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A study on the most Prevalent Bacterial cause of Corneal ulcer and their Susceptibility to five common types of Ophthalmic Antibiotics

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Abstract

Proper diagnosis of the corneal ulcer is one the most important efforts in the eyes medical urgencies. The aim of this study was to determine the bacteriological profile and in vitro antibiotic resistance of the bacteria isolated from the eyes of patients with infectious corneal ulcers In this study, 94 patients with corneal ulcer disease participated. After differential diagnosis of potentiated corneal ulcer infection and sampling of the active area of the wounds, the sample was transferred to the laboratory for cultivation in the suitable culture medium and finally incubatedin proper temperature. In cases of positive culture, the type of bacteria and antibiotic sensitivity test of the broth micro-dilution method was performed and evaluated for five antibiotics including ciprofloxacin, levofloxacin, gentamycin, erythromycin, and chloramphenicol.Our results indicated that patients consisted of 55% male and 45% female, 51% of whom were positive and the most common bacterium was staphylococcus negative coagulase with 48% prevalence. The isolated bacteria sensitivity for levofloxacin, ciprofloxacin, gentamycin, erythromycin and chloramphenicol was 94%, 79%, 67%, 33%, 27%, respectively. *In vitro* study for levofloxacin and ciprofloxacin showed ahigher percentage of antibiotic sensitivity in patients with corneal infectious ulcers in comparisonto other antibiotics. However, Erythromycin and chloramphenicol were not suitable for the bacterial corneal ulcer treatment due to the high microbial resistance. Accurate and precise training of physicians in the prescribing of ocular antibiotics as well as the prevention of arbitrary use of these drugs is important for reducing the microbial resistance.

Keywords: Antibacterial Agent, Gram-Negative Bacterial Infections, Corneal Ulcer, Drug Resistance, Ophthalmic Solutions

1. Introduction

DMicrobial keratitis is a severe ocular infection that lead to corneal ulcer or even in some cases lead to loss of sight (1-3). Corneal ulcer, an infective or more seriously, inflammatory disease of the cornea involving disruption of its epitheliome layers along with involvement of the stroma of corneal, is one of the main causes of monocular blindness after cataract in several of the developing countries in Africa, Asia and the Middle East (4, 5). In addition, It is also a sight threatening condition that affects both females and males and all age ranges (6). The annual economic burden in USA in direct health care expenditures related to patients with keratitis and corneal ulcer is estimated to be $175 million (7). In the developing nations, the financial burden due to cases related to this diesease is uncertain but speculated to be tragic (8).

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Corneal ulcer may occur due to various factors such as bacteria, fungi, viruses and parasite (2). Depending on the geographical location, the main etiology of corneal infection may be different. For instance, in Singapore, North America, Australia and Netherlands the most causative agent of corneal ulcer is bacterial, while in Nepal and India the most causative agent is fungal (9). Nevertheless, Streptococcus pneumonia, Pseudomonas, other coagulase-negative Staphylococcus species, Staphylococcus epidermidis, and Staphylococcus aureus remain the most frequently encountered agents in bacterial keratitis (9).

The common management approach if bacterial infection is suspected is to collect specimen of corneal tissue for culture and subsequently to initiate antibiotics therapeutic options empirically (10). The clinical outcome in corneal ulcer is possible to be dependent on the infecting bacteria virulence, host factors including the host's immune system or the presence of ocular surface disorder, as well as the minimum inhibitory concentration (MIC) of the antibiotic against the bacteria (11).There is inadequate data about the in vitro response of common bacterial isolated from cases of corneal ulcer to concentrations of antibiotics that can be expected with frequent topical use, and hence the proper disc susceptibility breakpoint for each antibiotic and bacterial isolated has not been revealed (11). Drug resistance poses a major challenge to the management of various infection disease, and corneal ulcer is not an exception (12).

Corneal ulcer is a disorder requiring timely medical attention. Thus accurate knowledge of the causative agents and their susceptibility issue is essential for deciding the appropriate course of therapeutic options. To the best of our knowledge, the microbial etiology of corneal ulcer and its management in Iran has not fully understood. Thus, the purpose of this study was to determine the bacteriological profile of patients with corneal ulcer at the Khalili hospital in Shiraz and determine the incidence of antibiotic resistance during the study period.

