Molecular identification of antibiotic-resistant bacteria isolated from used contact lens cases

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ABSTRACT

Aims: The contact lens (CL) has become one of the most convenience refractive devices used in vision correction, occupational and in cosmetics purposes. Despite the convenience of CL usage, poor hygiene might cause eye infections due to microbial contamination. In this work, a random collection of used CL cases among Universiti Malaysia Terengganu (UMT) students had shown the emergence of antibiotic-resistant bacteria towards commonly used antibiotics to treat eye infections.

Methodology and results: The study was carried out from 28 CL cases samples with the duration of one to three months of use. Bacteria that were successfully isolated from the CL cases were then exposed to the commonly prescribed antibiotics followed by identification through the partial 16S rDNA sequencing. Our finding exhibited that the rate of contamination is over 50% where 32 bacteria were isolated, with 20 (62.5%) of the isolates were Gram-positive bacteria. Approximately 31% of the isolated bacteria are resistant and intermediate resistant to the commonly used antibiotics to treat eye infection, especially erythromycin and chloramphenicol. The isolated bacteria were genotypic identified as *Bacillus cereus*, *B. anthracis*, *Acinetobacter variabilis*, *Klebsiella pneumoniae*, and *Serratia marcescens*. These bacteria are known as a common cause for microbial keratitis, except for *A. variabilis*, where the association of this bacteria in causing microbial keratitis is relatively rare.

Conclusion, significance and impact of study: This study highlights the emergence of antibiotic-resistant bacteria that can cause severe eye infections among CL wearer. The high percentage of contamination (>50%) found from the isolates reflected on the lack of hygiene practice on the CL handling. Thus, it is crucial to perceive this study as microbial contamination will lead to more serious eye infection disease such as conjunctivitis and keratitis.

Keywords: Antibiotic-resistant bacteria, eye infection, contact lens solution, 16S rDNA

INTRODUCTION

Nowadays, the use of a conventional eyeglass seems to be less practical (Willcox *et al.*, 2001; Emina and Idu, 2011; Thakur and Gaikwad, 2014). It is estimated that the number of contact lens (CL) wearer has been exceed more than 140 million worldwide (Morgan *et al.*, 2015; Fleiszig *et al.*, 2020). A survey conducted on optometric CL prescribing in Malaysia recorded an average of 90 new wearer each year and the numbers are expected to be increasing across the years with the rises of myopia patients (Mohidin and Fung, 2009; Mohd-Ali and Tan, 2019).

Even though CL has become one of the most useful devices for vision correction, poor care and lack of hygiene of the lenses may lead to the microbial contamination which may contribute to microbial keratitis (MK) that caused by bacteria (Tabbara *et al.*, 2000). A study by Lim *et al.* (2016) in Singapore showed that patients handling the CL with unwashed hand were 13 times higher in risk of developing MK. It is indisputable evidence that lack of hand hygiene and hand washing before wearing the CL were one of the factors contributing to MK (Fonn and Jones, 2019). Besides that, the sources of MK might due to contamination from lens cases and CL solutions (Bourcier *et al.*, 2003). In the case of the lens case contamination, the bacteria from the lens

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also can contaminate the lens solution, which serves as a reservoir for the microbial growth (Szczotka-Flynn *et al.*, 2010). The sources of the microbes that related with CL wearer were apparently originated mostly from the water supply, care solutions, hand and lens cases (Fonn and Jones, 2019). A study by Carnt and Stapleton (2016) on the CL related diseases has establish that there is a relationship between the use of lens solution and microbial contamination. The study also highlighted the significance of avoiding the unsterilized water from exposed to any part of the CL care and storage case or even when handling the lenses with wet hands.

Microbial keratitis is a type of vision-threatening infection that may lead to corneal ulcer among the CL wearers (Wu *et al.*, 2015). It is commonly related to the use of CL and caused by bacteria, fungi, virus, and a protozoan commonly reported from the genus of *Acanthamoeba* (Zimmerman *et al.*, 2016). A study by Spernovasilis *et al.* (2020), showed that out of 240 isolated microorganisms from 131 keratitis patients associated with CL, Gram-negative bacteria were the major etiological microbes of CL-related keratitis when 84% of the isolates were Gram-negative bacteria, while only 16% of it is Gram-positive bacteria. The *Pseudomonas* sp. is the Gram-negative bacteria that commonly lead to infections, where 60% of the eye infections caused by this particular species of bacteria. In regards to the increase of bacterial resistance towards antibiotics, the treatment for this bacterium, especially *Pseudomonas aeruginosa*, has become a great challenge, due to the ability of this bacteria to cooperate, and communicate to each other in its microbial community to form biofilms (Chadha, 2014; Subedi *et al.*, 2018).

