RESEARCH ARTICLE

Emerging Bacterial Eye Infection: Identification, Susceptibility and Immunotherapy of *Kocuria* Species in Patients of Basrah, Iraq

Israa Asaad Aziz[1,2] Marwan Y. Al-Maqtoofi[2] Ahmed A. Burghal[2*]

[1]*Basrah Health Department, Basrah Teaching Hospital, Ministry of Health, Basrah, Iraq*
[2]*Department of Biology, College of Science, University of Basrah, Basrah, Iraq*

Received: 20th October, 2022; Revised: 10th November, 2022; Accepted: 02nd December, 2022; Available Online: 25th December, 2022

ABSTRACT

Ocular infections are common and considered sight-threatening consequences Non-pathogenic commensal bacteria can cause a serious opportunistic pathogen among immunocompetent and immunocompromised individuals leading to blindness. The aim of this study was the identification bacteria isolated from ocular infections, investigate the antibiotic susceptibility, and using interleukin 17 (IL-17) as the immunotherapeutic agent to support the patient immune system. 33 eye swab samples were collected from people with microbial eye infections including (conjunctivitis, dacrocystis, corneal ulcers, and post-traumatic). Recovered isolates were gram stained and tested for antibiotic susceptibility. Out of 36 swabs, 14 bacterial isolates were identified. Gram positive bacteria were predominant isolates, pathogenic *Staphylococcus* species 6 (42.85%) followed by non-pathogenic commensal bacteria *Kocuria spp*. 5 (35.71%). Gram negative bacteria including only *Moraxella* species 2 (14.28%). Antibiotic susceptibility testing indicated high sensitivity of recovered isolates to tetracycline and Ofloxacin and significant resistance to both benzylpenicillin and erythromycin. The recovered bacteria exhibited significant resistance to IL-17 *via* Immunotherapeutic assay *in-vitro*. In conclusion, non- pathogenic commensal bacteria *Kocuria spp*. seems to be predominant and IL-17 lack to antimicrobial activity *in-vitro*.

Keywords: Antibiotic susceptibility, Bacterial profile, Conjunctivitis, IL-17, Ocular infection.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.15

How to cite this article: Aziz IA, Al-Maqtoofi MY, Burghal AA. Emerging Bacterial Eye Infection: Identification, Susceptibility and Immunotherapy of Kocuria Species in Patients of Basrah, Iraq. International Journal of Drug Delivery Technology. 2022;12(4):1571-1575.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The eyes are sterilizing organs due to the present antimicrobial agent in tear flowing. Human eyes can become infected with microbes from the external environment. Inflammation that occurred may not be resolved but requires immediate management.[1] A physical breach of surface epithelium due to trauma, surgery, injury or suppression of immune response due to treatment or cancer therapy might be resulting in bacterial eye infections.[2-4] However, bacterial infections can lead to visual impairments, blindness and life-threatening. Conjunctiva, lid and cornea are the most parts of the eye that are frequently infected. The most common microorganisms involved in eye infection are bacteria, followed by fungi and viruses. Bacteria are well recognized as source of ocular diseases for example conjunctivitis, scleritis, keratitis, blepharitis, canaliculitis, dacryocystitis, addition to internal infections including preseptal cellulitis, necrotizing fascitis and intraocular.[5,6] Distribution of ocular infections associated with many sources such as cosmetic and medical contact lenses, surgery, trauma, eye tear and immune system status.[7,8] That is why early diagnosis and management of microbial eye infections is essential for saving value organs.[8,9]

Bacteria associate with an eye infection in adults and children are *Staphylococcus aureus, Streptococcus pneumoniae, S. epidermidis, S. pyogenes, Escherichia coli, Escherichia Faecalis, Pseudomonas aeruginosa, and Haemophilus influenzae, Klebsiella pneumoniae, Acinetobactor, Serratia, Aeromonas, Fusobacterium, Proteus mirabilis, Pasteurella multocida, Moraxella catarrhalis* and H. influenza have been described as causing keratitis.1,[10–18] Bacterial ocular infection by *Kocuria spp.* are rare cases but might be unexpected source of infection resulting in serious health consequence.[19,20] For example, Kocuria rosea is commonly a non-pathogenic commensal inhabiting the skin. However, it can convert to the opportunistic pathogen in immunocompromised or even immunocompetent individuals under certain health conditions. For this reason, this study highlighted the consideration of *Kocuria spp.* as unusual ocular pathogenic agents. To our acknowledgment, this study reports the first case of ocular infection by *Kocuria spp.* in patients of Basrah, Iraq and provides a clinic-microbiological view along with antibiotic sensitivity and immunotherapy, and management.

