Antimicrobial susceptibility of bacterial isolates from the conjunctiva, storage cases and mobile phones of university students using contact lenses

Al Momani Waleed[a,*], Lua'i Abu-Ismail[b], Wisam Shihadah[b,c], Sana Janakat[d]

[a] Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, P.O Box 566, 21163, Irbid, Jordan
[b] Department of Clinical Medical Sciences, Faculty of Medicine, Yarmouk University, P.O Box 566, 21163, Irbid, Jordan
[c] Department of Special Surgery, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
[d] Department of Nutrition and Food Technology, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan

ARTICLE INFO

ABSTRACT

Purpose: : To investigate the presence of bacterial pathogens on the palpebral conjunctiva, mobile phones, and storage cases of contact lens wearers to study any possible correlation between types of bacteria isolated from the 3 sites and to determine their antibiotic profiles.

Methods: : One hundred and eighty nine swabs from the conjunctiva, mobile phones, and storage cases were collected from 63 contact lens wearing university students. The swabs were collected and transported to the microbiology laboratory within one hour and inoculated on nutrient agar, MacConkey agar, blood agar and mannitol salt agar. The subsequent bacterial isolates were identified by their cultural, morphological and bio-chemical characteristics.

Results: : Nine bacterial species were isolated and identified in the current study namely: *Staphylococcus. aureus, Streptococcus. pyogenes, Enterococcus. faecalis, Shigella dysentery, Pseudomonas aeruginosa, Proteus mirabilis, Citrobacter* spp*., Klebsiella pneumoniae* and *Escherichia coli*. Nine (26%) mobile phone and 7 (21%) conjunctival samples were contaminated with five different bacterial species. The highest level of contamination was detected in contact lens storage cases where 18 (52%) bacterial isolates were detected in cases.

Conclusions: : The storage cases and mobile phones of contact lens wearing university students were highly contaminated with pathogenic bacteria and may act as a carrier for the transmission of such bacteria to the eye causing eye infections which can be controlled by proper hygiene and using effective disinfectant for storage cases. Pathogenic bacteria were detected with multiple antibiotic resistance indices.

1. Introduction

Contact lenses are medical devices used for refractive, cosmetic, and lifestyle-oriented purposes. In recent years, many individuals are choosing to wear contact lenses for both cosmetic and optical purposes due to the many advantages they offer over wearing spectacles, not least of which is their aesthetic advantage. There are an estimated 140 million individuals who wear contact lenses worldwide [1].

In spite of the fact that contact lenses have many advantages, they also carry a risk of potential ocular complications and loss of vision. Several risk factors for corneal infection include extended wear, poor hand hygiene, and inadequate lens and lens-case care [2]. The promotion of good contact lens hygiene practices are essential to reduce the adverse effects of contact lens use [3]. Contact lenses alter the natural ocular environment by introducing a bio-burden of micro-organisms to the eye's surface from contaminated hands, lenses and lens

care solution [4]. The lens interferes with the protective function of the mucin layer, which is resistant to bacterial adherence, and it hinders the release of antimicrobial factors [3].

During insertion, removal, and cleaning, contact lenses are frequently touched by one's fingertips and palms, which in turn handle everything else in our surroundings, including the omnipresent cellular phone. As a result, the risk of colonization and infection of the eye is considerable [5].

Smartphone touchscreens are known pathogen carriers in clinical environments. However, despite a rapidly growing number of smartphone users worldwide, little is known about bacterial contamination of smartphone touchscreens in non-clinical settings. Such data are needed to better understand the hygienic relevance of these increasingly popular items [6]. Moreover, lens case hygiene still plays an important role in safe contact lens wear as shown in recent epidemiological studies [7]. It has also been demonstrated that identical organisms have been

identified from both the lens storage case and the corneal ulcer of the same patient [8]. Furthermore, another study found that the disease severity correlates with an increase in the diversity of bacterial types found in lens cases [9]. In another series, it was found that after removing contact lenses aseptically from the eye, between 56%–65% of contact lenses harbor microorganisms, almost exclusively bacteria, and approximately 10% of lenses harbored gram-negative and other highly pathogenic species [10].