Numerous clinical cases have been reported associated to the wearing of CL that continues to be a concern as the frequency rises every year. Although there are no cases were reported from patients who experienced severe infection from contaminated CL cases, the possibility of the incidences should be underscored. Hence, the goal of this study was to isolate and identify the antibiotic-resistance bacteria from a random collection of prolonged used CL cases. Understanding the emergence of antibiotic-resistance bacteria is important as an early prevention to reduce the number of incidences associated with contact lens usage.

MATERIALS AND METHODS

Sample collection

This study was carried out in the student's residential college at Universiti Malaysia Terengganu (UMT) between July until September 2016. The samples were collected from 28 asymptomatic CL wearers between the ages of 19 to 21 years. All samples were randomly collected from used CL cases that had already reached its recommended length of use of up to 3 months.

Bacteria isolation and phenotypic characterization

This experiment was done in a sterile condition where the outer part of the CL case was first sterilized with 70% alcohol prior to swabbing. A sterile cotton swab was dipped into the collected CL cases containing fresh CL solution and immediately streaked onto nutrient agar. All plates were incubated at 37 °C to allow for bacterial growth. The plates were also continued to incubate at room temperature for another 48 h to ascertain for growth and no growth. All grown bacteria (32 samples) were phenotypically characterized through the Gram staining procedure (Smith and Hussey, 2005).

Antibiotic susceptibility profile of bacterial isolates

All grown bacteria were kept in glycerol stock until further antibiotic susceptibility testing using the disk diffusion method according to the recommendation of the Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2017). Before antibiotic testing, the bacteria that were revived again on a nutrient agar. A sterile loop was used to pick a well-isolated colony and then suspended in saline and mixed to even turbidity. The density of the inoculum suspension was adjusted to 0.5 McFarland by increasing the bacteria concentration to its final turbidity reached a reading of 0.08-0.10 by spectrophotometer.

Subsequently, the suspension was subjected to the disk diffusion test according to the Kirby-Bauer disk diffusion-susceptibility test (Hudzicki, 2009). The number of disks on a plate was limited to only three sections to avoid overlapping of zones and interference. The test was done by using the advisable therapeutic guidelines of chloramphenicol (30 μg), erythromycin (15 μg), gentamycin (10 μg), kanamycin (30 μg), polymyxin B (300 U) and streptomycin (10 μg) with a maximum of three disks per agar plate (Watson *et al.*, 2018). Inhibition zones diameters were measured twice and interpreted into susceptibility categories (susceptible, intermediate and resistant) based on the guiding principle established by the CLSI (2017). The interpretive zone sizes for each of the antibiotics are as follows: chloramphenicol (30 μg) [susceptible, ≤12 mm; intermediate, 13-14 mm; resistant, ≥18 mm], erythromycin (15 μg) [susceptible, ≤13 mm; intermediate, 14-22 mm; resistant, ≥23 mm], gentamycin (10 μg) [susceptible, ≤12 mm; intermediate, 13-14 mm; resistant, ≥15 mm], kanamycin (30 μg) [susceptible, ≤13 mm; intermediate, 14-17 mm; resistant, ≥18 mm], polymyxin B (300 U) [susceptible, ≤8 mm; intermediate, 9-11 mm; resistant, ≥12 mm], and streptomycin (10 μg) [susceptible, ≤11 mm; intermediate, 12-14 mm; resistant, ≥15 mm] (CLSI, 2017).

Molecular identification of antibiotic-resistance isolates

The isolates with resistance- and intermediate susceptibility towards antibiotics following Kirby-Bauer disc diffusion test were further identified through Polymerase Chain Reaction (PCR) using with the

universal primer pair of 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R 5'-(GGTTACCTTGTTACGACTT-3'), amplifying approximately 1500 bp region of 16S rDNA genes (Edward *et al.*, 1989; Stackebrandt and Liesack, 1993). PCR components from PCR kit (GoTaq® G2 Flexi DNA Polymerase (Promega, USA) were mixed according to the manufacturer's recommendation. All the mixing reactions were conducted in a sterile condition on ice. The PCR reaction was performed under the following conditions: 94 °C for 3 min followed by 30 cycles of 94 °C for 1 min; 55 °C for 1 min and 72 °C for 1 min, with a final extension step at 72 °C for 5 min.

DNA sequencing and phylogenetic analysis

Purified PCR products were sent to Next Gene Sdn Bhd for Sanger sequencing services along with forward primer 8F to sequence the 16S rDNA (Edward *et al.*, 1989). The sequencing results obtained were then compared using Chromas DNA software, and the nucleotide sequence has been identified by using the Basic Local Alignment Search Tool (BLAST) by the National Center for Biotechnology Information (NCBI).

The phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA-X) and a phylogenetic tree was constructed with at least 950 nucleotides base pair, aligned using ClustalW together with other reference strain sequences retrieved from NCBI GenBank. The neighbour-joining method with Kimura-2 parameter model and 500 bootstrap replications was used to construct the phylogenetic tree (Li *et al.*, 2011).

RESULTS AND DISCUSSION

Bacterial isolation from used contact lens cases

Contact lens solutions are commercially designed to contain antimicrobial properties while conditioning the eyes to provide comfort during wear. This multipurpose CL solution typically removes accumulated biofilm by dissolving proteins and debris from CL. Any microbial growth in the solutions indicates contamination, perhaps due to lack of good hygienic practice while handling the CL, thus allowing bacterial colonization.

In this study, all the 28 CL cases were collected from UMT students with average of 18 to 21 years old with vision problems, which tends to replace their glasses with CL as this device is more practical for active campus life. From the samples collected, 16 samples (57.1%) were tested positive with the presence of bacterial growth, while 12 samples (42.9%) had no bacterial growth. Among the 16 CL samples with bacteria growth, 32 pure bacterial colonies were successfully isolated. Out of the 32 bacteria, 20 bacteria (62.5%) were identified as Gram-positive while the remaining 12 bacteria (37.5%) were Gram-negative.

Antibiotic susceptibility profile and molecular identification of antibiotic-resistant isolates

Antibiotics are drugs substances that inhibit bacteria from growing and it aids to move out the microorganisms that had resistance gene (Levy, 2002). Today, it was recorded that bacteria such as *Staphyloccocus aureus* has developed resistance towards methicillin and the bacteria is resistant towards the β-lactam antibiotics (Levy, 2002; Jindal *et al.*, 2015). Antibiotics resistance (AR) is microbiologically defined as resistant mechanism that is present genetically. The AR also characterized pathogens in laboratory whether it is resistant or susceptible, while in clinical definition, AR is an antimicrobial activity tested in therapeutics which can lead to increase of the effectiveness of the bacteria to become resistance (MacGowan and Macnaughton, 2017). According to Ventola (2015), there are many factors that can lead to AR which are mutation of gene of bacteria over the years, misused of antibacterial in certain country and availability of few new antibiotics. On the other hand, antimicrobial susceptibility tests are used to determine which specific antibiotics that a particular bacterium is sensitive to. The goals of this testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for a particular treatment of infections.

In this study, out of the six tested antibiotics, the isolates show resistant and intermediate to at least five antibiotics (Table 1). The highest resistance was observed on erythromycin (18.8%), followed by polymyxin B (6.3%), chloramphenicol (6.3%), streptomycin (3.1%) and kanamycin (3.1%). Amongst the tested isolates, 10 (31%) exhibited multiple resistance, resistant and intermediate against the tested antibiotics as shown in Table 2.

The bacteria with antibiotic resistance and intermediate profile were further subjected to 16S rDNA amplification by PCR (Figure 1). Partial sequence of 16S rDNA gene for each sample of previously categorized as antibiotic resistance bacteria were analysed using BLAST and further analysed by phylogenetic analysis, as shown in Table 3 and Figure 2, respectively. The phylogenetic tree has been constructed to observe the relationship between samples and between reference species with one outlier. Reference bacterial species were Gram-negative bacteria, including *Serratia marcescens* (AJ233431), *Klebsiella pneumoniae* (NR114506), *Acinetobacter variabilis* (KP278590), *Serratia liquefaciens* (AJ306725), *Klebsiella oxytoca* (AB004754), *Acinetobacter baumanni* (X81660), and *P. aeruginosa* (AB037545), Gram-positive bacteria including *Streptococcus pyogenes* (X59029), *Staphylococcus saprophyticus* (NR_074999), *S. aureus* (L37597), *Bacillus licheniformis* (MK6800064), *Bacillus anthracis* (AF176321, AY138375), *Bacillus cereus* (D16266, AF159952), and a virus, which is human poliovirus (AY184219) as an outlier group.

The BLAST results for sample S11 bacterial 16S rDNA gene sequence showed the similarity of the sample

Table 1: Number and percentage of overall antibiotic susceptibility patterns of bacteria isolated from contact lens cases.

Antibiotics	Antimicrobial category	Total of isolates tested	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Erythromycin (15 µg)	Macrolides	32	26 (81.2)	0 (0)	6 (18.8)
Polymyxin B (300 U)	Polymyxins	32	27 (84.3)	3 (9.4)	2 (6.3)
Chloramphenicol (30 µg)	Amphenicols	32	29 (90.6)	1 (3.1)	2 (6.3)
Streptomycin (10 µg)	Aminoglycoside	32	29 (90.6)	2 (6.3)	1 (3.1)
Kanamycin (30 µg)	Aminoglycoside	32	31(96.9)	0 (0)	1 (3.1)

Table 2: Antibiotic susceptibility profile of bacteria isolated from contact lens cases.