**Author for Correspondence: ahmed.burghal@uobasrah.edu.iq*

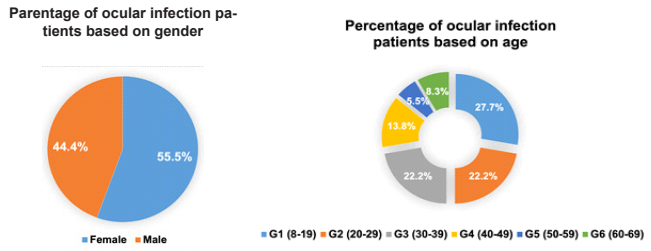

Figure 1: Percentage of ocular infection patients.

Table 1: Number of Bacterial isolates recovered from eye infections swabs.

Eye infection case	*Total samples*	*Bacterial isolates*
Conjunctivitis	23	11
Keratitis	8	1
Dacryocystitis	3	0
Post-trauma	2	2
Total	36	14

MATERIAL AND METHODS

Sample collection

Collection of samples was between October 2020 to March 2021 from Basrah Teaching Hospital, Basrah, Iraq. A total number of collected swab sample was 36 from eye infections. Patient group was 20 female and 16 males. Based on age, patients were divided into six groups G1 (8 -19), G2 (20 – 29), G3 (30 – 39), G4 (40 – 49), G5 (50 – 59) and G6 (60 – 69) in addition to a control group of 15 healthy individuals. All patients were clinically diagnosed with (conjunctivitis, dacrocystis, corneal ulcers, and post-traumatic) by the ophthalmologist with contact lens or without. Patients who had antibacterial treatment were excluded from the study.

Bacterial Isolation and purification

All the swabs were inoculated immediately on blood agar, MacConkey's agar and nutrient agar. The inoculated media were incubated in 37°C for 24 hours aerobically. Recovered colonies were sub-cultured to new plates for purification. The total number of recovered bacteria was 14 axenic isolates. Fungal growth was also purified and sub-cultured, then excluded from this study.

Identification of Recovered Bacteria

Bacterial axenic cultures were stained by Gram stain for first identification. Then were identified by standard laboratory procedures using biochemical identification of Vitek-2 Compact (Biomerux, VK2C11613, France) (system to diagnosis all bacterial isolates following.[21]

Antibiotic Susceptibility testing

Manual susceptibility testing of bacterial isolates were performed by Kirby-Bauer procedure for some isolates that didn't appearance by Vitek-2 system. The Kirby-Bauer method was used for the establisher test.[22] In this study, the values of sensitivity and resistance to antibiotics were determined based on the Clinical and Laboratory Standard Institute C.L.S.I. (2017).

Immunotherapy Assay

Testing human IL-17A usage for immunotherapy as a natural immune system production of human was conducted against bacterial isolates. Human IL-17A was provided by (Al-shkairate establishment for medical supply. RDEP0797. Amman, Jordon) as a powder, processed through dilution and aliquot for preparation final concentrations 10, 50, 100 µg/mL under manufacture instructions.

RESULTS

Sample details

Total patient samples were 20 female and 16 males located in six age groups as presented in Figure 1.

Microbial Growth

A total of 14 axenic bacterial isolates were identified. Details of sample sources of ocular infections were shown in Table 1.

Among 23 swabs from conjunctivitis, only 10 swabs which yielded growth. Details of recovered and identified bacterial species were presented in (Figure 2 and Table 2) (Sharma, 2010).

Sensitivity tests

Susceptibility testing results of axenic bacterial isolates using Vitek-2 Compact that include 20 kind of antibiotics presented in Table 3 and Table 4. The results of susceptibility testing using 6 types of antibiotics through Kirby-Bauer method shown in Table 5 and Figure 3.

Table 2: Recovered bacteria isolates from swabs of infected eyes.

