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Multivariate Analysis of Microbial Keratitis in Egypt

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Abstract: Microbial keratitis is a potentially sight threatening disorder and the leading cause of monocular blindness worldwide. This study analyzed the prevalence, microbiology, clinical risk factors and treatment of infectious microbial keratitis. A prospective review of all cases presenting with keratitis at Cornea Outpatient department of Research Institute of Ophthalmology from September 2008 to September 2012, were included in this study. Full ophthalmic examination with slit lamp bio-microscopy was performed and corneal scrapings were sent for microbiological diagnosis. Results revealed that of the 380 patients who attended the outpatient department 213 (56.05%) patients were confirmed to be positive for microbial keratitis whereas 167(43.95%) showed no growth.123 (57.75%) of positive cases were males with age ranged from (4 to 88 years) and 90 (42.25%) of cases were females with age ranged from (2 to 77 years). The most common predisposing cause of ulceration was trauma 45 (21.12%) followed by contact lens wearing 40 (18.78%). Pure bacterial cultures were obtained from 122 (32.10%) eyes, whereas pure fungal cultures were obtained from 48 (12.63 %) eyes. 43 (11.31%) eyes showed mixed growth. In conclusion, this limited study has revealed and reinforced that suppurative corneal ulcers are caused by both bacterial and fungal agents. The most commonly isolated bacteria was *Staph. epidermidis* 40(25.64%). Early stage of diagnosis and formulation of an uncompromising management protocol can prevent profound visual morbidity. Gentamicin (10µg), Amikacin (30µg), Gatifloxacin (5µg), Ceftazidime (30µg), Erythromycin (10µg) Chloramphenicol (30µg) and Vancomycin (30µg), Voriconazole (1µg), Fluconazole (25µg), Itraconazole (50µg), Ketoconazole (10µg),) Metronidazole (5µg) and Amphotercin B (20µg), were found to be better efficacious drugs against most of the bacterial and fungal pathogens noted in in-vitro susceptibility testing.

Key words: Suppurative corneal ulcer · Microbial keratitis · Infective keratitis · Etiological agent

INTRODUCTION

Corneal ulcer is a potentially sight threatening ocular condition and the leading cause of monocular blindness in developing countries [1]. Scarring of the cornea develops as a result of suppurative corneal ulcer which is the second commonest cause of preventable blindness after un- operated cataract among people in Asia, Africa and in the Middle East [2].

Corneal ulcer is an ocular emergency presenting clinically with pain, photophobia, redness, infiltration, corneal edema and anterior chamber reaction. If left untreated, apart from scar formation, it can lead to endophthalmitis and even cornea perforation and blindness. Perforation due to pseudomonas keratitis can occur in less than 24 hours after onset. Identification of pathogens, selection of fortified antibiotics and their early administration is the mainstay management [3].

Corneal ulcer can be caused by various pathogens i.e., bacteria, fungi, viruses and parasites. Etiologic and epidemiologic pattern of corneal ulceration varies with the patient population, geographic location and climate and it tends to vary somewhat over time. Although any organism can invade the corneal stroma if the corneal protective mechanisms such as blinking, tear dynamics and epithelial integrity are compromised but microbial causes of suppurative corneal ulcers vary considerably in different geographical areas. Bacteria and fungi are frequently responsible for suppurative corneal ulcers especially in the developing countries [4].

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Most of the organisms cultured from corneal infections are of the same species that are normally present on the lids and periocular skin, in the conjunctival sac or in adjacent nasal passage. However, both Gram-positive and Gram-negative bacteria are responsible for causing suppurative corneal ulcers with *Staphylococcus, Streptococcus* and *Pseudomonas* are the most frequent isolates [5]. While among the fungal causes of suppurative corneal ulcers, *Fusarium* and *Aspergillus species* are the predominant agents reported by many investigators [6].

Corneal ulceration is a vision-threatening disease that varies in incidence worldwide. Variation in incidence of infectious keratitis is a multi-factorial issue that includes a tight mix of different factors, such as geographical and other location-related factors [7], degree of development of the country concerned, the predominant predisposing factors and the type of infection commonly present in this community [8].