2. Material and Methods

2.1. Study setting and design

This hospital based prospective study was carried out between October 2012 to March 2013 At the Khalil ophthalmology hospital which is the largest referral ophthalmology center in the south of Iran. 94 corneal scarping samples collected during the period of 6 months were included in the study. Informed consent was obtained from all individual participants included in the study. The Institutional Review Board and the Medical Ethics Committee of the hospital approved the study. Not being pregnant, not being monocular and not using antibiotics 48 hours prior to referral to emergency ward were the inclusion criteria. The data were collected by a clinical pharmacist and an by ophthalmologist.

2.2. Sample collection and laboratory processing

After taking a thorough history and clinical examination, the patient's corneal ulcer biopsy was collected under aseptic conditions by ophthalmologists, using 1-2 drops of tetracain without using any eyelid disinfectant. Biopsy was obtained from the deep part of the active ulcer by using number 15 sterile surgical blade. Each sample was transferred to tioglycolate medium and sent to the microbiology laboratory. After transition to the laboratory, the medium was incubated for 48 hours in 37 °C.

Briefly,the samples were also cultured in routine culture media (Blood agar and Chocolate agar) and inoculated 24 hours and subjected for microscopic examination. According to the colonies' shape and hemolysis, gram staining and diagnostic test were also performed. For gram positive cocci: Catalase test, Coagulase test by slide and tube method using rabbit plasma, Mannitol fermentation test in Mannitol salt agar medium, Dnase enzyme production test, Sensitivity determining test in bacitracin disk with unit of 0.04 were done for final diagnosis. For gram negative bacilli Oxidase test,Sulfide-Indole-Motility Agar-Merck Co, TSI medium culture, Citrate test, Urea disintegration test, Methyl red-Vagous,Prauskauser, and Oxidative were done for final diagnosis.

2.3. Antibiotic susceptibility test

Minimum inhibitory concentration (MIC) testing was performed by broth microdilution method according to Clinical and Laboratory Stan-

dards Institute (CLSI) guidelines. The antibiotics used were levofloxacin, ciprofloxacin, erythromycin, chloramphenicol and gentamicin. Various concentrations of antibiotic (0.01, 0.1, 1, 10, 50, 100, and 200 µg/mL) were added to equal volumes of LB broth medium with various concentrations of bacteria (~10^5, ~10^6 and ~10^7 Colony Forming Units (CFU)/mL). Absorbance (Optical Density (OD) λ 600 nm) as a measure of bacterial growth was assessed hourly up to 9 h and then at 24 h of incubation at 37 °C with shaking (200 rpm). Comparison of the 24 h OD readings for bacterial growth with and without antibiotics was used to calculate the lowest concentration of antibiotic that completely reduced the OD growth by 100% and concentration of antibiotic that reduced the OD growth by 50%.

2.4. Data analysis

The data obtained was analyzed by Statistical Package for Social Sciences (SPSS) version 19.0. Frequency and percentages were calculated.

3. Result

94 patients with infectious keratitis were enrolled in this study. Information on the patients' gender and age is presented in table 1. Of the 94 samples, 48(51%) samples were culture positive. 32 (66.7%) bacterial isolates were Gram positive, and 16 (33.3%) were Gram-negative. Coagulase negative Staphylococci(CONS) was the most common microorganisms isolated followed by *Pseudomonas aeruginosa* (Table 2). This is also worth mentioning that in another patients corneal ulceration may occur due to aother factors such as fungi, viruses and parasite.

Table 1. Demographic factors of corneal ulceration.

Demographic variables		N (%)
Sex	Male	52 (55.32%)
	Female	42 (44.68%)
Age (year)	1-9	1 (2.1)
	10-19	3 (6.2)
	20-29	3 (6.2)
	30-39	5 (10.4)
	40-49	8 (16.7)
	50-59	11 (22.9)
	60 ≥	17 (35.5)

Table 2. Distribution of most commonly isolated bacteria from cultures

Species Isolated	Number(%)
Coagulase negative	23(47.9)
Pseudomonas aeruginosa	10(20.8)
Staphylococcus aerus	9(18.7)
Escherichia coli	3(6.3)
Gram positive	32(66.7)
Gram negative	16(33.3)