Bacterial species	*Conjunctival*	*Corneal*	*Post-trauma*	*Total*
Staphylococcus aureus	3	0	0	3
Staphylococcus haemolyticus (CoNS)	1	0	0	1
Staphylococcus hominis (CoNS)	1	0	0	1
Staphylococcus xylosus (CoNS)	1	0	0	1
Kocuria rosea	4	0	0	4
Kocuria varians	0	1	0	1
Granulicatella adiacens	0	0	1	1
Moraxella group	1	0	1	2
Total isolates	11	1	2	14

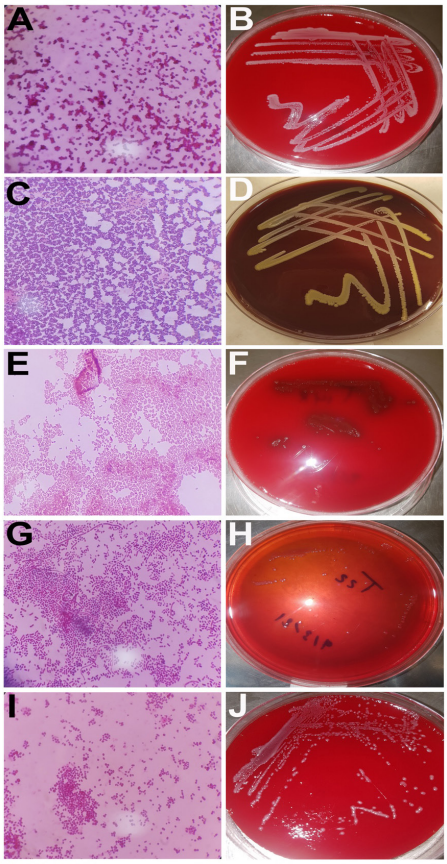

Figure 2: Microscopic examination of recovered bacteria with gram staining (left column) and growth on blood agar media (right column) as (A and B) Coagulase Negative *Staphylococci* (CoNS), (C and D) *Staphylococcus aureus*, (E and F) *Koucoria rosea*, (G and H) *Moraxella group*, (I and J) *Granulicatella adiacens.*

Immunotherapy Assay

All bacterial isolates were exhibited a significant resistance to human IL-17A aliquot *in-vitro* using NA plates as shown in Figure 4.

DISCUSSION

Ocular infection is one of the main causes of eye redness and discharge. The frequent of this infection varies according to many factors including gender, age and immune status. Bacteria are the major source of ocular infections worldwide. Early diagnosis of these infections is essential step for treating the source. However, untreated infected eye can lead to serious damage of ocular structures and resulting in blindness or visual impairments or even death at advance stage of dissemination. To the best of our knowledge, there is little known about ocular infections caused by opportunistic bacterial species and commensal bacteria including *Koucoria*, *Moraxella* and *Granulicatella* with far too little attention has been paid. Meanwhile, these microbes can be life threatening causing serious health consequences if not highlighted and managed. For this reason, this project aimed to scan and highlight unusual bacterial species in patients with clinical eye infections in order to increase awareness and consideration in term of diagnosis and medicine for saving value organs, eyes and life. The distribution of each bacterial isolate among the different type of ocular infections might be determined by variety of factors. For instance, using contact lens could be a serious factor in bacterial keratitis. In addition, the immune system status and age are also a critical factor of infections.[9]

Table 3: Susceptibility testing done by Vitek-2 system for Gram positive bacteria

Antibiotics	*S. aureus (3)*	*S. haemolyticus*	*S. hominis*	*S. xylosus*
Benzylpenicillin	3R	R	S	R
Oxacillin	2 S / 1R	R	S	S
Gentamicin	3S	S	S	S
Ciprofloxacin	1S /--		S	S
Erythromycin	1S/ 1I / 1R	R	I	R
Clindamycin	3S	I	S	S
Linezolid	3S	S	S	S
Daptomycin	1S/--		S	S
Teicoplanin	3S	R	S	S
Vancomycin	3S	R	S	S
Tetracycline	3S	R	S	S
Tigecycline	3S	S	S	S
Fosfomycin	1S/--	--	S	R
Fusidic acid	1S/--	--	S	S
Trimethoprim	3S	S	S	S
Tobramycin	-/ 2S	S	--	--
Levofloxacillin	-/ 2S	S	--	--
Moxifloxacin	-/ 2S	S	--	--
Nitrofurantoin	-/ 2S	S	--	--
Rifampicin	-/ 1S / 1R	R	--	--

Table 4: Susceptibility testing done by Vitek-2 system for gram negative bacteria

Antibiotics	*Moraxella species*
Piperacillin	S
Ceftazidime	S
Aztreonam	S
Imipenem	S
Meropenem	S
Amikacin	S
Gentamycin	S
Netilmicin	S
Tobramycin	S
Ciprofloxacin	S
Levofloxacin	S
Tetracycline	S
Tigecycline	S
Colistin	S
Trimethoprim/Sulamethoxazole	S

Table 5: Susceptibility testing using Kirby-Bauer method.