However, variations in incidence of infectious keratitis have been reported in different keratitis studies conducted in different countries even though these were similar in the degree of development, geographical location and the most prevalent type of corneal infection and related predisposing factors [9, 10].

It is widely accepted that bacterial and fungal keratitis have far higher incidence in the developing world than that in the developed world [11, 12]. In the developing world, corneal ulcers appear to be occurring in epidemic proportions, being 10 times more common than in the developed countries. As trachoma and vitamin A deficiency become less common, suppurative keratitis is becoming the major cause of corneal blindness in the developing world. While contact lens use is a major risk factor for corneal ulceration in the developed world, a high prevalence of fungal infections, agriculture related trauma and use of traditional eye medicines is unique to the developing world [13]. Also it is thought that viral keratitis is more prevalent in developed countries, such as the United States where *Herpes Simplex* Keratitis (HSK) is considered the leading cause of corneal blindness [14,15] However, predominance of bacterial [9,16] and fungal [17] keratitis in developed countries has been reported.

Although several studies have investigated different aspects of infectious keratitis, little information is available about incidence of different types of infectious keratitis in large populations [18].

A wide spectrum of microbial organisms can produce corneal infections and consequently the therapeutic strategies may be variable .One of the key elements in this effort is a proper understanding of the microbiological and clinical characteristics of this disease entity which will enable the ophthalmologist to initiate appropriate antimicrobial therapy [19].

The bacterial sensitivity to various antimicrobial agents varies from place to place and in the same place from time to time .Therefore, the changing spectrum of microorganisms involved in ocular infections and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy. The empirical choice of an effective treatment is becoming more difficult as ocular pathogens are increasingly becoming resistant to commonly used antibiotics [20]

This research study was undertaken to analyze the prevalence, microbiology, clinical risk factors and treatment of microbial keratitis and this would be of help in the planning of keratitis and corneal ulcer management strategy to hopefully avoid any permanent ocular damage.

MATERIALS AND METHODS

Patients: A total of 380 clinically diagnosed patients of suppurative corneal ulcers of different age and sex who attended the Cornea Outpatient Department and also admitted in the Ophthalmology ward of Research Institute of Ophthalmology during a four years study from 2008 till 2012 were included in the study.

Collection of Samples: Demographic details like name, age, sex, clinical history and associated findings were recorded onto standard clinical history form. It was ensured that clinical microbiology material was collected before giving antibiotic therapy or 48 hours after discontinuing local antibiotics.

All patients underwent thorough slit-lamp bio microscopic examination by an ophthalmologist. After a detailed ocular examination, corneal scrapings were taken by an ophthalmologist with all aseptic precautions. Five minutes after instillation of local anesthetic to the affected eye, corneal scrapings were taken. The material obtained was spread onto labeled slides for Gram stain and was also inoculated onto the surfaces of agar plates for bacterial and fungal growth.

Bacterial Culture and Sensitivity Test [21, 22]: Gram's staining was performed. The swab was inoculated onto Blood agar, MacConkey's agar, Chocolate agar and Wilkins media. The inoculation technique consisted of multiple "C" shape streaks on the culture plate, with the idea to localize the site of implantation of the corneal scraping on the agar media, then incubated at 37°C for 24 hours. The culture media were inspected for growth. If organism had not grown, plates were further incubated and finally declared as culture negative after 5 days. To insure 5-10% CO2, Chocolate agar plates were incubated in- CO2 incubator and Wilkins media were incubated anaerobically. Culture positive growth was identified by their colony, morphology, Gram staining and motility testing by hanging drop preparation, pigment production and relevant biochemical tests. All bacterial isolates were tested for susceptibility by disc diffusion method against Gentamicin (10µg), Amikacin (30µg), Gatifloxacin (5µg), Ceftazidime (30µg), Erythromycin (10µg) Chloramphenicol (30µg) and Vancomycin (30µg). The results of susceptibility were recorded as sensitive, intermediate or resistant.