MICs of *E. coli* and enterobacterand pseudomonas bacteria are listed in table 3; it is shown that the majority of bacterial isolates were susceptible to levofloxacin (141, 89.2%), amikacin (137, 93.2%), gentamicin (131, 89.1%), chloramphenicol (106, 70.2%) and doxycycline (100, 71.9%). However, bacterial isolates were less susceptible to erythromycin and chloamphenicol (79, 51.3%) levofloxacin has the best coverage against *E. coli* and enterobacter. Erythromycin and chloramphenicol revealedthe worst coverage against *E. coli* and enterobacter, and their resistance was 67% and 50%, respectively. Table 3 also shows the MIC of *Pseudomonas bacteria*. Levofloxacin had the best coverage against pseudomonas bacteria. Erythromycin and chloramphenicol had the worst coverage against pseudomonas, with a 80% resistance rate.

Table 4 represents that Levofloxacin had the best coverage against coagulase-negative CNS bacteria and only 4% of these bacteria were resistant to it. Chloramphenicol and erythromycin had the worst coverage against these bacteria, with 78% and 65% resistance to them, respectively. 22% of theCNS bacteria were resistant to ciprofloxacin and 35% to gentamicin. The MIC of Staphylococcus aureus. Levofloxacin had the best coverage against Staphylococcus aureus. Chloramphenicol and erythromycin, with 67% and 56% resistance to them, respectively, had the worse coverage. 22% of the coagulase negative staphylococci were resistant to ciprofloxacin and 33% to gentamycin.

Table 3 also shows that MIC for the gram-negative bacteria had the best protection against gram-negative bacteria, levofloxacin, and subsequently ciprofloxacin, and the worst coverage was for erythromycin and chloramphenicol.

Table 3. MICs of Coagulase Negative *Staphylococcus* Bacteria, *Staphylococcus aureus*, Enterobacteriaceae and *Pseudomonas*, Gram negative and positive bacteria.

Species isolated	Antibiotics used	Min IC µg/ml	Mac IC µg/ml	MIC50 µg/ml	MIC90 µg/ml	% susceptible
Coagulase Negative *Staphylococcus*	Ciprofloxacin	0.25>	32	0.5	8	78
	Gentamicin	0.5>	128	2	64	65
	Erythromycin	0.25>	128<	16	128	35
	Chloramphenicol	1	256<	64	256<	22
	Levofloxacin	0.25>	64	1	2	96
Staphylococcus aureus	Ciprofloxacin	0.25>	128	1	4	78
	Chloramphenicol	4	256<	32	256	33
	Erythromycin	0.25>	128<	4	32	44
	Levofloxacin	0.25>	4	0.5	2	89
Gram negative	Ciprofloxacin	0.25>	64	0.5	4	81
	Gentamicin	0.5>	128	2	32	69
	Erythromycin	0.5	128<	16	128<	25
	Chloramphenicol	2	256<	32	256<	31
	Levofloxacin	0.25>	8	0.5	2	94
Gram positive	Ciprofloxacin	0.25>	32	0.5	8	78
	Gentamicin	0.5>	256	2	64	66
	Erythromycin	0.25>	128<	8	128<	38
	Chloramphenicol	1	256<	32	256<	25
	Levofloxacin	0.25>	64	0.5	2	94
Enterobacteriaceae	Ciprofloxacin	0.25>	32	0.5	32>	83
	Gentamicin	0.5>	128	1	128>	67
	Erythromycin	0.5	128<	8	128>	33
	Chloramphenicol	2	256<	8	256	50
	Levofloxacin	0.25>	2	0.5	2	100
Pseudomonas	Ciprofloxacin	0.25>	64	1	4	80
	Gentamicin	0.5>	128	2	32	70
	Erythromycin	0.5	128<	64	128	20
	Chloramphenicol	8	256<	64	256	20

It also indicates the MIC susceptibility of gram-positive bacteria, which shows the best coverage against gram-positive bacteria, Levofloxacin and subsequently ciprofloxacin, and represents that the worst coverage was for chloramphenicol and erythromycin. Comparison of the predisposing factors of corneal ulcer in all patients in the present and previous studies are presented in Table 4.