Antibiotics	*Kocuria rosea*	*Kocuria varians*	*Granulicatella adiacens*	*Moraxella group*
Penicillin	4S	S	R	R
Tetracycline	4S	S	S	S
Ampicillin	4S	S	S	I
Erythromycin	3S/ 1I	S	I	S
Ofloxacin	4S	S	S	S
Rifampicin	4S	S	R	R

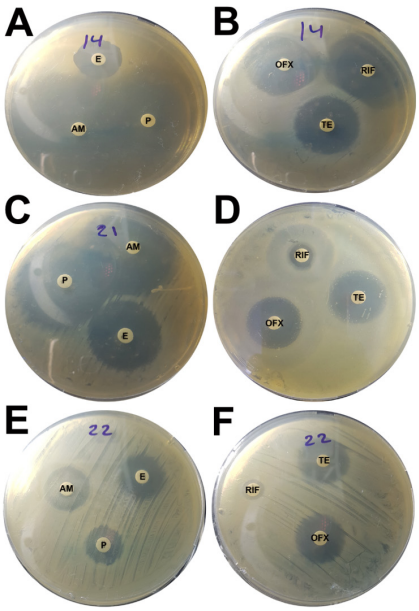

Figure 3: Antibiotic susceptibility testing using manual Kirby-Bauer method for *Koucoria rosea* (A and B), *Moraxella* group (C and D) and *Granulicatella adiacens* (E and F) where P: penicillin, AM: Ampicillin, E: erythromycin, TE: tetracycline, RIF: rifampicin, OFX: Ofloxacin.

Therefore, using extensive immune suppression drugs such as corticosteroids leading to a significant association with ocular infections through proinflammatory cytokine production inhibition such as IL-17A.23,24 During this study, the high occurrence of ocular infection was reported in female 20 (55.55%) comparing to male 16 (44.44%) patients who attended to eye infection unit at the hospital in Basrah. This finding might be due to using contaminated contact lenses by women more than men in addition to dry eye, dirtiness or even hosting bets. Previous studies reported the coagulase-negative *Staphylococci* and *S. aureus* were the most prevalent pathogenic bacterial species isolated from ocular infections, including conjunctivitis.[16,17,25–27] In this study, the predominant source of ocular infection was by pathogenic Staphylococcus species 6 isolates (42.8%) followed directly by floral bacterial including *Koucoria* spp. 5 (35.71%). In addition, another less frequent causative bacteria such as *Moraxella* and *Granulicatella* were also isolated. These results provide an obvious evidence about non-pathogenic commensals microbes found on skin and mucous membrane of human can be a serious sight threatening agent. For this reason high attention

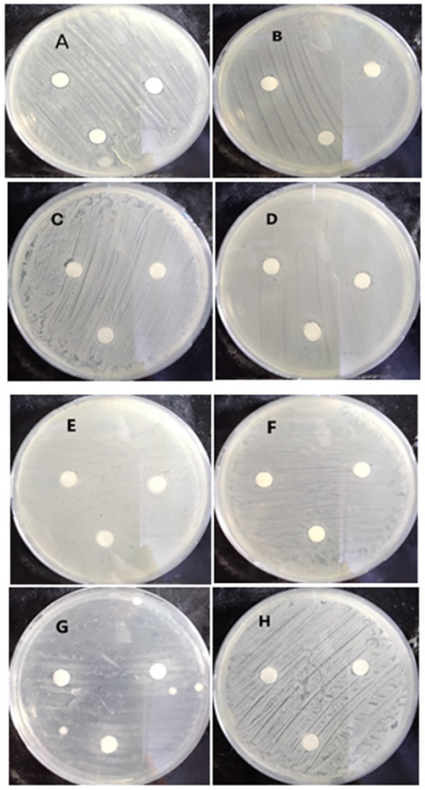

Figure 4: Activity of IL-17 as immunotherapeutic agents against bacterial isolates which (**A** and **B**) *Koucoria rosea*, (**C** and **D**) *Staphylococcus aureus*, **E**: *Granulicatella adiacens*, **F**: *Moraxella* group, **G**: *Staphylococcus haemolyticus* and **H**: *Koucoria varians*