Detection of Fungal Agents: Three corneal scrapings were used for fungal detection. First corneal scraping was used for wet preparation in 10% KOH, second scraping for fungus culture and third scraping for lacto phenol cotton blue staining. Materials obtained by second scraping were spot inoculated on plain Sabouraud's dextrose agar medium (SDA). Inoculated SDA media was incubated at 25-°C and observed daily for the first 7 days and on alternate days for next 7 days for observing slow growing fungi. Only growth occurring on the "C" streaks was considered as significant and out growth away from the "C" streak was discarded as contaminants.

The plates which did not show any evidence of growth after 14 days were discarded. Fungal growth was grossly identified by its colony morphology, pigment production and microscopically by lacto-phenol cotton blue stain.

All fungal isolates were tested for their antifungal susceptibility by disc diffusion method against Voriconazole (1µg), Fluconazole (25µg), Itraconazole (50µg), Ketoconazole (10µg),) Metronidazole (5µg) and Amphotercin B (20µg), the results of susceptibility were recorded as sensitive, intermediate or resistant.

RESULTS

Of all the 380 keratitis patients who attended the outpatient department reviewed from 2008 to 2012 a total of 213 (56.05%), cases were culture positive. Five cases had bilateral ocular involvement, the distribution of patients having corneal ulcer according to the various age groups is shown in Table (1) which reveals that the highest frequency belonging to the 30 to 40 years (middle age group) 22.53%.

Table 1: Distribution of patients with corneal ulcer according to age groups

Age Groups (Years)	Number of Patients	Percentage (%)
0-10	4	1.87
10-20	18	8.45
20-30	36	16.90
30-40	48	22.53
40-50	34	15.96
50-60	26	12.20
60-70	34	15.96
70-80	13	6.1
Total	213	100

Table 2: Distribution of patients with corneal ulcer according to Gender

Gender	Number Percentage (%)	Age Group (Years)
Male	123 57.75%	4-88
Female	90 42.25%	2-77
Total	213 100%	

Table 3: Microbial growth patterns in culture of corneal ulcer patients

Culture Result	No. of cases	Percentage (%)
Positive Culture	213	56.05
Pure bacterial growth	122	32.10
Pure fungal growth	48	12.63
Mixed Microbial Growth	43	11.31
No Growth	167	43.95
Total	380	100

Table 4: Predisposing risk factors for culture-positive microbial keratitis

Predisposing Factor	No. of Cases	Percentage (%)
Trauma	45	21.12
Organic		
Stick	3	1.41
Soil and dust	4	1.87
Stone	4	1.87
Plant leaf and thorn	19	8.92
Insect	1	0.46
Fingernail	8	3.75
Non Organic		
Metal	3	1.41
Ball	1	0.46
Hard paper	2	0.93
Miscellaneous		
Contact lens	40	18.78
Rubbing eye	1	0.46
Ocular operation	7	3.28
Unknown	120	56.33
Total	213	100

Table (2) depicts the distribution of culture positive patients according to gender. It is seen that 57.75% of the cases were males and their age ranged from 4 to 88 years, while the females were 42.25% of cases and their age were between 2 and 77 years, so, in our study males had a tendency to get corneal ulcer more than females.

The culture was positive in more than half of the cases, a total of 213 cases with a 56.05% rate and 167 cases were culture negative with almost 43.95% rate .We found that the map of organisms isolated in culture ranged from pure bacterial or fungal 'single' growth or mixed microbial growth. The highest number of cases were a pure bacterial growth culture with 122(32.10%) cases, out of the 213 culture positive cases, pure single fungal isolates were observed in 48 (12.63%) cases, mixed microbial isolates were 43 (11.31%) Table (3).

Table (4), shows the predisposing factors of culture positive cases which ranged from trauma (whether it is organic or non-organic) to miscellaneous causes due to contact lenses or eye rubbing or even postoperative. Interestingly, 56.33% of cases were not able to specify a cause for their eye affection and we stated them under ' unknown'. In the other half of the cases, trauma was the predominant predisposing factor in 45 (21.12%) of cases, most of them were plant origin especially in rural areas with agricultural work. Contact lens as a predisposing factor was found in 40 cases with an (18.78%) rate.

