either long-lasting lenses or daily use lenses. They were not taking any antibiotic nor eye medications (Mohamed et al., 2017) and no one was suffering from any eye disease, inflammation or infection at the time of sampling. Samples were taken from right and left CL cases (CL storage cases), disinfectant solution bottles, and mouth rims of solution bottles.

Ethical approval to conduct the study was obtained from the Ethical committee at Mosul Technical Institute

Sampling

From each contact lens unit used by the individual, 33 samples were obtained. These comprised the following solutions: (1) Disinfectant solution from its original bottle; (2) Right and Left Contact Lens Cases (RCLC and LCLC, respectively); and (3) Mouth Rim Swabbing of Disinfectant Solution Bottle.

In order to counteract the disinfectant's effects (Kelsey, 1974; Dennyer et al., 2008), 0.5 ml of solutions from each RCLC, LCLC, SB, and swabs were suspended in 4.5 ml of Trypticase Soy Broth (TSB, biolab). This mixture was then incubated at 35 °C for 1-2 hours in order to allow stressed microbial cells to recover. Using a sterile L-shaped solid glass rod (dipped in spirit and flamed), duplicate Trypticase Soy Agar (TSA, biolab) plates were covered with two-fold dilutions of each sample (100 µL and 50 µL). For 24 to 48 hours, plates were incubated at 35 °C.

Additionally, loopfuls of the four sources' TSB inoculums were inoculated onto the subsequent selective and differential media. These are Sabouraud dextrose agar (SDA biolab), Mannitol Salt Agar (MSA, biolab), and MacConkey's (MAC) agar (Scharlau). Then incubated for 24 to 48 hours at 35 °C. In order to determine if growth was there or not, SDA plates were incubated for at least one week. Colony Counter (WTW, Keimzählgerät BZG 28) was used to count isolated colonies grown on TSA in order to determine the initial CFU in each solution (Denyer et al., 2008).

On Nutrient Agar (NA, Scharlau) plates, colonies were purified before being Gram stained. For use in identification research, pure colonies were also cultivated on nutrient agar slants and refrigerated. According to their development response in these media, colonies produced on selective and differential media were also initially described and recognized (Subhash, 2012). The AUTOCLAVI DA PAVIMENTO ATV80 gadget was used for all autoclaving procedures.

Identification of Microorganisms

Culture media: Characteristics of grown bacterial colonies were determined by examining colonial morphology on TSA. These include size, edge, elevation, consistency and pigmentation (Subhash, 2012).

Staining: According to Barrow and Feltham (1993), the initial genus-level identification of isolates was performed by analyzing the growth characteristics of colonies on differential medium, as well as by using Gram stain for bacteria and simple stain for yeast.

Biochemical Identification: The following biochemical examinations are carried out to verify colony identification at the generic level. The production of catalase, oxidase, and deoxyribonuclease (Dnase) is one of these. Other abilities include the ability to perform the mixed acids pathway (Methyl red test), use citrate as a carbon source, use Triple Sugar Iron (TSI, HIMEDIA®) agar with the production (or not) of hydrogen sulfide, and the production of coagulase enzyme (to differentiate between CoNS and CoPS) to enable the conversion of fibrinogen to fibrin (Rakotovao-Ravahatra et al., 2019). To distinguish between S. marcescens and S. liquefaciens species, Serratia Differential Medium (HIMEDIA®, Twin Pack, M1288) was used (Faddin, 1985). S. liquefaciens species relied on their capacity to ferment larabinose and decarboxylate ornithine. For the purpose of identifying Acinetobacter species, HiCrome TM Acinetobacter Agar Base (HIMEDIA, M1938) was utilized.