According to the our results, levofloxacin had the best coverage against enterobacteriaceae bacteria and all of these bacteria were sensitive to it. Erythromycin and chloramphenicol revealed the worst coverage against this bacterium, which were 67% and 50% resistant, respectively. 17% of the enterobacteriacea bacteria were resistant to ciprofloxacin and 33% to gentamicin. Levofloxacin had the best coating against pseudomonas bacteria and 90% of these bacteria were sensitive to it. Erythromycin and chloramphenicol had the worst cover against this bacterium that has been 80% resistant to them. 20% of the pseudomonas bacteria were resistant to ciprofloxacin and 30% to gentamicin. Levofloxacin had the best coating against CONS bacteria and only 4% of these bacteria had been resistant to it. Chloramphenicol and erythromycin had the worst coverage against these

Table 4. Comparison of clinical presentations of corneal ulcersin the current study with another studies

Risk factors	No. (current study)	% (current study)	%In 1999	%In 2001	%In 2004
Blepharite	41	44	-	47.5	47
Trauma	22	23	57	20	-
Dry eye	35	37	12	45	41
Underlying diseases (ectropion-entropion-trichiasis-chronic dacryocystis)	19	20	9	10	24
Surgical style (Pearl - Corneal transplantation – Pterygium eye surgery)	15	16	-	-	22
An external object	10	11	-	7.5	-
Contact lens consumptuion	8	9	9	-	5

bacteria that were 78% and 65% resistantto them, respectively. 22% of the coagulase negative staphylococcal bacteria were resistant to ciprofloxacin and 35% to gentamicin. Levofloxacin had the best coating against *Staphylococcus aureus* bacteria, chloramphenicol and erythromycin which had the worst coverage, which was 67% and 56% resistant to them, respectively. 22% of the staphylococcal coagulase bacteria were resistant to ciprofloxacin and 33% to gentamicin. Levofloxacin and then ciprofloxacin had the best coverage against gram negative bacteria and erythromycin and chloramphenicol showed the worst coverage. Levofloxacin and then ciprofloxacin revealed the best protection against gram positive bacteria and chloramphenicol and erythromycin showed the worst coverage. All CONs, *Pseudomonas*, Enterobacteriaceae and *Staphylococcus aureus* were susceptible to ciprofloxacin and *S. pneumonia* was resistant and semi-resistant to these drugs.

4. Discussion

Appropriate treatment and management of corneal ulcers requires completed identification of the etiology (13). Identification and isolation of a bacterial species in corneal ulcers can direct the selection of an proper antibiotic drug so that this antibiotic targeting the microrganism responsible can be administered on time (4). However, the inconsistency in frequency and causes of corneal ulcers across geographical regions and ethnic populations make it challenging to administer a standard set of protocols in order to reduce the incidence and frequency of corneal blindness (4). Given these milieu, a comprehensive data including causative factors, epidemiological features, and etiological agents concerning this ophthalmic condition is needed. The purpose of current study was therefore, to explore the etiology of corneal ulcer and antimicrobial susceptibility of bacterial isolates identified. It is interesting to note that a majority of our patients (89%) treated by various kinds of antibiotic drops, and in some cases patients were treated by topical steroid therapy, before referring to the hospital. The data realated to history of culturing and sampling before the hospital referral was available for less than 10% of the patients.

The prevalence of 77% of antibiotic use in patients with positive culture in the present study can indicate the inadequate effect of antibiotics prescribed in sterilization of corneal ulcer. Generally, the problem with the correct prescription of antibiotics before cultivation can lead to problems such as increase in the risk of microbial resistance, occurance of misleading changes in the clinical outline of the wound due to drug toxicity and the need for discontinuation of the drug for a period before cultivation, all of which are causing trouble and disruption in the diagnosis and treatment of the disease. It is noteworthy that the concentration and effect of the prescribing agent are different *in vivo* than *in vitro* (14). The drug tissue level after topical administration is influenced by such factors as drug concentration, time of consumption, penetration of the drug into the epithelium and cornea stroma, and degree of binding to the stromal proteins. Therefore, after initiating the consumption,

the clinical response is preferred comparing to the sensitivity test.

Consistent with other results (14-20), in terms of the underlying causes of corneal ulcer, these factors include blepharitis, dry eye, trauma, anatomical disorder inthe eyelid, presence of foreign body in the cornea, history of surgery, and contact lens utilization. Our results indicate that belpharitis and dry eye were the most common risk factors in our patients. This is consistent with the findings from India (21). which showed that Gram-positive bacteria contributed to the majority (66.7%) of the total bacterial isolates.