and consideration should pay during handling any recovered microfloral bacteria from clinical eye swab samples. Based on that, early and accurate diagnosis and management could prevent an outbreak eye infections in Basrah, Iraq or even worlwide. To manage control non-pathogenic bacteria that isolated, antibiotic sensitivity profiling was inverstigating, in addition to using IL-17A as an immunotherapeutic agent. The result indicated to the possible control and management of non-pathogenic commensals bacterial and preventing causing ocular infections via treating with tetracycline or oflocxacin that work through blocking nucleic acid synthesis of bacterial cells such as *Koucoria* spp., *Moraxella* and *Granulicatella* exhibited a sensitivity toward these antibiotics. Meanwhile, *Moraxella* and *Granulicatella* showed resistance to both penicillin and rifampicin. These antibiotics should eliminate from the list of treating these kinds of bacteria. In addition, there was a significant resistance of bacterial isolated in this study to IL-17A. This could be due to previous exposure to IL-17A by the patient immune system as all these tested bacteria were directly isolated from human ocular infections. This suggests, previous exposure to IL-17A might give a chance to bacterial cells developing a certain resistance mechanism against IL-17A.

CONCLUSION

Non-pathogenic commensal bacteria such as *Koucoria* spp were predominantly isolated from human ocular infections

in both adult and children after *Staphylococcus* species. This outcome grape attention to consider any growth of normal flora bacteria in eye swab samples. Antibiotic sensitivity profile for control and managing *Koucoria* spp., *Moraxella* spp. and *Granulicatella* spp. showed treating these infectious agents by tetracycline or oflocxacin as exhibited a high sensitivity to these drugs. Penicillin and rifampicin should be avoided for treating *Moraxella* spp. and *Granulicatella spp.* due to their significant resistance. In terms of immunotherapeutic, IL-17A does not show any antibacterial activity to these microbes.