Antibiotic Susceptibility

Agar Dilution Method: The antibiotic oxacillin was used to test Staphylococcus isolates for methicillin susceptibility or resistance. Using an N4S UV-Vis spectrophotometer, isolates were subcultured on NA for 18 to 24 hours. A small number of colonies

were then moved to TSB and standardized to produce turbidity equivalent to 0.5 McFarland (McF) standard (Cockerill et al., 2013). MSA and Mueller-Hinton Agar (MHA, HIMEDIA®) with 4% NaCl were the two media that were developed. They were allowed to cool to around 50°C after being sterilized. Each medium was combined with 6 µg/ml of oxacillin, gently mixed, and then poured into sterile petri plates (Pillai et al., 2012). A portion of the produced TSB was put onto the MSA and MH agar plates (containing 4% NaCl) together with 6 µg/ml Oxacillin. The plates were then incubated for 48 hours at 33–35°C (Thornsberry and McDougal, 1983, Cockerill et al., 2013). Growth on either medium was noted as indicating methicillin resistance.

Disc Diffusion Method: Clinical and Laboratory Standards Institute (CLSI) 2013 (Patel et al., 2013) guidelines were followed in examining the antibiotic susceptibility of the most common Gram negative and Gram positive bacteria from contaminated CL units using the disc diffusion test. Pure colonies that had grown on TSA for 18 to 24 hours were subcultured on TSB to create suspensions that were equal to 0.5 McFarland (as stated above). Bacterial solutions were equally distributed and inoculated onto MHA using sterile cotton brushes. The antibiotic disc dispenser (Oxoid) was used to evenly space the antibiotic discs on MHA plates after the plates had been let to dry for five to ten minutes. The plates were then incubated at 35°C for eighteen to twenty-four hours.

The following antibiotics (Oxoid) were tested against gram negative bacteria: ceftriaxone 30 µg, levofloxacin 5 µg, tetracycline 30 µg, ceftazidime 30 µg, cefotaxime 10 µg, and gentamicin 10 µg (Sohail et al., 2016, Carvalheira et al., 2017).

According to Patel et al. (2013), gram-positive samples were tested against penicillin (10 units), amoxicillin-calavulanic acid (30 µg), and erythromycin (15 µg). Millimeter-based measurements of the zones of inhibition were made, and the findings were classified as sensitive or resistant (with intermediate values included with the resistant ones). The data were then interpreted using standard tables derived from the CLSI criteria (Patel et al., 2013). The zone diameter's Standard Deviation (SD) was computed after the test was conducted twice.

Statistics Analysis

Data storage and graph creation were done using Microsoft Excel. SPSS software, version 25, was used for all statistical studies on Windows. A p-value of 0.05 or less was designated as the significance threshold. For continuous variables, participant characteristics were reported using means and standard deviations; for categorical variables, frequencies with percentages were utilized. Using the Chi square test ($\chi 2$), contaminated and noncontaminated samples were compared using a dichotomous variable that represented the isolate status.

Result

Eye-related Health Status

Table (1) demonstrates eye-related health status. Almost two-thirds (63.3%) of participants denied having any pervious eye-related medical conditions/diseases. Out of eleven CL wearers who had a previous eye-related conditions/disease (36.7%), nine received medical examination. Diagnoses included: infection (n=4), inflammation (n=3) and dryness of eyes (n=2), out of these diagnostic cases, microbial contamination was detected at least in one item of CL units belonging to 2, 3 and 2 participants respectively. Reported conjunctivitis and keratitis were treated by antibiotics. None of participants reported active eye infections at the time of the study. Eye redness after wearing CLs is almost significant sign associated with microbial contamination of CL units. All CL wearers, who continuously or intermittently suffered from eyelid boils, have microbial contamination in their CL units.

Identification of microbial contamination

All colonies grown on MAC, MSA, SDA, and TSA were primarily characterized and identified. Sixty-four isolates were obtained from contaminated samples. These included: 60 (93.8%) bacteria and 4 (6.3%) yeasts. Forty-six (71.9%) isolates of bacteria were Gm-ve and the remaining 14 isolates (21.9%) were Gm+ve. Table (2) demonstrates distribution frequency of M.O. in each item of CL units.

Identification of Gram-positive bacteria

Fourteen isolates were Gm+ve bacteria cluster shaped cocci, six of them ferment mannitol when grown on MSA, oxidase negative and produced catalase and coagulase They were identified as CoPS. The six CoPS produced DNase, which is indicative for identification as S. aureus. The 7th mannitol fermenting isolate did not produce coagulase.The other seven cluster shaped cocci were oxidase negative producing catalase but not coagulase, were identified as CoNS (Table 3).