Bacterial corneal ulcer were mostly caused by gram-positive bacteria. Nevertheless, unlike other investigations from Africa (22) and Asia (23)where infections by Streptococcus pneumoniae were most frequent; in our study, negative staphylococcus coagulase -related bacterial corneal ulcer predominated (47.9%) followed by Pseudomonas aeruginosa (20.8%). A review of literature showed that most of the studies from developed nations such as the Australia (24) and United States (25) described coagulase-negative staphylococci or S. epidermidis as the leading cause of this condition. These results are also consistent with conducted by Suekeet al. (26). It is not obvious whether the tendency to consider coagulase-negative staphylococci or *S. epidermidis* as a common commensal of the conjunctiva may have led to underreporting in some of the investigation. This is also worth mentioning that in another studies, data have shown that *S. pneumoniae* is the major biological agent causing corneal ulcer in developing as well as industrial nations (27, 28).

The infection ratio of male: female was found to be 1:0.8. This results is in consistency with several studies conducted elsewhere which have revealed a high susceptibility of male toward infection in comparison with to female (29-31). Nevertheless, the role of gender in corneal ulceration is always contradictory and further precise research is necessary. The highest number of patients, 40% (18/45) from corneal ulcer positive case belonged to age group 60≥. It is due to the fact that people of age 60≥ years have many predisposing factors like CDK (climatic droplet keratopathy), cataract surgery, dryness of the eyes, macular degeneration, glaucoma, previous ocular surgeries and lid deformities due to trachomatous scarring which most likely predispose this age group to ulceration of corneal more than the other age ranges (32). The mean age of our patients was about 52 years, which is consistent with previous studies in Shiraz .

Although the culture positivity of 51% that we observed in our populations is comparable to several previous studies that reported more than 50%, culture positivity (26, 33), another studies detected lower positivity (29, 34, 35). The reason for such lower prevalence could be due to differences in sample size and difference in methods used to ascertain positivity. A significant reduction in the incidence of streptococcal bacteria has been also observed in previous studies, which reduced from 13% in the first study to 5% and 4% in subsequent studies. In our study, none of the patients had positive culture with streptococcal bacteria.

In the view of numerous reports of changing pattern of bacteria susceptibility to antibiotics, testing of clinical isolates for their susceptibility to antibiotic drugs is required for choice of appropriate drugs or for changing an already administered antibiotic. In the study of Nejabat *et al*., in Shiraz, antibiotic susceptibility testing was performed with gentamicin, ciproforoxacin, erythromycin, and chloramphenicol antibiotics. The following results were reported: all bacteria were susceptible to ciprofloxacin and no resistance was observed. It was reported that 50% of pseudomonas bacteria were resistant to gentamicin, chloramphenicol and erythromycin, and all Staphylococcus aureus bacteria were susceptible to these antibiotics (36). In this study, the isolated bacteria were tested against five different antibiotics including Ciprofloxacin, Gentamicin, Erythromycin, Chloramphenicol and Levofloxacin. Data have revealed the resistance to ciprofloxacin. Moreover, 22% of the coagulase negative staphylococcus were resistant to ciprofloxacin and 35% were resistant to gentamicin; this is not in the same line with the results of our study.

These studies show that antibiotic resistance is higher in the developing countries than developed countries. World reports show that,

unfortunately, the issue of emerging antibiotic resistance, especially in the developing countries, remains unresolved, due to lack of proper care programsand inappropriate, overuse and irregular consumption of antibiotics in hospitals . The results of these studies was similar to that another studies reported by Nejabat *et al.* in Shiraz that determined there has been increasing resistance to antibiotics over time in corneal isolates (36). In those studies, first reports have revealed that ciprofloxacin, because of 100% effectiveness, can be an appropriate therapeutic option in treatment of bacterial corneal ulcer cases. But in subsequent studies ciprofloxacin resistance level reached 8%, and in our study the overall resistance reached about 20%. Resistance to gentamicin has also increased from about 25% in previous studies to about 33% in our study. Resistance to erythromycin and chloramphenicol antibiotics was also observed in the past and increased to a high level and are practically ineffective in the treatment of corneal ulcer. Resistance to erythromosin has been also increased from 50% to 70% in our study. In addition, resistance to chloramphenicol has grown from 40% in previous studies to more than 70% in our study, as the most resistant to this drug over the years (36).