This current study provides evidence that the same pathogenic bacteria were detected on the conjunctiva, smart phone screens, and lens storage cases of contact lens wearing university students. It highlights the need for awareness of the serious risk of contaminated smart phones on the eyes' health of contact lens users using standard diagnostic techniques and investigating the antibiogram characteristics of the isolated bacteria.

2. Methods

2.1. Sample collection

The present study included a convenience sample of 63 students who volunteered to participate from a public university in northern Jordan. A station was established to collect 189 swabs from 63 volunteers as per standard aseptic procedures. One sample was collected from each of the concave surface of the inner palpebral conjunctiva (n = 63) and the surface of the participants mobile phone (n = 63). A sterile cotton-wool swab moistened with sterile isotonic sodium chloride solution was used to swab the interior of the contact lens solution storage case (n = 63) of each student. The sample population was from different colleges belonging to the same university.

2.2. Bacterial isolation

The swabs were collected in Stuarts transport medium and transported to lab within one hour. The inoculations were cultured on nutrient agar, MacConkey agar, blood agar and mannitol salt agar (Bio lab, Hungary), then incubated aerobically at 37 C for 24 hr C for 24 h. All culture media were prepared following the manufacturer's instructions and sterilized by autoclaving at 121 °C for 20 minutes.

2.3. Identification of bacterial isolates

The bacterial isolates were identified by studying their cultural, morphological and biochemical characteristics according to Baron et al. and Forbes et al. [11,12]. Biochemical tests were used to confirm the identity of each isolate. The identification tests vary according to the Gram reactivity exhibited by the isolate. If upon Gram stain the isolate was confirmed as Gram positive bacteria, then catalase and coagulase tests were performed, followed by assessment of the ability of the organism to grow on bile esculin agar and then its tolerance to novobiocin was established. *Micrococci* spp. was differentiated from Staphylococci by using the bacitracin susceptibility test. For Gram negative bacteria, the reaction pattern on triple sugar iron slant was performed followed by testing the isolate for motility, triple sugar iron agar, indole, methyl red, voges proskauer and citrate tests to confirm the diagnosis.

2.4. Antibiotic susceptibility test

Antibiotic susceptibility test was performed on each of the isolates by using the disc diffusion method on Muller-Hinton agar as recommended by Clinical Laboratory Standards Institute "CLSI" using the following antibiotic disks: ciprofloxacin (5 μg), ampicillin (10 μg), norfloxacin (10 μg), erythromycin (15 μg), chloramphenicol (30 μg), gentamicin (10 μg), tetracycline (30 μg), vancomycin (30 μg), amoxicillin/clavulanic acid (20/10 μg), cefepime (30 μg), penicillin (10 μg), clindamycin (2 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg)

and oxacillin (1 μg). Briefly, microorganisms were suspended in saline to turbidity of 0.5 McFarland standards. A swab of the cell suspension was then spread in three directions on the entire surface of a Mueller Hinton Agar plate (MHA), and left for 15 min to air dry at room temperature before antibiotic disks were applied onto the agar. The agar plates were then incubated at 35 °C for 18–24 h. *S. aureus* (ATCC # 25922) was bought as lyophilized from a local supplier and used as a control. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [13].

2.5. Multiple antibiotic resistance

Multiple antibiotic resistance was determined to investigate the level of resistance among the isolated bacteria and was calculated according to the following equation:

Multiple antibiotic resistance index = no. of antimicrobials to which the isolate is resistant/no. of antibiotics to which the isolate is subjected.

2.6. Statistical analysis

Cross tabulation analysis using the Chi-square test was performed to assess the difference in the number of bacterial isolates from the conjunctiva, the surface of mobile phone and the contact lens storage case. Fisher's-Exact test was used in cases where n < 5 leading to violation of Chi-square assumptions. A *P*-value of < 0.05 was considered statistically significant in all cases.

2.7. Ethical approval

The study protocol was approved by the Institutional Ethics Committee and each participant gave written informed consent.