The bacterial growth pattern in the culture positive cases is shown in Table (5). It revealed that out of 156 bacterial isolates 122 were pure isolates, 20 were mixed with another species of bacteria and 14 were mixed with a single fungal species. The predominant organism isolated were Gram positive cocci which were isolated in 102 cases (65.38%) with *staphylococcus epidermidis* followed by *staphylococcus aureus* being the commonest species 40 and 35 cases respectively. Gram negative bacilli were isolated in 27(17.30%) cases only and *Pseudomonas aeruginosa* was the predominant species isolated in 17(10.89%) cases. The anaerobic *Propionibacterium* was anaerobically isolated in 4 cases only.

Table 5: Bacterial growth patterns in culture of corneal ulcer patients

Name of Bacteria Isolates	Pure Isolates	Mixed with other Bacteria	Mixed with Fungi	Total (%)
Total Gram-positive cocci	80	12	10	102(65.38%)
Staph. epidermidis	33	3	4	40(25.64%)
Staph. Aureus	29	3	3	35(22.43%)
Strept.pneumoniae	11	4	2	17(10.89%)
Strept.viridans	4	1		5(3.20%)
Strept. pyogenes	3	1	1	5(3.20%)
Total Gram-negative cocci	6	1	-	7(4.48%)
Neisseria	6	1	-	7(4.48%)
Total Gram-positive bacilli	17	1	2	20(12.82%)
Diphtheroids	14	-	2	16(10.25%)
Propionibacterium	3	1		4(2.56%)
Total Gram-negative bacilli	19	6	2	27(17.30%)
Pseudomonas Aeruginosa	12	3	2	17(10.89%)
Klebsiella Species	3	2	-	5(3.20%)
Moraxella Species	4	1	-	5(3.20%)
Total	122	20	14	156(100%)

Table 6: Fungal growth patterns in culture of corneal ulcer patients

Name of the Fungal Isolate	Pure Isolates	Mixed with other Fungi	Mixed with Bacteria	Total(%)
Aspergillus flavus	9	2	2	13(18.31)
Aspergillus fumigatus	3	-	1	4(5.63)
Aspergillus niger	7	-	1	8(11.26)
Candida	15	3	3	21(29.57)
Fusarium	7	4	6	17(23.94)
Rhizopus	4	-	1	5(7.04)
Mucor	3	-	-	3(4.22)
Total	48	9	14	71(100)