The clinical outcome in corneal ulceration is probable to be dependent on the infecting bacteria virulence, the minimum inhibitory concentration (MIC) of the antibiotic against the isolates. Based on the MIC systemic breakpoints, all bacteria were interpreted to be resistant, intermediate, or susceptible. For reference, reported breakpoint concentrations derived for systemic infections were used, that is, the MIC above or below which microrganisms are classified as resistant or susceptible, respectively (37). The ciprofloxacin had the lowest MIC50 for all organisms. In most instances the MIC50 concentration for these microorganisms was below the systemic breakpoint, and these microorganisms would therefore usually be reported as susceptible by laboratories. The MIC for ciprofloxacin against Coagulase Negative Staphylococcus, *Staphylococcus aureus*, Enterobacteriaceae and *Pseudomonas* spp. was similar to the range (0.12-0.25 g/L) reported by Lomholt and Kilian (38). The MIC90 for ciprofloxacin were similar to those reported for streptococci and staphylococci by Oliveira *et al*. (11) for corneal ulceration in Brazil. For *Staphylococcus aureus* in the present study, Ciprofloxacin, Levofloxacin, and Erythromycin exhibited the lowest MIC90 s, which were all below the systemic breakpoints. The MIC90s for the Chloramphenicol against Coagulase Negative *Staphylococcus*, *Staphylococcus aureus*, Enterobacteriaceae and *Pseudomonas* suggest a reduced susceptibility of these organisms to this group of antibiotics. The our data have also shown that All the bacterial isolates (Gram positive and negative) were 25-31% and 25-38% susceptible to the Chloramphenicol and Erythromycin antibiotics, respectively. These results indicate that chloramphenicol should not be used routinely as the topical antibiotic of choice for corneal infection in Iran, a view supported by studies in Australia, Singapore, London (39) and Nepal (6).

According to the results obtained from this study, the following points should be suggested:

1. Sampling for culture prior to the start of antibiotic treatment of corneal ulcer is very necessary. Sampling should be done by an ophthalmologist, from the active area at the edge and depth of the wound and shouldbe directly transferred to the environment.
2. In the case of absence for clinical response to treatment, a more appropriate drug shouldbe prescribed based on an antibiotic susceptibility test.
3. Due to the low microbial resistance to levofloxacin and even ciprofloxacin in our study, these drugs are still effective in the treatment of corneal ulcer. Precaution to correctly administer and prevent inappropriate prescription of these drugs helps us to prevent the development of bacterial resistant strains.
4. Accurate and precise training of patients in the use of ocular antibiotics as well as the prevention of arbitrary use of these drugs is important for reducing the microbial resistance.

5. Conclusion

The inappropriate, irrational and irregular use of these antibiotics in hospitals and in the community has led to antibiotic resistance. Due to the fact that we still do not see the extensive use

of levofloxacin, resistance to this drug was lower than other drugs. Resistance to ciprofloxacin was also very low on its early in the drug market, but due to its irrational consumption, we see increasing resistance to it. Therefore, preventing the use of these drugs in unnecessary cases is recommended to prevent resistance to levofloxacin and new-generation fluoroquinolones, such as moxifluxacin and gatifloxacin.

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Conflict of Interest

None declared.

Informed consent

Informed consent was obtained from each participant in this study.

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Ethical approval

Ethical approval to conduct the study was granted by Medical Ethics Committee of Shiraz University of Medical Sciences.