Frequency distribution

Frequency distribution of identified M.O. in CL cases and rims of solution bottles is shown in Table (4). illustrates distribution of identified M.O.

Antibiotics Sensitive

This method was applied for Staphylococcus spp. only, whether CoPS or CoNS to examine their resistance to methicillin. Two (25%) CoNS isolates, one from CL case and the other from the rim of one bottle of the same CLs unit. Both slowed resistance to 6 µg/ml oxacillin, hence termed methicillin resistance (MRCoNS). Two S. aureus from different CL cases also showed resistance to 6 µg/ml oxacillin, thus termed MRSA. (Table 5).

Hygienic Habits Associated with Contact Lens Unit

Few numbers of participants (10.0%) use a special plastic forceps to apply CLs. More than one half of them (56.7%) rinse their CLs with CLs solution, while only one third rub CLs while rinsing them. Rubbing lens with CL solution is a significant sign associated with

Table 1. Eye-related medical problems

Eye complications	Response	N=30 n (%)	Microbial contamination n =25 (%)	*P*-value
Eye medical condition / disease	Yes	11 (36.7%)	9 (81.8%)	0.865
	No	19 (63.3%)	16 (84.2)	
Eye redness after CL wearing	Always/Often	5 (16.6%)	5 (100%)	0.088
	Sometimes	18 (60%)	16 (88.9%)	
	Rarely/Never	7 (23.3%)	4 (57.1%)	
Eyelid boils	Always/Often	2 (6.7%)	2 (100%)	0.401
	Sometimes	5 (16.7%)	5 (100%)	
	Rarely/Never	23 (76.7%)	18 (78.3%)	

Table 2. Microbial contamination of immersion solutions in contact lens cases and Rims of solution bottles.

Microbial contamination	Right cases n = 21 (%)	Left cases n= 17 (%)	Rims n= 6 (%)
Type of contamination: Monomicrobial	12 (57.1%)	11 (64.7%)	6 (100%)
Polymicrobial	9 (42.9%)	6 (35.3%)	-
Colony Forming Unit: < 30 CFU/ml	6 (28.6%)	6 (35.3%)	-
>30-300 CFU/ml	7 (33.3%)	2 (11.8%)	
> 300 CFU/ml	8 (38.1%)	9 (52.9%)	

Table 3. Frequency distribution of microorganisms in contact lens cases and rims of Solution bottles.

No. of Isolates	Right case n=33 (%)	Left case n = 25(%)	Rim n = 6 (%)	Total n = 64 (%)
Gram-negative bacteria	25 (75.8%)	19 (76%)	2 (33.3%)	46 (71.9%)
Gram-positive bacteria	5 (15.2%)	6 (24%)	3 (50%)	14 (21.9%)
Yeast	3 (9.1%)	-	1 (16.7%)	(6.3%)

Table 4. Frequency distribution of microorganisms isolated from indicated contact Lenses units.

Organisms	Right cases n= 33 (%)	Left cases n= 25 (%)	Rim n= 6 (%)	Total N=64
Pseudomonas spp.	6 (18.2%)	5 (20%)	-	11 (17.2%)
S. marcescens	3 (9.1%)	5 (20%)	-	8 (12.5%)
CoNS	3 (9.1%)	2 (8%)	3 (50%)	8 (12.5%)
S. aureus	2 (6.1%)	4 (16%)	-	6 (9.4%)
Pseudomonas aeruginosa	1 (3%)	3 (12%)	1 (16.7%)	5 (7.8%)
Shewanella putrefaciens	2 (6.1%)	-	1 (16.7%)	3 (4.7%)
Burkholderia pseudomallei	1 (3%)	1 (4%)	-	2 (3.1%)
Acinetobacter spp.	1 (3%)	1 (4%)	-	2 (3.1%)
A.calcoaceticus	2 (6.1%)	-	-	2 (3.1%)
Shigella spp.	2 (6.1%)	-	-	2 (3.1%)
S. liquefaciens	1 (3%)	-	-	1 (1.6%)
Salmonella spp	-	1 (4%)	-	1 (1.6%)
E. coli	1 (3%)	-	-	1 (1.6%)
Filamentous bacteria*	1 (3%)	-	-	1 (1.6%)
Short-rod shaped*	1 (3%)	1 (4%)	-	2 (3.1%)
Yeast	3 (9.1%)	-	1(16.7%)	4 (6.3%)
Non-Lactose fermenting**	3 (9.1%)	2 (8%)	-	5 (7.8%)