REFERENCE

1. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. Bacterial profile of ocular infections: a systematic review. BMC ophthalmology. 2017 Dec;17(1):1-9.
2. Kowalski RP, Nayyar SV, Romanowski EG, Shanks RM, Mammen A, Dhaliwal DK, Jhanji V. The prevalence of bacteria, fungi, viruses, and acanthamoeba from 3,004 cases of keratitis, endophthalmitis, and conjunctivitis. Eye & contact lens. 2020 Sep 1;46(5):265-8.
3. Sharma S. Ocular infections: research in India. Indian journal of medical microbiology. 2010 Apr 1;28(2):91.
4. Song X, Xu L, Sun S, Zhao J, Xie L. Pediatric microbial keratitis: a tertiary hospital study. European journal of ophthalmology. 2012 Mar;22(2):136-41.
5. Bertino Jr JS. Impact of antibiotic resistance in the management of ocular infections: the role of current and future antibiotics. Clinical ophthalmology (Auckland, NZ). 2009;3:507.
6. Cervantes LJ, Mah FS. Clinical use of gatifloxacin ophthalmic solution for treatment of bacterial conjunctivitis. Clinical Ophthalmology. 2011 Apr 18:495-502.
7. Grzybowski A, Brona P, Kim SJ. Microbial flora and resistance in ophthalmology: a review. Graefe's Archive for Clinical and Experimental Ophthalmology. 2017 May;255(5):851-62.
8. Willcox MD. Pseudomonas aeruginosa infection and inflammation during contact lens wear: a review. Optometry and Vision Science. 2007 Apr 1;84(4):273-8.
9. Cao J, Yang Y, Yang W, Wu R, Xiao X, Yuan J, Xing Y, Tan X. Prevalence of infectious keratitis in Central China. BMC ophthalmology. 2014 Dec;14(1):1-6.
10. Assefa Y, Moges F, Endris M, Zereay B, Amare B, Bekele D, Tesfaye S, Mulu A, Belyhun Y. Bacteriological profile and drug susceptibility patterns in dacryocystitis patients attending Gondar University Teaching Hospital, Northwest Ethiopia. BMC ophthalmology. 2015 Dec;15(1):1-8.
11. Getahun E, Gelaw B, Assefa A, Assefa Y, Amsalu A. Bacterial pathogens associated with external ocular infections alongside eminent proportion of multidrug resistant isolates at the University of Gondar Hospital, northwest Ethiopia. BMC ophthalmology. 2017 Dec;17(1):1-0.
12. Iwalokun BA, Oluwadun A, Akinsinde KA, Niemogha MT, Nwaokorie FO. Bacteriologic and plasmid analysis of etiologic agents of conjunctivitis in Lagos, Nigeria. Journal of ophthalmic inflammation and infection. 2011 Sep;1(3):95-103.
13. Kaliamurthy J, Kalavathy CM, Parmar P, Nelson Jesudasan CA, Thomas PA. Spectrum of bacterial keratitis at a tertiary eye care centre in India. BioMed research international. 2013 Oct;2013.
14. Karsten E, Watson SL, Foster LJ. Diversity of microbial species implicated in keratitis: a review. The open ophthalmology journal. 2012;6:110.
15. Mshangila B, Paddy M, Kajumbula H, Ateenyi-Agaba C, Kahwa B, Seni J. External ocular surface bacterial isolates and their antimicrobial susceptibility patterns among pre-operative cataract patients at Mulago National Hospital in Kampala, Uganda. BMC ophthalmology. 2013 Dec;13(1):1-6.
16. Shiferaw B, Gelaw B, Assefa A, Assefa Y, Addis Z. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia. BMC ophthalmology. 2015 Dec;15(1):1-8.
17. Shimizu Y, Toshida H, Honda R, Matsui A, Ohta T, Asada Y, Murakami A. Prevalence of drug resistance and culture-positive rate among microorganisms isolated from patients with ocular infections over a 4-year period. Clinical Ophthalmology (Auckland, NZ). 2013;7:695.
18. Wallin Ö, Al-ahramy AM, Lundström M, Montan P. Endophthalmitis and severe blebitis following trabeculectomy. Epidemiology and risk factors; a single-centre retrospective study. Acta Ophthalmologica. 2014 Aug;92(5):426-31.
19. Ali MJ, Pujari A, Motukupally S, Naik MN. Kocuria rosea canaliculitis: a clinicomicrobiological correlation. Ophthalmic Plastic & Reconstructive Surgery. 2014 Nov 1;30(6):e139-40.
20. Pedro-Aguilar L, Ramirez-Miranda A, Bautista-de Lucio VM, Navas A, Ortiz-Casas M, Graue-Hernandez EO. Epidemiology and outcomes of kocuria keratitis. Eye & Contact Lens. 2016 Sep 1;42(5):e20-4.
21. Pincus DH. New Vitek 2 Colorimetric GN Card for Identification of Gram-Negative Nonfermentative Bacilli. Journal of clinical microbiology. 2007 Dec;45(12):4094-5.
22. Bauer AW. Antibiotic susceptibility testing by a standardized single disc method. Am J clin pathol. 1966;45:149-58.
23. Henry CR, Flynn Jr HW, Miller D, Forster RK, Alfonso EC. Infectious keratitis progressing to endophthalmitis: a 15-year study of microbiology, associated factors, and clinical outcomes. Ophthalmology. 2012 Dec 1;119(12):2443-9.
24. Ly CN, Pham JN, Badenoch PR, Bell SM, Hawkins G, Rafferty DL, McClellan KA. Bacteria commonly isolated from keratitis specimens retain antibiotic susceptibility to fluoroquinolones and gentamicin plus cephalothin. Clinical & experimental ophthalmology. 2006 Jan;34(1):44-50.
25. Long C, Liu B, Xu C, Jing Y, Yuan Z, Lin X. Causative organisms of post-traumatic endophthalmitis: a 20-year retrospective study. BMC ophthalmology. 2014 Dec;14(1):1-7.
26. Mascarenhas J, Srinivasan M, Chen M, Rajaraman R, Ravindran M, Lalitha P, Oldenburg CE, Ray KJ, Glidden DV, Costanza S, Lietman TM. Differentiation of etiologic agents of bacterial keratitis from presentation characteristics. International ophthalmology. 2012 Dec;32(6):531-8.
27. Petrillo F, Pignataro D, Di Lella FM, Reibaldi M, Fallico M, Castellino N, Parisi G, Trotta MC, D'Amico M, Santella B, Folliero V. Antimicrobial susceptibility patterns and resistance trends of staphylococcus aureus and coagulase-negative staphylococci strains isolated from ocular infections. Antibiotics. 2021 May;10(5):527.