3. Results

In total, one hundred and eighty nine swabs from the conjunctiva, mobile phones and contact lens storage cases from 63 university students were examined. Bacterial species were isolated from 22 (35%) of the 63 tested students. Thirty four bacterial species were identified and tested for their antibiotic susceptibility. The isolated bacteria including, 9 (26%) from mobile phone and 7 (21%) from conjunctival samples were contaminated with five different bacterial species. The highest contamination was detected in storage case where 18 (52%) bacterial isolates were detected in cases.

Nine bacterial species were isolated and identified in the current study namely:

S. aureus 7 (21%), *S. pyogenes* 6 (18%), *E. faecalis* 2 (6%), *S. dysentery* 3 (9%), *P. aeruginosa* 1 (3%), *P. mirabilis* 4 (12%), *Citrobacter* spp. 2 (6%), *K. pneumoniae* 1 (3%) and *E. coli* 8 (23%). The frequency of Gram positive and Gram negative bacteria isolated from the conjunctiva, storage cases and mobile phones swabs studied is shown in Table 1. Fifty-six percent of isolates were Gram negative and 44% were Gram negative. The antibiotic profile of the isolated bacteria showed a large variation. Although some bacterial species were susceptible to 12 antibiotics, other species were highly resistant to the antibiotics used as shown in Table 2. The *S. aureus* isolates were sensitive to most of the antibiotics used and 57% of isolates (4 of 7) were resistant to erythromycin and penicillin. The *S. pyogens* isolates showed higher rates of antibiotic resistance compared to the *S. aureus*. Four isolates were resistant to trimethoprim/sulphamethoxazole and oxacillin. Three of the six isolated S. pyogens were resistant to vancomycin, tetracycline, penicillin and clindamycin. Two isolates were resistant to chloramphenicol and erythromycin while a single isolate was resistant to ciprofloxacin.

The two isolates of *E. faecalis* were sensitive to eight antibiotics and resistant to 4 of the antibiotics tested namely: ampicillin, clindamycin,

A.M. Waleed, et al.

Table 1

The isolated bacteria from the conjunctiva, mobile phones and washing solution of the university students.

Bacteria spp.	Type of specimen			Total no. of isolates
	C	P	S	
Staphylococcus aureus	0	4	3	7
Streptococcus. pyogenes	3	2	1	6
Shigella dysentery	1	0	2	3
Pseudomonas aeruginosa	0	0	1	1
Entrococcus faecalis	1	1	0	2
Proteus mirabilis	1	1	2	4
Citrobacter spp.	1	0	1	2
Klebsiella pneumoniae	0	0	1	1
Escherichia coli	0	1	7	8
Total	7	9	18	34

C = Conjunctiva, P = Phone, S = Storage case.

penicillin and oxacillin as shown in Table 2.

The three *Shigella* isolates also showed a complex profile and were sensitive to norfloxacin, chloramphenicol, gentamicin, tetracycline and cefepime. One isolate was sensitive to ciprofloxacin, ampicillin, amoxicillin/clavulanic acid, clindamycin, penicillin and oxacillin. One of the *E. faecalis* isolates was resistant to trimethoprim/sulphamethoxazole and ciprofloxacin.

Two isolates were resistant to ampicillin, amoxacillin/clavulanic acid, clindamycin, penicillin and oxacillin. A single isolate showed intermediate susceptibility to ciprofloxacin. Seven of the *E. coli* isolates were sensitive to gentamicin, amoxicillin/clavulanic acid, cefepime, and trimethoprim/sulphamethoxazole, while 6 of them were sensitive to chloramphenicol, and tetracycline, 5 isolates were sensitive to norfloxacin only and two isolates were sensitive to ciprofloxacin. All *E. coli* isolates were resistant to ampicillin, clindamycin, penicillin and oxacillin. Three isolates were resistant to norfloxacin, 2 isolates were resistant to chloramphenicol, and tetracycline and one isolates was resistant to ciprofloxacin, gentamicin, amoxicillin/clavulanic acid, cefepime and trimethoprim/sulphamethoxazole. Five *E. coli* isolates showed an intermediate susceptibility to ciprofloxacin. A single *P. aeruginosa* isolate was sensitive to ciprofloxacin, gentamicin, and cefepime with variable resistance to eight of the other antibiotics. The two isolated *Citrobacter* spp. were sensitive to norfloxacin, chloramphenicol, gentamicin, tetracycline and cefepime while these isolates were resistant to ampicillin, amoxicillin/clavulanic acid, clindamycin, penicillin, trimethoprim/sulphamethoxazole, oxacillin and showed