References

1. Green MD, Apel AJ, Naduvilath T, Stapleton FJ. Clinical outcomes of keratitis. *Clin Exp Ophthalmol.* 2007;35(5):421-426. doi:10.1111/j.1442-9071.2007.01511.x
2. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol.* 2003;87(7):834-838. doi:10.1136/bjo.87.7.834
3. Gudmundsson OG, Ormerod LD, Kenyon KR, et al. Factors influencing predilection and outcome in bacterial keratitis. *Cornea*. 1989;8(2):115-121.
4. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. *Bull World Health Organ.* 2001;79(3):214-221.
5. Bhadange Y, Sharma S, Das S, Sahu SK. Role of liquid culture media in the laboratory diagnosis of microbial keratitis. *Am J Ophthalmol*. 2013;156(4):745-751. doi:10.1016/j.ajo.2013.05.035
6. Suwal, S.; Bhandari, D.; Thapa, P.; Shrestha, M. K.; Amatya, J., Microbiological profile of corneal ulcer cases diagnosed in a tertiary care ophthalmological institute in Nepal. BMC Ophthalmol. 2016; 16 (1), 209.
7. Collier SA, Gronostaj MP, MacGurn AK, et al. Estimated burden of keratitis--United States, 2010. *MMWR Morb Mortal Wkly Rep.* 2014;63(45):1027-1030.
8. Cao J, Yang Y, Yang W, et al. Prevalence of infectious keratitis in Central China. *BMC Ophthalmol.* 2014;14:43. Published 2014 Apr 2. doi:10.1186/1471-2415-14-43
9. Shah A, Sachdev A, Coggon D, Hossain P. Geographic variations in microbial keratitis: an analysis of the peer-reviewed literature. *Br J Ophthalmol.* 2011;95(6):762-767. doi:10.1136/bjo.2009.169607
10. Rodman RC, Spisak S, Sugar A, Meyer RF, Soong HK, Musch DC. The utility of culturing corneal ulcers in a tertiary referral center versus a general ophthalmology clinic. *Ophthalmology.* 1997;104(11):1897-1901. doi:10.1016/s0161-6420(97)30010-4
11. Kaye S, Tuft S, Neal T, et al. Bacterial susceptibility to topical antimicrobials and clinical outcome in bacterial keratitis. *Invest Ophthalmol Vis Sci.* 2010;51(1):362-368. doi:10.1167/iovs.09-3933
12. Al-Dhaheri HS, Al-Tamimi MD, Khandekar RB, Khan M, Stone DU. Ocular Pathogens and Antibiotic Sensitivity in Bacterial Keratitis Isolates at King Khaled Eye Specialist Hospital, 2011 to 2014. *Cornea*. 2016;35(6):789-794. doi:10.1097/ICO.0000000000000844
13. Ple-plakon P.A., Hood C.T. (2016) Diagnosis and Management of Corneal Ulcers in Pediatric Patients. In: Traboulsi E., Utz V. (eds) Practical Management of Pediatric Ocular Disorders and Strabismus. Springer, New York, NY
14. Stoffelns, B.M. Daniel M. Albert, Joan W. Miller, Dimitri T. Azar, Barbara A. Blodi (eds): Albert & Jakobiec's Principles and Practice of Ophthalmology. *Graefes Arch Clin Exp Ophthalmol*