Table 7: Susceptibility of the bacterial isolates to different antibiotics

		Vancomycin			Erythromycin			Chloramphenicol			Ceftazidim			Gentamicin			Amikacin			Gatifloxacin		
		S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Organism	Total	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Staph.aureus	35	29	3	3	10	19	6	11	16	8	11	22	2	16	15	4	27	4	4	29	5	1
		82.8	8.6	8.6	28.5	54.3	17.2	31.4	45.7	22.9	31.4	62.9	5.7	45.7	42.9	11.4	77	11.5	11.5	82.8	14.3	2.9
Staph. Epidermidis	40	33	6	1	24	10	6	35	5	0	11	29	0	19	15	6	33	4	3	33	4	3
		82.5	15	2.5	60	2	15	87.5	12.5	0	27.5	72.5	0	47.5	37.5	15	82.5	10	7.5	82.5	10	7.5
Strept.pneumonaie	17	9	8	0	16	1	0	13	4	0	10	7	0	6	5	6	11	4	2	15	2	0
		53	47	0	94	6	0	76.5	23.5	0	58.8	41.2	0	35.3	29.4	35.3	64.7	22.5	11.8	88.2	11.8	0
Diphtheroids	16	13	2	1	6	6	4	11	3	2	5	9	2	12	2	2	8	7	1	15	1	0
		81.3	12.5	6.2	37.5	37.5	25	68.7	18.8	12.5	1.2	56.3	12.5	75	12.5	12.5	50	43.8	6.2	93.8	6.2	0
Strept. Viridans	5	4	1	0	2	3	0	4	1	0	2	3	0	4	1	0	2	3	0	4	1	0
		80	20	0	40	70	0	80	20	0	40	70	0	80	20	0	40	70	0	80	20	0
Strept.pyogenes	5	5	0	0	3	0	2	0	5	0	2	3	0	0	4	1	2	0	3	5	0	0
		100	0	0	60	0	40	0	100	0	40	60	0	0	80	20	40	0	60	100	0	0
Propionibacterium	5	3	1	1	2	3	0	3	1	1	3	2	0	2	1	2	5	0	0	4	0	1
		60	20	20	40	60	0	60	20	20	60	40	0	40	20	40	100	0	0	80	0	20
Neisseria	7	2	4	1	6	1	0	3	3	1	4	1	2	4	1	2	3	2	2	6	1	0
		28.6	57.1	14.3	85.7	14.3	0	42.8	42.8	14.3	57.1	14.3	28.6	57.1	14.3	28.6	42.8	28.6	28.6	85.7	14.3	0
Moraxella	5	1	3	1	0	2	3	3	1	1	1	3	1	4	0	1	4	1	0	3	1	1
		20	60	20	0	40	60	60	20	20	20	60	20	80	0	20	80	20	0	60	20	20
Pseudomonas	16	2	14	0	1	15	0	4	9	3	12	4	0	13	2	1	16	0	0	15	1	0
		12.5	87.5	0	6.3	93.7	0	25	56.3	18.7	75	25	0	81.2	12.5	6.3	100	0	0	93.7	6.3	0
Klebsiella	5	1	4	0	1	4	0	3	2	0	4	1	0	4	1	0	5	0	0	4	1	0
		20	80	0	20	80	0	60	40	0	80	20	0	80	20	0	100	0	0	80	20	0

Table 8: Antifungal susceptibility pattern

Antifungal	Suspected pattern	Asp.fum. n=8 %		Asp.flavus n=16 %		Asp.niger n=11 %		Fusarium n=9 %		Mucor n=1 %		Rhizopus n=2 %		Candida n=29 %	
Fluconazole	S	2	25	0	0	3	27.3	0	0	0	0	0	0	4	13.8
	I	0	0	5	31.3	3	27.3	0	0	0	0	0	0	4	13.8
	R	6	75	11	68.7	5	45.4	9	100	1	100	2	100	21	72.4
Ketoconazole	S	1	12.5	3	18.7	3	27.3	1	11.1	1	100	0	0	24	82.8
	I	0	0	1	6.3	0	100	8	88.9	0	0	0	0	2	6.9
	R	7	87.5	12	75	8	72.7	0	0	0	0	2	100	3	10.3
Voriconazole	S	7	87.5	16	100	11	100	3	33.3	1	100	2	100	5	17.2
	I	0	0	0	0	0	0	0	0	0	0	0	0	2	6.9
	R	1	12.5	0	0	0	0	6	66.7	0	0	0	0	22	75.9
Itraconazole	S	4	50	11	68.7	6	54.5	2	22.2	0	0	2	100	2	6.9
	I	3	37.5	3	18.8	4	36.4	2	22.2	0	0	0	0	0	0
	R	1	12.5	2	12.5	1	9.1	5	55.6	1	100	0	0	27	93.1
Amphotercin B	S	4	50	7	43.7	1	9.1	6	66.7	1	100	1	50	1	3.4
	I	3	37.5	4	25	6	54.5	2	22.2	0	0	0	0	0	0
	R	1	12.5	5	31.3	4	36.4	1	11.1	0	0	1	50	28	96.6
Metronidazole	S	0	0	0	0	2	18.2	0	0	0	0	0	0	24	82.8
	I	0	0	0	0	0	0	0	0	0	0	0	0	2	6.9
	R	8	100	16	100	9	81.8	9	100	1	100	2	100	3	10.3

Of all the 71 fungal pathogens isolated from patients with corneal ulcers, 48 were pure isolates, 9 were mixed with another species of fungus and 14 were mixed with a single species of bacteria. *Aspergillus* was the predominant fungus isolated in 25cases, (*Aspergillus flavus* being the commonest species). *Candida* was isolated in 21(29.57%) cases, next common to *Aspergillus*. *Fusarium* was also a common fungus cultured as well in 17 (23.94%) cases. The other fungal isolates were *Rhizopus species* in 5 (7.04%) and *Mucor species* in 3 (4.22%) cases, Table (6).