Table 3

Multidrug resistance profile of the isolated bacteria to the tested antibiotics (n = 14).

Parameter	Frequency	MAR index
S. aureus		
R2 = E, P	2	0.14
S. pyogenes		
R5 = TE, VA, P, DA, OX	1	0.357
R8 = E, C, TE, VA, P, DA, SXT, OX	1	0.571
R9 = CIP, E, C, TE, VA, P, DA, SXT, OX	1	0.642
***Citrobacter* spp**		
R6 = AML, AMC, P, DA, SXT, OX	2	0.5
P. aeruginosa		
R6 = AML, NOR, C, AMC, P, DA, SXT, OX	1	0.545
K. pneumoniae		
R5 = AML, AMC, P, DA, OX	1	0.416
E. coli		
R4 = AML, P, DA, OX	2	0.333
R5 = AML, TE, P, DA, OX	1	0.416
R5 = AML, AMC, P, DA, OX	1	0.416
R5 = AML, NOR, P, DA, OX	1	0.416
R5 = AML, C, P, DA, OX	1	0.416
R8 = AML, NOR, C, CN, TE, P, DA, OX	1	0.666
R8 = CIP, AML, NOR, FEP, P, DA, SXT, OX	1	0.666
E.faecalis		
R4 = AML, P, DA, OX	1	0.285
R6 = AML, AMC, P, DA, SXT, OX	1	0.428
S. dysentery		
R6 = AML, AMC, P, DA, SXT, OX	1	0.5
R6 = CIP, AML, P, DA, OX	1	0.5
P. mirabilis		
R4 = AMC, P, DA, SXT, OX	1	0.333
R5 = AML, P, DA, SXT, OX	1	0.416
R6 = AML, CN, P, DA, SXT, OX	1	0.5

*CIP: ciprofloxacin, AML: Ampicillin, NOR: norfloxacin, E: Erythromycin, C: Cloramphenicol, CN: Gentamicin, TE: Tetracycline, VA: Vancomycin, AMC: Amoxacillin/clavulanic acid, FEP: Cefepime, P: Penicillin, DA: Clindamycin, SXT: Trimethoprim/sulphamethoxazole, OX: Oxacillin*MAR index = multiple antibiotic resistance index = no. of antimicrobials to which the isolate is resistant/no. of antibiotics to which the isolate is subjected.

intermediate susceptibility to ciprofloxacin.

All *P. mirabilis* isolates were sensitive to norfolxacin and cefepime while three isolates were sensitive to five antibiotics namely, ciprofloxacin, gentamicin, chloramphenicol, amoxicillin/clavulanic acid and tetracycline Two *P. mirabilis* isolates were sensitive to ampicillin and the other two were resistant to the same antibiotic. The four isolated *P.*

Table 2

Antimicrobial profile of the isolated bacteria from the eye, mobile phones and washing solution among the university students.