Antibiotics	Bacterial samples									
	S11	S12	S41	S72	SA3	SB2	SF2	SG4	SH1	SH3
Chloramphenicol (30 µg)	S	S	I	R	S	S	R	S	S	S
Erythromycin (15 µg)	R	R	R	S	S	R	S	S	R	R
Kanamycin (30 µg)	S	S	S	S	S	S	S	S	R	S
Gentamicin (10 µg)	S	S	S	S	S	S	S	S	S	S
Polymyxin B (300 U)	R	S	R	I	I	S	S	I	S	S
Streptomycin (10 µg)	I	S	I	S	R	S	S	S	S	S

S: Susceptible; I: Intermediate; R: Resistance
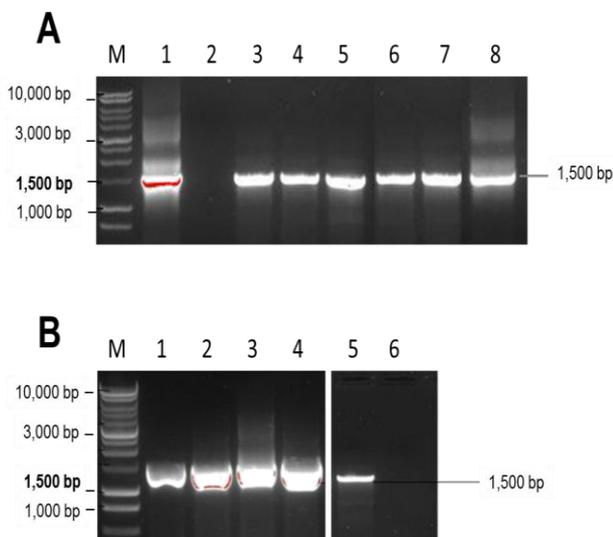
Figure 1: PCR products of the 16S rDNA gene of Gram-negative bacteria (A) and Gram-positive bacteria (B) on 1.2% agarose gel stained with ethidium bromide. In (A), Lane M: 1kb DNA marker, lane 1: positive control (*E. coli*), lane 2: negative control, lane 3: sample S11, lane 4: sample S12, lane 5: sample S41, lane 6: sample SH1, lane 7: sample SH3, lane 8: sample SA3. In (B), M: 1kb DNA marker, 1: sample SG4, 2: sample SF2, 3: sample SB2, 4: sample S72, 5: positive control (*S. aureus*), 6: negative control.

to an uncultured bacterium which was further analysed with phylogenetic analysis. Through the analysis, sample S11 was identified as identical to *S. marcescens* bacteria species, however, for sample S41, it was identified as *S. marcescens* in both BLAST and phylogenetic results. *S. marcescens* is a Gram-negative bacterium, belongs to *Enterobacteriaceae* family and commonly isolated from the environment, especially soil and water (Pinna *et al.*, 2011), where it might infect eyes of CL wearer due to lack of hygiene during CL handling. Antibiotic resistance event occurred in this bacterium is due to the presence of drug resistance gene in the *S. marcescens* bacteria species. This species was reported to exhibit inherent resistance towards some antimicrobial groups such as beta-lactam and tetracycline (Livermore and Brown, 2001; Stock *et al.*, 2003; Moradigaravand *et al.*, 2016). Polymyxin B that were commonly used to treat eye infections also seems to be ineffective against *S. marcescens* as observed in Table 2. According to a study by Lin *et al.* (2014), the emergence of antibiotic resistance traits in *S. marcescens* towards polymyxin B is due to a mutation of a gene that acts as a regulator to polymyxin B susceptibility in this bacteria species, causing it to unable to be expressed. A finding reported by Chaidaroon and Supalaset (2016) also shows that *S. marcescens* can cause keratitis followed with ring infiltrates favours to CL wearing in a corneal scrap sample obtained from a patient with immunocompromised disease and was reported with overnight used of CL. Another study reported by Pinna *et al.* (2011) also found *S. marcescens* from corneal ulcers

Table 3: Sequence analysis by BLAST.