Table 5. Methicillin Resistant Staphylococcus spp

Antibiotic	Susceptibility	*S. aureus* N= 6	CoNS N= 8
Oxacillin 6 µg/ml	Resistant	2 (33.3%)	2 (25%)
	Sensitive	4 (66.7%)	6 (75%)

Table 6. Duration of used disinfectant solutions and lenses

CLs units	Duration Periods (Month)	N=30 n (%)	Microbial Contamination n =25 (%)	*P*-value
CLs Solution	<1	11 (36.7%)	10 (90.9%)	0.020
	1-3	4 (13.3%)	1 (25%)	
	4-6	2 (6.7%)	2 (100%)	
	7-12	4 (13.3%)	4 (100%)	
	>12	9 (30.0%)	8 (88.9%)	
Contact lenses	<1	3 (10.0%)	2 (66.7%)	0.105
	1-3	3 (10.0%)	3 (100%)	
	4-6	7 (23.3%)	7 (100%)	
	7-12	1 (3.3%)	-	
	>12	16 (53.3%)	13 (81.3%)	

Table 7. Hygienic habits of contact lens wearers' toward contact lenses and solutions.

Hygienic Habits	Responses	N= 30 n (%)	Microbial Contamination n =25 (%)	*P*-value
Using forceps for wearing CLs	Yes	3 (10%)	2 (66.7%)	0.414
	No	27 (90%)	23 (85.2%)	
Rinsing lens with CL solution	Always/ Often	17 (56.7%)	14 (82.4%)	0.603
	Sometimes	9 (30%)	7 (77.8%)	
	Rarely/ Never	4 (13.3%)	4 (100%)	
Rubbing lens with CL solution	Always/ Often	10 (33.3%)	8 (80%)	0.021
	Sometimes	6 (20 %)	3 (50%)	
	Rarely/ Never	14 (46.7%)	14 (100%)	
Using water for CLs storage	Always/ Often	4 (13.8%)	4 (100%)	0.316
	Sometimes	4 (13.8%)	4 (100%)	
	Rarely/ Never	21 (72.4%)	16 (76.2%)	
Duration of adding solution to CL cases	Daily	17 (56.7%)	14 (82.4%)	0.494
	Weakly	8 (26.7%)	6 (75%)	
	Monthly	5 (16.7%)	5 (100%)	
Frequency of washing CL cases	Always/ Often	18 (60%)	16 (88.9%)	0.157
	Sometimes	4 (13.3%)	4 (100%)	
	Rarely/ Never	8 (26.7%)	5 (62.5%)	
Washing CL cases by:	Solution	12 (40%)	10 (83.3%)	0.845
	Water	11 (36.7%)	9 (81.8%)	
	Both	3 (10%)	3 (100%)	
	Not washing	4 (13.3%)	3 (75%)	
CLs cases replacement	Always/ Often	15 (50%)	12 (80%)	0.852
	Sometimes	6 (20%)	5 (83.3%)	
	Rarely/ Never	9 (30%)	8 (88.9%)	
Addition of residual old solution to the new one	Always/ Often	4 (13.3%)	3 (75%)	0.494
	Sometimes	1 (3.3%)	1 (100%)	
	Rarely/ Never	25 (83.3%)	21 (84%)	

microbial contamination of CL units. Eight participants (27.6%) reported using tap water instead of the recommended solution to store contact lenses at some points. A summary of contact lenses wearers' hygienic habits toward contact lenses and solutions are shown in Table (6). It should be noted that percentage of participants using water or using water and CL solution alternatively for washing CL cases always was (38.9%) and (16.7%), respectively

Discussion

Microbiological contamination of commercial contact lenses poses a significant threat to ocular health, elevating the risk of ocular inflammation and related complications. This contamination stems from various factors, such as inadequate hygiene practices, improper lens care, prolonged wear durations, and environmental exposure. To mitigate these risks and safeguard optimal eye health, adherence to recommended cleanliness protocols, timely replacement of lenses, and prompt medical attention at the first sign of irritation are imperative.