The susceptibility of the bacterial isolates to the different antibiotics used is illustrated in Table (7). *Staphylococcus aureus* had the same sensitivity to Vancomycin and Gatifloxacin 82.8% and to a lesser extent to Amikacin 77%. Both *Staphylococcus aureus* and *Staphylococcus epidermidis* were moderately resistant to Ceftazidime (62.9% and 72.5% respectively) followed by Gentamicin (42.9 and 37.5% respectively). While *Staphylococcus epidermidis* was highly sensitive to Chloramphenicol 87.5% and had the same sensitivity to Vancomycin, Amikacin and Gatifloxacin 82.5%.

Pseudomonas was highly sensitive to Amikacin with a 100% sensitivity, followed by Gatifloxacin and Gentamicin (94.12% and 82.35% respectively) and was resistant to both erythromycin and Vancomycin. The anaerobic *Propionibacterium* showed an absolute sensitivity to Amikacin (100%) Followed by Gatifloxacin (80%) and Vancomycin (60%) and was resistant to Erythromycin (60%).

The susceptibility of the fungal isolates to six antifungals is shown in Table (8). The three *Aspergillus species* isolated in the culture positive cases were highly sensitive to Voriconazole, ranging from 87.5% and 100% sensitivity. Amphotericin B gave a moderate sensitivity to *Fusarium spp*. (66.7%), while had an absolute sensitivity to the single *Mucor spp*. isolated together with Voriconazole. *Candida spp.* was equally sensitive to Ketoconazole and Metronidazole with an 82.8% sensitivity.

DISCUSSION

Infectious keratitis affects both males and females. A male preponderance has been noted. In our study males had a tendency to get corneal ulcer more than females, which may be explained by males having more chances of accident or trauma than females due to more outdoor activities. This is similar to the other studies conducted in Peshawar [23], Thailand [24], India [25] and Oman [26]. Also in Shoja and Manaviat study [3] 57.5% of cases, in Behboody and Mohammadi study [27] 64.2% and in Ormerds' series [28] 71% of the cases were male. In observations made by Srinivasan *et al.* [29], this frequency was 61.3%. Norina *et al*. [30] concluded that male patients in their mid-40s contributed to the majority of their patients, with a male-to-female ratio of 3:2. In contrast there is female preponderance in studies from Bahrain [31] and England [16].

The result of this study showed the highest frequency for corneal ulcer belonging to age group of 30-40 years, this is similar to the observations made by Upadahyay, in Nepal [32] Shoja and Manaviat in Iran [3] as well as in others series [33,34]. Our study also revealed a high frequency in other age groups that ranged from 20 to 70 years and these findings are similar to studies from Thailand [24] and India [25] where corneal ulcer was common in middle age group and also in harmony with the study from Oman [26] where corneal ulcer was more common in the elderly. A bimodal age distribution of patients presenting with microbial keratitis has been documented, which may be attributed to ocular trauma and contact lens related keratitis in younger group and poor immunity and predisposing ocular disease in the older age group [9, 35].

Ocular trauma is a major risk factor for corneal ulcers in the developing countries [36]. In this study, a history of ocular trauma, especially with organic matter is the main predisposing factor, this is in accordance with a study concluded that in regions where agricultural work is more common, vegetative material-induced corneal trauma is the major cause of microbial keratitis [37]. In another study trauma was the leading predisposing factor (39%) [24] and 77.5% in some areas [38].