Antibiotic	S. aureus			S. pyogens			E. faecalis			S. dysentery			E. coli			P. aeruginosa			Citrobacter spp			P. mirabilis			K. pneumoniae		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
CIP	7	0	0	5	1	0	2	0	0	1	1	1	2	1	5	1	0	0	2	0	0	3	0	1	0	0	1
AML	7	0	0	6	0	0	0	2	0	1	2	0	0	8	0	0	1	0	0	2	0	2	2	0	0	1	0
NOR	7	0	0	6	0	0	2	0	0	3	0	0	5	3	0	1	0	0	2	0	0	4	0	0	1	0	0
E	3	4	0	4	2	0	2	0	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
C	7	0	0	4	2	0	2	0	0	3	0	0	6	2	0	0	1	0	2	0	0	3	0	1	1	0	0
CN	7	0	0	6	0	0	2	0	0	3	0	0	7	1	0	1	0	0	2	0	0	3	1	0	1	0	0
TE	7	0	0	3	3	0	2	0	0	3	0	0	6	2	0	N	N	N	2	0	0	3	0	1	1	0	0
VA	7	0	0	3	3	0	2	0	0	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
AMC	7	0	0	6	0	0	1	1	0	1	2	0	7	1	0	0	1	0	0	2	0	3	1	0	0	1	0
FEP	7	2	0	6	0	0	2	0	0	3	0	0	7	1	0	1	0	0	2	0	0	4	0	0	1	0	0
P	3	4	0	3	3	0	0	2	0	1	2	0	0	8	0	0	1	0	0	2	0	0	4	0	0	1	0
DA	7	0	0	3	3	0	0	2	0	1	2	0	0	8	0	0	1	0	0	2	0	0	4	0	0	1	0
SXT	7	0	0	2	4	0	1	1	0	2	1	0	7	1	0	0	1	0	0	2	0	0	4	0	1	0	0
OX	7	0	0	2	4	0	0	2	0	1	2	0	0	8	0	0	1	0	0	2	0	0	4	0	0	1	0

*S = Sensitive, R = Resistant, I = Intermediate, N = Not applicable.

CIP: ciprofloxacin, AML: Ampicillin, NOR: norfloxacin, E: Erythromycin, C: Chloramphenicol, CN: Gentamicin, TE: Tetracycline, VA: Vancomycin, AMC: Amoxacillin/clavulanic acid, FEP: Cefepime, P: Penicillin, DA: Clindamycin, SXT: Trimethoprim/sulphamethoxazole, OX: Oxacillin.

mirabilis were resistant to clindamycin, penicillin, trimethoprim/sulphamethoxazole, oxacillin. Only one isolate showed resistant to amoxicillin/clavulanic acid and gentamicin. Three isolates showed intermediate susceptibility to ciprofloxacin, tetracycline and chloramphenicol. A single *K. pneumonia*e isolate was sensitive to six antibiotics these are: norfolxacin, gentamicin, chloramphenicol, gentamicin, cefepime and trimethoprim/sulphamethoxazole. The same isolate was resistant to ampicillin, amoxicillin/clavulanic acid, clindamycin, penicillin, oxacillin and showed intermediate susceptibility to ciprofloxacin.

It is apparent from the antibiotic resistance profile in Table 3 that *E. coli* and *S. pyogens* isolates were the most resistant bacteria to the studied antibiotics, and showed resistance to multiple antibiotics used in this study with a ratio of 0.67 and 0.64 respectively. The most effective antibiotics against the isolated bacteria were gentamicin, cefepime and norflaxacin.

The multiple antibiotic resistance (MAR) indices of the isolated resistant bacteria were determined with reference to fourteen different antibiotics used in this study. The values of MAR indices are shown in Table 3. Analysis of the MAR index of isolates showed that 22 of the total 34 resistant bacteria studied were above 0.2 indicating the highly resistant nature of these isolates. The highest MAR indices (Table 3) were detected in *E. coli* (0.66) and *S. pyogenes* bacterial isolates (0.64) followed by *P. aeruginosa* (0.54), *S. dysentery* (0.5) and *Citrobacter* spp. (0.5). *K. pneumoniae*, *E. fecalis* and *S. aureus* had MAR index less than 0.5

Cross tabulation analysis was performed to assess the relationship between bacterial species and the 3 sites of isolation. As illustrated in Table 4, about 37% of microorganisms isolated from the storage case were *E. coli*, followed by *S. aureus* and *P. mirabilis* (16% for each). *S. aureus* was the dominant microorganism isolated from phones (50%) followed by *S. pyogenes* (25%). However, about 43% of microorganisms isolated from the conjunctiva were *S. pyogenes* compared to 14% for each of *S. dysentery*, *E. fecalis*, *P. mirabilis* and *Citrobacter* spp. The contamination rate varied considerably across the three sites, and was highest in the contact lens storage case. The observed differences in the proportion of species isolated from the palpebral conjunctiva, surface of mobile phone and the contact lens solution storage case approached statistical significance ($P = 0.05$) as shown in Table 4.