Sample ID	Species	Accession no.	Similarity (%)	Query cover (%)
S11	Uncultured bacterium	KF081635	92	82
S12	Uncultured *Klebsiella* sp.	KC907122	94	84
S41	*Serratia marcescens*	MF773753	93	98
SH1	*Acinetobacter variabilis*	MF582331	96	99
SH3	*Acinetobacter variabilis*	MF582331	96	99
SA3	*Acinetobacter variabilis*	MF582331	99	100
SG4	*Bacillus cereus*	MK696420	98	98
SF2	*Bacillus cereus*	MH916584.1	99	99
SB2	*Bacillus anthracis*	JX307685	98	98
S72	*Bacillus cereus*	KF475795	99	98
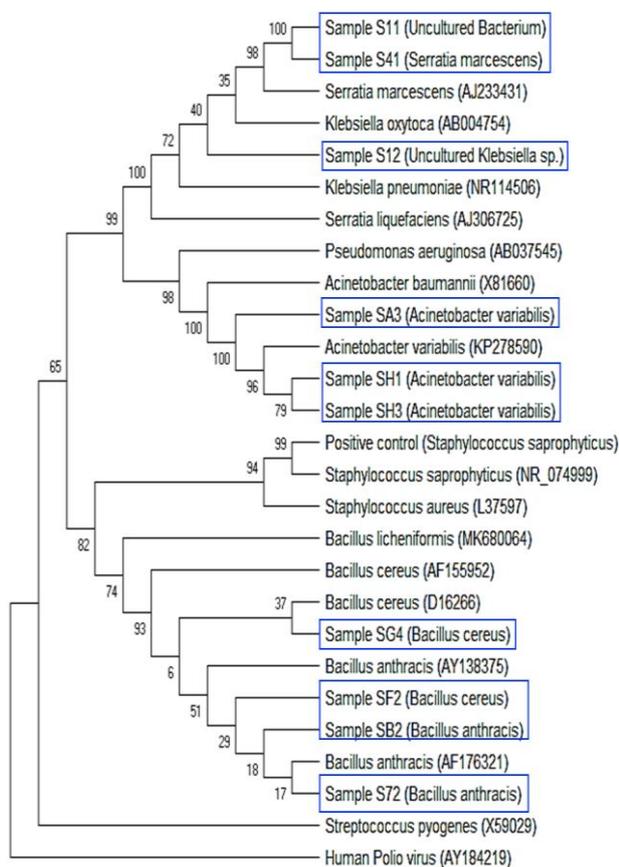
Figure 2: Phylogenetic analysis showing relationships of 16S rDNA gene sequences of samples (shown in boxes) with close homologs of the species.

due to soft CL wear. Collectively, these proved that *S. marcescens* has been recognized as one of the causes that lead to eye infections, especially due to CL wear. A study conducted by Chen *et al.* (2003) shows these bacteria were resistance towards certain antibiotics, such as erythromycin, which is corroborated with the findings in the antimicrobial profile of the bacterial sample S11 and S41.

The BLAST result for sample S12 16S rDNA gene sequence shows this bacterium was identified as uncultured *Klebsiella* sp. and through phylogenetic analysis of 16S rDNA, the result shows that this bacterium is identical to *K. pneumoniae*. *Klebsiella pneumoniae* is a Gram-negative bacterium that belongs to *Enterobacteriaceae* family categorized as rod-shape bacteria (Ashurst and Dawson, 2019). *Klebsiella pneumoniae* is commonly found in soil and normally cause pneumoniae, however, it also becomes a common cause for MK and other eye infections. According to Cumurcu *et al.* (2011), a case was reported where *K. pneumoniae* was identified in an eye infection associated to CL wear from corneal scrap of a 19 years old boy, where the CL worn by the patient with an extended period. A study by Bokaeian *et al.* (2014) also supports the finding in this study where 70% of *K. pneumoniae* isolated was also resistant towards erythromycin. The emergence of this antibiotic-resistant bacteria might be due to the ability of this bacteria to encoded a gene called erythromycin ribosome methylase (*erm*), where it leads to methylation of antibiotic ribosomal target and impaired the binding ability of the erythromycin to its target site (Depardieu *et al.*, 2007; Sun *et al.*, 2018).

The SH1, SH3, and SA3 sample were identified to be identical to *A. variabilis*. The manifestation of *A. variabilis* in CL-related eye infection is rarely found, however, the genus of *Acinetobacter* bacteria was found to cause keratitis that associated with CL wear as reported by Obrubov and Slonimskii (2018). *Acinetobacter* sp. bacteria not only cause CL-related keratitis, but it also was isolated from cataract patients, where it becomes a misleading cause of eye conjunctiva (Deephti *et al.*, 2018). Then, bacterial samples SH1 and SH3 used in this study shown to be antibiotic-resistance towards erythromycin, which is contradicting to a study reported by Cho *et al.* (2018), where *Acinetobacter* sp. used in that study was susceptible towards this particular antibiotic.

Cho *et al.* (2018) also shows that half of the *Acinetobacter* sp. in that study was resistance towards streptomycin, which is identical to bacteria sample SA3 used in this study.