247, 1437-1438 (2009). doi: 10.1007/s00417-008-0918-0
15. Baum J, Barza M. The evolution of antibiotic therapy for bacterial conjunctivitis and keratitis: 1970-2000. *Cornea*. 2000;19(5):659-672. doi:10.1097/00003226-200009000-00011
16. Kowalski RP, Karenchak LM, Gordon YJ. Comparison of ciprofloxacin and ofloxacin using human corneal susceptibility levels. *Cornea*. 1998;17(3):282-287.
17. Ormerod LD, Heseltine PN, Alfonso E, et al. Gentamicin-resistant pseudomonal infection. Rationale for a redefinition of ophthalmic antimicrobial sensitivities. *Cornea*. 1989;8(3):195-199.
18. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y. Bacterial keratitis: a prospective clinical and microbiological study. *Br J Ophthalmol.* 2001;85(7):842-7. doi:10.1136/bjo.85.7.842
19. Vajpayee RB, Dada T, Saxena R, et al. Study of the first contact management profile of cases of infectious keratitis: a hospital-based study. *Cornea*. 2000;19(1):52-6. doi:10.1097/00003226-200001000-00011
20. Graves A, Henry M, O'Brien TP, Hwang DG, Van Buskirk A, Trousdale MD. In vitro susceptibilities of bacterial ocular isolates to fluoroquinolones [published correction appears in Cornea 2001 Jul;20(5):546]. *Cornea*. 2001;20(3):301-5. doi:10.1097/00003226-200104000-00012
21. Kowalski RP, Kowalski TA, Shanks RM, Romanowski EG, Karenchak LM, Mah FS. In vitro comparison of combination and monotherapy for the empiric and optimal coverage of bacterial keratitis based on incidence of infection. *Cornea*. 2013;32(6):830-4. doi:10.1097/ICO.0b013e318268d6f4
22. Carmichael TR, Wolpert M, Koornhof HJ. Corneal ulceration at an urban African hospital. *Br J Ophthalmol.* 1985;69(12):920-6. doi:10.1136/bjo.69.12.920
23. Srinivasan M, Gonzales CA, George C, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. *Br J Ophthalmol.* 1997;81(11):965-71. doi:10.1136/bjo.81.11.965
24. McClellan KA, Bernard PJ, Billson FA. Microbial investigations in keratitis at the Sydney Eye Hospital. *Aust N Z J Ophthalmol.* 1989;17(4):413-6. doi:10.1111/j.1442-9071.1989.tb00564.x
25. Ormerod LD, Hertzmark E, Gomez DS, Stabiner RG, Schanzlin DJ, Smith RE. Epidemiology of microbial keratitis in southern California. A multivariate analysis. *Ophthalmology.* 1987;94(10):1322-33. doi:10.1016/s0161-6420(87)80019-2
26. Sueke H, Kaye S, Neal T, et al. Minimum inhibitory concentrations of standard and novel antimicrobials for isolates from bacterial keratitis. *Invest Ophthalmol Vis Sci.* 2010;51(5):2519-24. doi:10.1167/iovs.09-4638
27. Karthikeyan RS, Priya JL, Leal SM Jr, et al. Host response and bacterial virulence factor expression in Pseudomonas aeruginosa and Streptococcus pneumoniae corneal ulcers. *PLoS One*. 2013;8(6):e64867. Published 2013 Jun 4. doi:10.1371/journal.pone.0064867
28. Leck AK, Thomas PA, Hagan M, et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *Br J Ophthalmol*. 2002;86(11):1211-5. doi:10.1136/bjo.86.11.1211
29. Lavaju P, Arya SK, Khanal B, Amatya R, Patel S. Demograhic pattern, clinical features and treatment outcome of patients with infective keratitis in the eastern region of Nepal. *Nepal J Ophthalmol*. 2009;1(2):101-6. doi:10.3126/nepjoph.v1i2.3683
30. Basak SK, Basak S, Mohanta A, Bhowmick A. Epidemiological and microbiological diagnosis of suppurative keratitis in Gangetic West Bengal, eastern India. *Indian J Ophthalmol.* 2005;53(1):17-22. doi:10.4103/0301-4738.15280
31. Keshav BR; Zacheria G; Ideculla T; Bhat V; Joseph M. Epidemiological characteristics of corneal ulcers in South sharqiya region. *Oman Med J.* 2008;23(1):34-39.
32. Mallika P, Tan A, Asok T, Faisal H, Aziz S, Intan G. Pattern of ocular trauma in kuching, malaysia. *Malays Fam Physician.* 2008;3(3):140-145. Published 2008 Dec 31.
33. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea*. 2008;27(1):22-7. doi:10.1097/ICO.0b013e318156caf2
34. Feilmeier MR, Sivaraman KR, Oliva M, Tabin GC, Gurung R. Etiologic diagnosis of corneal ulceration at a tertiary eye center in Kathmandu, Nepal. *Cornea*. 2010;29(12):1380-1385. doi:10.1097/ICO.0b013e3181d92881

35. Dhakhwa K, Sharma MK, Bajimaya S, Dwivedi AK, Rai S. Causative organisms in microbial keratitis, their sensitivity pattern and treatment outcome in western Nepal. *Nepal J Ophthalmol.* 2012;4(1):119-127. doi:10.3126/nepjoph.v4i1.5863
36. Nejabat M RM, Alborzi A. Comparison of therapeutic effect and side effects of ciprofloxacin 0.3% with cefazolin-gentamicin-enhanced drops in patients with corneal bacterial ulcer. Feiz. 2005;6(1):1-9.
37. Andrews JM; BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 8). *J Antimicrob Chemother*. 2009;64(3):454-489. doi:10.1093/jac/dkp244
38. Lomholt JA, Kilian M. Ciprofloxacin susceptibility of Pseudomonas aeruginosa isolates from keratitis. *Br J Ophthalmol.* 2003;87(10):1238-1240. doi:10.1136/bjo.87.10.1238
39. Badenoch PR, Halliday CL, Ellis DH, Billing KJ, Mills RA. Ulocladium atrum keratitis. *J Clin Microbiol.* 2006;44(3):1190-1193. doi:10.1128/JCM.44.3.1190-1193.200