4. Discussion

In the current study bacteria were isolated from 22 (35%) of the 63 students tested showing the same type of bacterial species isolated by Liaqat et al. [14] including *K. pneumoniae., S. aureus, E. coli, S. pyogens., P. aeruginosa. E. faecalis, S. dysentery*, *P. mirabilis* and *Citrobacter* spp. In the present study 56% of bacterial species were Gram negative organisms compared to only 35% detected by Liaqat et al. [14]. *E. coli* (23%) was the most commonly isolated bacteria followed by *S. aureus* (21%) and *S. pyogens* (18%) which was higher than reported previously [14]. In this study *P. aeruginosa* and *K. pneumoniae* comprised 3% of total bacterial isolates comparing to Üstüntürk and Zeybek [15] who determined that aerobic mesophilic bacteria were isolated in 45 (90%)

while Gram negative rod bacteria were isolated in 20 (40%) and *Pseudomonas spp*. were isolated in 2 (4%) out of 50 contact lens storage cases.

Despite a rapidly growing number of smartphone users worldwide, little is known about bacterial contamination of smartphone touchscreens in non-clinical settings. Such data are needed to better understand the dangers of using smartphones on contact lens wearers. In this study, smartphone touchscreens from the investigated student community were contaminated by bacteria of mostly human skin origin mainly *S. aureus* and *S. pyogens* in agreement with Meadow et al. [15] who stated that 'it appears unlikely that smartphone surfaces represent a greater microbial threat for healthy persons than other commonly touched surfaces or gadgets' [16]. The result of current study indicates that the rate of bacterial contamination of mobile phones in our sample was much lower than previous studies; 96% of Saudi medical students' mobile phones were found to be contaminated with bacteria [17] and similar results were reported from Egypt revealing a contamination rate of 97% for mobile phones and hands [18]. Moreover, 100% mobile phones of college students in India showed high degree of bacterial contamination [19]. In Mauritius, mobile phones of volunteers in the general community revealed a bacterial contamination rate of 92% [20].

In a previous study bacteria were isolated from 45 out of 57 (79%) lens cases collected from university students showing a high rate of bacterial contamination in contact lens wearers [14]. Recent epidemiological studies showed that lens case hygiene still plays an important role in safe contact lens wear. It has also been demonstrated in another study that identical organisms have been identified from both a lens storage case and cornea ulcer [7]. According to Szczotka-Flynn et al. [10], in storage cases all types of care solutions can become contaminated, including up to 30% of preserved products with approximately 10% of lenses harbor Gram- negative and highly pathogenic species, even in asymptomatic subjects. In the current study 78% of the isolated bacteria from the contaminated storage cases tested (63) were Gram negative, similar to previous studies [10].

Szczotka-Flynn et al. [10] stated that in particular, contact lens acute red eye has been associated with *P. aeruginosa*. Infiltrative keratitis and contact lens peripheral ulcer have been associated with *S. aureus* and *S. pyogens*. Four Gram positive and three Gram negative bacteria were detected in the palpebral conjunctiva of the students examined in the current study.

According to Ramachandran et al., [21] the common contaminants in contact lens cases and preserving solutions were identified as *Bacillus spp., S. epidermidis, S. aureus, Klebsiella spp. E. coli, P. auruginosa,*. The presence of *S. aureus, Streptococci* spp. and Gram negative bacteria, even in small numbers, is considered an important bioburden, since these species are reported to be linked with corneal infection and swelling and are hardly ever isolated in asymptomatic subjects.