Phylogenetic relationship of the samples SF2, S72, SG4 and SB2 were clustered together which indicates that it is closely related to *Bacillus* sp. The isolates SB2 and SF2 were clustered under the same group as seen in Figure 2. *Bacillus cereus* is often associated with an eye infection. *Bacillus cereus* causes a devastating disease that results in vision loss and inflammation within a short time. Even with prompt surgical and antimicrobial agent treatment, enucleation of the eye and blindness are common when eyes are severely infected with *B. cereus* (Drobniewski, 1993). It is also shown that *B. cereus* was susceptible to vancomycin, gentamicin, and chloramphenicol (Ikeda *et al.*, 2015). In contrast to our study as SF2 (*B. cereus*) sample was resistance towards chloramphenicol and possible reason for this situation is *B. cereus* might have developed resistance towards chloramphenicol overtime.

Another *Bacillus* group identified in this study is *B. anthracis*. *Bacillus anthracis* is soil bacteria that can cause anthrax (Amraoui *et al.*, 1992; Carlson *et al.*, 2018). *Bacillus cereus*, *B. anthracis*, *B. thuringiensis* and *B. mycoides* are grouped by phenotype within the *B. cereus* group. *Bacillus anthracis* is rarely found associated with eye infections. *B. anthracis* isolates were susceptible to all of the tested antibiotics except erythromycin. The result obtained was comparable similar to the study conducted by Luna *et al.* (2007) where sample SB2 that were identified as *B. anthracis* was resistant towards erythromycin.

Although this study has provided significant information on the possibilities of antibiotic-resistant bacteria to be present on CL case when it is already reached its recommended length of use, it is acknowledged that there are few limitations that should be overcome like the limited number of samples being taken. A larger sample size should be considered in the future where it may provide an opportunity to determine the relationship between the types of bacterial contamination and its microbial burden to the specific types of CL cases, where such information may be useful in predicting the mechanisms to prevent bacterial colonization and contamination in CL cases.

CONCLUSION

In conclusion, this study has shown the highlight of antibiotic-resistant bacteria isolated from CL cases that were contaminated with bacteria due to poor hygiene and mishandling among CL wearer. The bacteria were identified as *B. cereus*, *B. anthracis*, *S. marcescens*, *K. pneumoniae*, and *A. variabilis* where some bacteria were shown to be resistant and intermediate to commonly used antibiotics in treating eye infections. High percentage of bacteria isolated from the sample indicated that the high potential of microbial contamination in CL wearers. Despite assuming that most CL users are aware on the

basic CL care and handling, the contamination rate as presented in this study, is relatively high. The identification of CL microbial contamination in broader studies should also be underscored to increase awareness on the importance of proper CL handling avoiding severe microbial contamination in eyes.

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REFERENCES

Amraoui, A., Tabbara, K. F. and Zaghloul, K. (1992). Anthrax of the eyelids. *British Journal of Ophthalmology* **76(12), 753-754.**

Ashurst, J. V. and Dawson, A. (2019). *Klebsiella pneumonia*. Stat Pearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK519004/ [Retrieved at 21st July 2020].

Bokaeian, M., Saeidi, S., Shahi, Z. and Kadaei, V. (2014). TetA and tetB genes in *Klebsiella pneumoniae* isolated from clinical samples. *Gene, Cell and Tissue* **1(2), e18152.**

Bourcier, T., Thomas, F., Borderie, V., Chaumeil, C. and Laroche, L. (2003). Bacterial keratitis: Predisposing factors, clinical and microbiological review of 300 cases. *British Journal of Ophthalmology* **87(7), 834-838.**

Carlson, C. J., Getz, W. M., Kausrud, K. L., Cizauskas, C. A., Blackburn, J. K., Bustos Carrillo, F. A., Colwell, R., Easterday, W. R., Ganz, A. H., Kamath, P. L., Økstad, O. A., Turner, W. C., Kolsto, A. B. and Stenseth, N. C. (2018). Spores and soil from six sides: Interdisciplinarity and the environmental biology of anthrax (*Bacillus anthracis*). *Biological Reviews* **93(4), 1813-1831.**

Carnt, N. and Stapleton, F. (2016). Strategies for the prevention of contact lens-related *Acanthamoeba* keratitis: A review. *Ophthalmic and Physiological Optics* **36(2), 77-92.**

Chadha, T. (2014). Bacterial biofilms: Survival mechanisms and antibiotic resistance. *Journal of Bacteriology and Parasitology* **5(3), 1-4.**

Chaidaroon, W. and Supalaset, S. (2016). Corneal ring infiltrates caused by *Serratia marcescens* in a patient with human immunodeficiency virus. *Case Rep Ophthalmology* **7(2), 359-363.**