In this study, we collected 33 samples of commercial contact lens solution to isolate bacteria. Table 2 presents the distribution of isolated bacteria, with Pseudomonas aeruginosa and Staphylococcus epidermidis being the most prevalent at 27.2% and 21.2%, respectively. Staphylococcus aureus followed at 18.1%, while Escherichia coli, Bacillus spp., and Streptococcus pneumoniae were all found at 12.1%. Klebsiella spp. constituted 3% of the isolates. Bacterial identification relied on phenotypic characteristics and the use of the Vitik 2 system.

Contaminated bacteria

Lens preservation solutions are among the primary contaminants of lenses, with their misuse and repetitive use posing significant contamination risks. Additionally, handling lenses with unwashed hands is a major contributor to eye injuries (Lipener et al., 2000). Holden et al. (1996) note that Gram-negative bacteria are particularly adept at contaminating lens care solutions due to their ability to thrive in environments with minimal nutritional requirements. Our study concurs with Aljanabi et al. (2013) in identifying Pseudomonas aeruginosa as the most common bacteria found in contact lens storage solutions, often associated with biofilm formation on lenses. P. aeruginosa possesses various virulence factors, including exotoxins, proteases, elastases, and biofilm formation, making it a leading cause of ocular infections such as keratitis and corneal ulcers associated with contact lens wear (Sandel et al., 2013). Similarly, Staphylococcus epidermidis, or coagulase-negative staphylococci, are frequently encountered bacteria on contact lenses, consistent with findings by Waghmare and Jeria (2022). Bacterial contamination of contact lens solutions can occur from hands or contaminated lens storage environments. These bacteria, often considered normal ocular flora, can be transferred to accessories during handling (Choby et al., 2020). Factors such as contact lens wear, immune system status, and organism transfer to other parts of the body can influence the prevalence and distribution of bacterial agents, potentially leading to infections, particularly by normal flora like staphylococci (Eguchi et al., 2013).

Staphylococcus aureus was isolated in this study, consistent with findings by Abadi et al. (2021), who associated it with eye colonization and subsequent infections. S. aureus is known for its wide-ranging infections and ability to secrete numerous enzymes, including coagulase, proteinase, hyaluronidase, gelatinase, lipase, phosphatase, lactamase, staphylokinase, and fibrinolysin, as well as production of exotoxins (Li et al., 2014). Escherichia coli strains can be harmless commensals of the intestinal tract or major pathogens in humans and animals (Gomes et al., 2016). Transmission typically occurs via the fecal-oral route, making it a significant route for pathogenic strains (Issa et al., 2014). E. coli can cause intestinal and extraintestinal diseases in both healthy and immunocompromised individuals (Mahmood, 2021). Gram-positive bacilli, including Bacillus species, are also found on contact lenses and are known to cause ocular infections such as conjunctivitis and post-traumatic endophthalmitis (Choby et al., 2020). Streptococcus pneumoniae, a normal inhabitant flora of the nasopharynx, was also detected in contact lens solutions, particularly in children, highlighting the importance of immunization to reduce its spread to other sites, including the eyes. Klebsiella spp. was isolated in small numbers in the current study. It is a pathogenic bacterium known for its virulence factors such as capsules made of complex acidic polysaccharides and B-lactamase or Carbapenemases enzymes, making antibiotic selection challenging for treatment. Klebsiella spp. also exhibits the ability to adhere to various host cell surfaces and spread from one organ to another, contributing to the development of infectious diseases (Ashurst, 2023; Li et al., 2014).