The study of normal conjunctival flora by Keshav and Basu [22] found that coagulase-negative *Staphylococci* (82%) and *S. aureus* (4%) are the normal flora inhabiting the lid margin and conjunctiva of normal populations [22]. In the current study *S. aureus* was found in 21% of the isolated samples.

Table 4
Cross tabulation analysis of the relationship between bacterial species and the site of isolation.

Isolate Site	Type of microorganism									p.value*
	S. aureus No (%)	*S. pyogenes* No (%)	*S. dysentery* No (%)	*P. aeruginosa* No (%)	*E. fecalis* No (%)	*P.mirabilis* No (%)	*Citrobacter* spp. No (%)	*K. pneumoniae* No (%)	*E. coli* No (%)	
Storage case	3 (15.8)	1 (5.3)	2 (10.5)	1 (5.3)	0 (0.0)	2 (10.5)	1 (5.3)	1 (5.3)	7 (36.8)	0.05
Phone	4 (50.0)	2 (25.0)	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	1 (12.5)	
Conjunctiva	0 (0.0)	3 (42.9)	1 (14.3)	0 (0.0)	1 (14.3)	1 (14.3)	1 (14.3)	0 (0.0)	0 (0.0)	
Total Count %	7 (20.6)	6 (17.6)	3 (8.8)	1 (2.9)	2 (5.9)	4 (11.8)	2 (5.9)	1 (2.9)	8 (23.5)	

* Fisher's-Exact test was used.

Pathogenic bacteria were detected with multiple antibiotic resistance indices. Storage cases and mobile phones were highly contaminated with pathogenic bacteria and may act as a carrier for transmission of that bacteria to the eye which may lead to eye infection. Proper hand hygiene, disinfection of mobile phone surfaces, as well as contact lens storage case compliance may reduce the possibility of eye infections. The present work provides evidence that contact lens storage cases are a potential major source of pathogenic bacteria, consistent with previous studies, since they displayed higher bacterial contamination (52%) than the mobile phones and the conjunctiva itself which approached statistical significance ($P = 0.05$). The current study highlights the need for the development of awareness about the high risk of unsuitable disinfectants used in storage cases hygiene leading to serious eye infections. In previous studies Anaerobic species, predominantly *Propionibacterium*, were obtained from 27% of conjunctival cultures [23]. The predominant recovered anaerobes were *Clostridium* spp., Gram-negative anaerobic *bacilli*, and *Peptostreptococcus* spp. Anaerobic bacteria were also recovered from patients who wore contact lenses and developed conjunctivitis, and were reported in cases of keratitis [24] A limitation of this research was the lack of detection of anaerobes and the convenience sample size. Further studies using a larger sample size should investigate anaerobes among students who use contact lenses.

Pathogenic bacteria were detected in palpebral conjunctiva, storage cases and mobile phones with multiple antibiotic resistance indices. The highest level of contamination was detected in contact lens storage cases (52%) followed by mobile phones (26%). The transmission of infections from these sites could be controlled proper hygiene and using effective disinfectant for storage cases.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript

Acknowledgment

References

[1] A. Sulley, G. Young, C. Hunt, Factors in the success of new contact lens wearers, Cont Lens Anterior Eye 40 (1) (2017) 15–24.
[2] E. Mark, Contact-lens-related microbial keratitis: case report and review, J Optom 4 (4) (2011) 122–127.
[3] F. Tariq, P. Koay, The risk of contact Lens wear and the avoidance of complications, Int J Med Stud 1 (2) (2013) 80–85.
[4] Y. Jiang, M. Jacobs, S. Bajaksouzian, A.N. Foster, S.M. Debanne, R. Bielefeld, et al., Risk factors for microbial bioburden during daily wear of silicone hydrogel contact lenses, Eye Cont Lens 40 (3) (2014) 148–156.
[5] M. Boost, P. Cho, Z. Wang, Disturbing the balance: effect of contact lens use on the ocular proteome and microbiome, Clin Exp Optom 100 (5) (2017) 459–472.
[6] M. Egert, K. Späth, K. Weik, H. Kunzelmann, C. Horn, M. Kohl, et al., Bacteria on smartphone touchscreens in a German university setting and evaluation of two popular cleaning methods using commercially available cleaning products, Fol Microbiol. 60 (2) (2015) 159–164.
[7] Y.T.Y. Wu, M. Willcox, H. Zhu, F. Stapleton, Contact lens hygiene compliance and lens case contamination: a review, Cont Lens Anterior Eye 38 (5) (2015) 307–316.
[8] Z. Mohajernezhadfard, P. Vahedi, M. Mohammadpour, A. Khodabande, Antibiotic susceptibility patterns of pseudomonas corneal ulcers in contact lens wearers, Mid Eas Afr J Ophthal. 18 (3) (2011) 228–231.
[9] L. Wiley, D.R. Bridge, L.A. Wiley, J.V. Odom, T. Elliott, J.C. Olson, Bacterial biofilm diversity in contact lens-related disease: emerging role of achromobacter, stenotrophomonas, and delftia, Inves Ophthal Vis Scien. 53 (7) (2012) 3896–3905.
[10] L.B. Szczotka-Flynn, E. Pearlman, M. Ghannoum, Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review, Eye Cont Len. 36 (2) (2010) 116–129.
[11] E.J.O. Baron, L.R. Peterson, S.M. Finegold, Enterobacteriaceae, Bailey and Scotts-diagnostic microbiology, Mosby, Missuri, USA, 1994, pp. 62–385.
[12] B.A. Forbes, D.F. Sahm, A.S. Weissfeld, Bailey and Scott's. Diagnostic microbiology 12th edition, Mosby Elsevier, St. Louis, MO: USA, 2007, pp. 778–781.
[13] Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing; Twenty-sixth informational supplement. CLSI document M100-S26, Wayne, PA: Clin Lab Stand Inst, USA, 2016.
[14] I. Liaqat, S. Qurat-ul-Ain, H.M. Tahir, M. Arshad, N. Arshad, Identification of virulence factors in contact lens associated bacteria: a physiological approach, Cont Lens Anterior Eye 42 (2) (2019) 159–164.
[15] M. Üstüntürk, Z. Zeybek, Microbial contamination of contact lens storage cases and domestic tap water of contact lens wearers, Wien Klin Wochenschr 124 (Suppl. 3) (2012) 17–22.
[16] J.F. Meadow, A.E. Altrichter, J.L. Green, Mobile phones carry the personal microbiome of their owners, Peer J 2 (2014) e447.
[17] S. Zakai, A. Mashat, A. Abumohssin, et al., Bacterial contamination of cell phones of medical students at King abdulaziz university, Jeddah, Saudi Arabia, J Microsc Ultrastruct. 4 (3) (2016) 143–146.
[18] M.T. Elkholy, I.E. Ewees, Mobile (cellular) phone contamination with nosocomial pathogens in intensive care units, Med J Cairo Univ 78 (2) (2010) 1–5.
[19] P. Kumar, M.L. Aswathy, Identification of mobile phone-associated pathogens, Keral J Orthop 27 (1) (2014) 69–72.
[20] A. Bhoonderowa, S. Gookool, S.D. Biranjia-Hurdoyal, The importance of mobile phones in the possible transmission of bacterial infections in the community, J Commun Health 39 (5) (2014) 965–967.
[21] L. Ramachandran, S. Sharma, P.R. Sankaridurg, C.M. Vajdic, J.A. Chuck, B.A. Holden, et al., Examination of the conjunctival microbiota after 8 hours of eye closure, CLAO J 21 (3) (1995) 195–199.
[22] B.R. Keshav, S. Basu, Normal conjunctival flora and their antibiotic sensitivity in Omanis undergoing cataract surgery, Oman J Ophthal 5 (1) (2012) 16–18.
[23] T.R. Singer, S.J. Isenberg, L. Apt, Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects, Br J Ophthalmol 72 (6) (1988) 448–451.
[24] I. Brook, Ocular infections due to anaerobic bacteria in children, J Pediatr Ophthalmol Strabismus 45 (2) (2008) 78–84.