Chen, J., Lee, E. W., Kuroda, T., Mizushima, T. and Tsuchiya T. (2003). Multidrug resistance in *Serratia marcescens* and cloning of genes responsible for the resistance. *Biological and Pharmaceutical Bulletin* **26(3), 391-393.**

Cho, G. S., Li, B., Rostalsky, A., Fiedler, G., Rösch, N., Igbinosa, E., Kabisch, J., Bockelmann, W., Hammer, P., Huys, G. and Franz, C. M. (2018). Diversity and antibiotic susceptibility of *Acinetobacter*

strains from milk powder produced in Germany. *Frontiers in Microbiology* **9(536), 1-12.**

Clinical and Laboratory Standards Institute (CLSI) (2017). Performance standards for antimicrobial susceptibility testing. 27th edn. CLSI supplement M100. Wayne, Pennsylvania.

Cumurcu, T., Firat, P., Ozsoy, E., Cavdar, M. and Yakupogullari Y. (2011). Contact-lens-related corneal ulcer caused by *Klebsiella pneumoniae*. *Clinics (Sao Paulo)* **66(8), 1509-1510.**

Deepthi, K. G., Jayasudha, R., Girish, R. N., Manikandan, P., Ram, R., Narendran, V. and Prabagaran, S. R. (2018). Polybacterial community analysis in human conjunctiva through 16S rRNA gene libraries. *Experimental Eye Research* **174, 1-12.**

Depardieu, F., Podglajen, I., Leclercq, R., Collatz, E. and Courvalin, P. (2007). Modes and modulations of antibiotic resistance gene expression. *Clinical Microbiology Reviews* **20(1), 79-114.**

Drobniewski, F. A (1993). *Bacillus cereus* and related species. *Clinical Microbiology Reviews* **6(4), 324-338.**

Edwards, U., Rogall T., Blöcker, H., Emde, M. and Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research* **17(19), 7843-7853.**

Emina, M. O. and Idu, F. K. (2011). Bacteria and parasites in contact lenses of asymptomatic wearers in Nigeria. *Journal of Optometry* **4(2), 69-74.**

Fleiszig, S. M., Kroken, A. R., Nieto, V., Grosser, M. R., Wan, S. J., Metruccio, M. M. and Evans, D. J. (2020). Contact lens-related corneal infection: Intrinsic resistance and its compromise. *Progress in Retinal and Eye Research* **76, 100804.**

Fonn, D. and Jones, L. (2019). Hand hygiene is linked to microbial keratitis and corneal inflammatory events. *Contact Lens and Anterior Eye* **42(2), 132-135.**

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. https: //asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro [Retrieved at 6th June 2020].

Ikeda, M., Yagihara, Y., Tatsuno, K., Okazaki, M., Okugawa, S. and Moriya, K. (2015). Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections. *Annals Clinical Microbiology and Antimicrobials* **14, 43.**

Jindal, B. A. K., Pandya, M. K. and Khan, M. I. D. (2015). Antimicrobial resistance: A public health challenge. *Medical Journal Armed Forces India* **71(2), 178-181.**

Levy, S. B. (2002). Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **49(1), 25-30.**

Li, B., Yu, R., Liu, B., Tang, Q., Zhang, G., Wang, Y., Xie, G. and Sun, G. (2011). Characterization and comparison of *Serratia marcescens* isolated from edible cactus and from silkworm for virulence potential and chitosan susceptibility. *Brazilian Journal of Microbiology* **42(1), 96-104.**

Lim, C. H. L., Carnt, N. A., Farook, M., Lam, J., Tan, D. T., Mehta, J. S. and Stapleton, F. (2016). Risk factors for contact lens-related microbial keratitis in Singapore. *Eye* **30(3), 447-455.**

Lin, Q. Y., Tsai, Y. L., Liu, M. C., Lin, W. C., Hsueh, P. R. and Liaw, S. J. (2014). *Serratia marcescens arn*, a PhoP-regulated locus necessary for polymyxin B resistance. *Antimicrobial Agents and Chemotherapy* **58(9), 5181-5190.**

Livermore, D. M. and Brown, D. F. J. (2001). Detection of β-lactamase-mediated gene. *Journal of Antimicrobial Chemotherapy* **48(1), 59-64.**

Luna, V. A., King, D. S., Gulledge, J., Cannons, A. C., Amuso, P. T. and Cattani, J. (2007). Susceptibility of *Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides* and *Bacillus thuringiensis* to 24 antimicrobials using Sensititre® automated microbroth dilution and Etest® agar gradient diffusion methods. *Journal of Antimicrobial Chemotherapy* **60(3), 555-567.**

MacGowan, A. and Macnaughton, E. (2017). Antibiotic resistance. *Medicine* **45(10), 622-628.**