The 16s rRNA gene was PCR-amplified to isolate *P. aeruginosa*, yielding a distinct gel electrophoresis band at 1495 bp, as depicted in Figure 2. We selected these isolates due to their highest rate of occurrence and notable resistance to antibiotics compared to diagnoses made by the Vitek device. Molecular diagnostics confirmed the identity of the bacterial isolates as P. aeruginosa, with a 98% similarity to global isolates identified in NCBI blasts. This discovery indicated a novel genotype, leading to their classification as new local isolates in the NCBI database, designated as RSR1, RSR2, and RSR3. Our findings parallel those of Altaai et al. (2014), who similarly isolated P. aeruginosa from pathological samples and validated their identification through molecular analysis of the 16s rRNA segment, as depicted in Figure 3.

Sensitivity to antibiotic test

The antibiotic sensitivity of the isolates was assessed through the Kirby–Bauer disc diffusion method on Muller Hinton agar, following the guidelines outlined by the Clinical Laboratory Standard Institute (CLSI, 2021). Table 3 displays a range of sensitivities among the bacteria to various antibiotics. This variability may be attributed to the overuse of antibiotics, resulting in the emergence of highly resistant bacterial strains.

Age effect on eye infection

The age distribution of females in the current study is depicted in Figure 4. The results reveal a notable prevalence of eye infections in the age group of 12-20 years, accounting for 41%, followed by 21-30 years with 22%, 31-40 years with 19%, 41-50 years with 13%, and a minimal percentage of 5% in the 51-60 years age range. These findings align with previous research by Aljanabi et al. (2013). This trend may be attributed to a potential lack of awareness regarding the risks associated with improper contact lens care among individuals in these age groups (Patel et al., 2022). Patel et al. (2022) noted that eye infections resulting from contact lens use tend to occur at a younger age due to improper handling of lens care fluids and the potential use of tap water instead of proper storage fluid, emphasizing the importance of hand hygiene before handling contact lenses.

Effect Education level on eye infection

The results depict the distribution of eye infections arising from contact lens usage across different education levels. Primary education level exhibits the highest percentage at 63%, followed by secondary education level at 27%, and higher education level at 10%. Factors such as poor hygienic practices and education level, coupled with the prevalence of contact lens wearers in the population, play crucial roles in the occurrence of serious bacterial infections associated with high rates of contamination on contact lenses (Yung et al., 2017).

The bacterial isolates identified in this study comprise both pathogens and normal flora originating from the gastrointestinal tract, skin, and the environment, all of which may contribute to contact lens-related microbial keratitis. The incidence of contamination is significantly influenced by the handling practices of contact lens wearers, improper hygiene practices, and the failure of certain preservative systems, all of which contribute to contamination development. For instance, when lens wearers handle lenses with bare fingers during immersion or removal from disinfecting solutions, faecal bacteria such as Enterobacter, Serratia, and Klebsiella species may become trapped in the lens case and subsequently transferred to the lenses. Notably, Serratia and Pseudomonas species exhibit resistance to some disinfecting solutions (Willcox, 2011).

In Figure 6, an association between eye infections resulting from contact lens usage and the duration of use is illustrated. A high percentage is observed for lenses worn for 24 hours, accounting for 57%, followed by 12 hours at 22%, 6 hours at 14%, and 1 hour at 7%. Prolonged wear of contact lenses increases the risk of exposure to eye infections. Various factors beyond lens care practices may also influence the rate of microbial contamination, including gender, age of the population, type of contact lens, and temperature. It is conceivable that bacterial growth may initially occur in the contact lens case, followed by secondary contamination of the lenses (Zainodin et al., 2021).

Conclusion

Our study demonstrated the diverse array of bacteria responsible for eye infections associated with contact lens wear, with Pseudomonas aeruginosa emerging as the predominant culprit, with three distinct strains documented within the National Center for Biotechnology Information. Furthermore, certain factors, including age, educational attainment, and duration of lens wear, were identified as contributors to increased infection rates. Moreover, continual efforts to minimize microbiological contamination and enhance contact lens safety necessitate ongoing research into advanced lens materials and disinfection techniques.

Author contribution

R.S.H., S.M.A. performed analysis, analyzed data, R.A.T., M.F.H. reviewed, edited, and prepared the manuscript. All authors approved the manuscuprt for publication.

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Competing financial interests

The authors have no conflict of interest.

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