Mohd-Ali, B. and Tan, X. L. (2019). Patterns of use and knowledge about contact lens wear amongst teenagers in rural areas in Malaysia. *International Journal of Environmental Research and Public Health* **16(24), 5161.**

Mohidin, N. and Fung, L. T. (2009). A survey of optometric contact lens prescribing in Malaysia. *Malaysian Journal of Health Sciences* **7(2), 59-72.**

Moradigaravand, D., Boinett, C. J., Martin, V., Peacock, S. J. and Parkhill, J. (2016). Recent independent emergence of multiple multidrug-resistant *Serratia marcescens* clones within the United Kingdom and Ireland. *Genome Research* **26(8), 1101-1109.**

Morgan, P. B., Woods, C. A., Tranoudis, I., Helland, M., Efron, N., Jones, L. *et al.* **(2015).** International contact lens prescribing in 2014. *Contact Lens Spectrum* **28-33.**

Obrubov, A. S. and Slonimskii, A. Y. (2018). Contact lens-related keratitis and purulent corneal ulcers. *Vestnik Oftalmologii* **134(4), 17-24.**

Pinna, A., Usai, D., Sechi, L. A., Carta, A. and Zanetti, S. (2011). Detection of virulence factors in *Serratia* strains isolated from contact lens-associated corneal ulcers. *Acta Ophthalmologica* **89(4), 382-387.**

Smith, A. C. and Hussey, M. A. (2005). Gram Stain Protocols. American Society for Microbiology. https: //asm.org/ getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3 / gram-stain-protocol-2886. pdf [Retrieved at 7th June 2020].

Spernovasilis, N., Maraki, S., Kokorakis, E., Kofteridis, D., Tsilimbaris, M., Siganos, C. and Samonis, G. (2020). Antimicrobial susceptibility of isolated pathogens from patients with contact lens-related bacterial keratitis in Crete, Greece: A ten-year analysis. *Contact Lens and Anterior Eye*. In-press.

Stackebrandt, E. and Liesack. W. (1993). Nucleic acids and classification. *In*: Handbook of New Bacterial Systematics. Goodfellow, M. and O'Donnell, A. G. (eds.). Academic Press, London, England. **pp. 152-189.**

Stock, I., Grueger, T. and Wiedemann, B. (2003). Natural antibiotic susceptibility of strains of *Serratia marcescens* and the *S. liquefaciens* complex: *S. liquefaciens sensu stricto*, *S. proteamaculans and S. grimesii*. *International Journal of Antimicrobial Agents* **22(1), 35-47.**

Subedi, D., Vijay, A. K. and Willcox, M. (2018). Overview of mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*: An ocular perspective. *Clinical and Experimental Optometry* **101(2), 162-171.**

Sun, X., Lin, Z. W., Hu, X. X., Yao, W. M., Bai, B., Wang, H. Y., Li, D. Y., Chen, Z., Cheng, H., Pan, W. G., Deng, M. G., Xu, G. J., Tu, H. P., Chen, J. W., Deng, Q. W., Yu, Z. J. and Zheng, J. X. (2018). Biofilm formation in erythromycin-resistant *Staphylococcus aureus* and the relationship with antimicrobial susceptibility and molecular characteristics. *Microbial Pathogenesis* **124, 47-53.**

Szczotka-Flynn, L. B., Pearlman, E. and Ghannoum, M. (2010). Microbial contamination of contact lenses, lens care solutions, and their accessories: A literature review. *Eye Contact Lens* **36(2), 116-129.**

Tabbara, K. F., El-Sheikh, H. F. and Aabed, B. (2000). Extended wear contact lens related bacterial keratitis. *British Journal of Ophthalmology* **84(3), 327-328.**

Thakur, D. V. and Gaikwad, U. N. (2014). Microbial contamination of soft contact lenses and accessories in asymptomatic contact lens users. *The Indian Journal of Medical Research* **140(2), 307-309.**

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics* **40(4), 277-283.**

Watson, S., Cabrera-Aguas, M. and Khoo, P. (2018). Common eye infections. *Australian Prescriber* **41(3), 67-72.**

Willcox, M. D. P., Harmis, N., Cowell, B. A., Williams, T. and Holden, B. A. (2001). Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. *Biomaterials* **22(24), 3235-3247.**

Wu, Y. T. Y., Willcox, M., Zhu, H. and Stapleton, F. (2015). Contact lens hygiene compliance and lens case contamination: A review. *Contact Lens and Anterior Eye* **38(5), 307-316.**

Zimmerman, A. B., Nixon, A. D. and Rueff, E. M. (2016). Contact lens associated microbial keratitis: Practical considerations for the optometrist. *Clinical Optometry* **8, 1-12.**