Of the non-traumatic risk factors, contact lens usage contributed to 18.78% of all cases. Lam *et al*.[39] reported that the incidence of bacterial keratitis was six fold higher in contact lens wearers than in the general population and an increased incidence of *Pseudomonas aeruginosa* (*P. aeruginosa*) infections coincided with the increased popularity of contact lens wear. Erie *et al*, reported, in a Minnesota study, that the cases of ulcerative keratitis associated with contact lens usage increased from zero percent in the 1950s and 1960s to 32% in the 1970s and 52% in the 1980s [40]. These figures were directly related to the increasing usage of contact lenses either for refractive correction or cosmetic purposes. So, contact lens wearing is one of the most, if not the most, important risk factor for infectious keratitis in the developed world [41]. Increasingly, overnight wear of orthokeratology lenses, which are used for the temporary reduction of myopic refractive error, is being implicated as a risk factor for infectious keratitis in East Asia, where these lenses have become popular [42,43]. In our study contact lens wearing occupied the second common risk factor (18.78%) which is more common in 20 to 40 years age group and this is in accordance with studies done by Shoja and Manaviat [3] and Norina *et al*. [30] but in contrast with Schaefer's study, which revealed that the leading risk factor was contact lens (36%) followed by trauma (20%) [44].

A total of 380 samples obtained from corneal ulcer patients were analyzed, of which 213 (56.05%) yielded growth of bacteria and fungi, while 167(43.95%) showed no growth. Lone bacterial and fungal growths were detected in 122 (32.10%) and 48 (12.63%) cases respectively. Taking the mixed growths into account, the total bacterial and fungal culture positive cases were 156 and 71 respectively. The sterile' ulcers may occur due to non-microbial causes, or may be of infectious origin with negative culture and virological results due to various reasons. It may not be possible to detect a

microorganism in around 35–60% of patients with suspected infectious keratitis, possibly because of scanty sample material, delay in performing investigations, prior use of antimicrobial agents or even the use of certain corneal stains such as rosebengal and lissamine green [45]. Prior use of topical antibiotics may only delay the time taken to grow organisms in culture without affecting culture-positivity rates [46, 47].

Positive cultures were observed in 56.05% of our cases, which agrees with Behboodi's study (49%) [27]. In positive cultures, *Staphylococcus epidermidis* was the most common pathogen, of cases in 40(25.64%) which is similar to the results of several other studies [33, 44], followed by *Staphylococcus aureus* in 35(22.43%) and *Streptococcus pneumonia* in 17(10.89%) of cases.

Further, fungi were identified as principal etiological agents of corneal ulcer, isolated from 58.93% cases in a study made by Akter *et al*. [2] is consistent with the findings of researchers from different parts of the world [29, 49] whereas pure fungal growth 48(12.63%) of our cases was obtained which is in harmony with Behboodi's series [27], where fungi caused 20.75% of the cases of keratitis. Also, in a study in Bangladesh, the most common strain was fungus (35.9%). These differences are due to the differences in epidemiologic factors [2].

In this study, the highest percentage of bacterial isolates were susceptible to Gatifloxacin 133 (85.2%) and Amikacin 116 (74.3%) (Table 7), while the highest percentage of bacterial isolates were resistant to Ceftazidime 84 (53.8%), Erythromycin 64 (41%) and Gentamicin 47 (30.1%). Similarly, Bharathi *et al*. [50] reported the highest percentage of bacterial isolates were susceptible to Gatifloxacin (96.5%) and Amikacin (91.1%) while the highest percentage of bacterial isolates were resistant to Norfloxacin (47.7%) and Gentamicin (41.3%). Similarly, of all antibacterial agents tested, Gatifloxacin and Amikacin showed lowest percentage of resistance to all categories of bacterial species recovered from bacterial keratitis. Sun *et al*. observed higher effectiveness of Amikacin against both Gram positive and Gram negative bacterial isolates [51].

Antibacterial susceptibility pattern of the bacterial isolates carried out by Akter *et al*. [2] revealed that lomefloxacin, Tobramycin and Gentamicin were better effective drugs against most of the gram positive and gram negative bacteria.

In this study, the efficacy of Chloramphenicol which is frequently used, was found to be poorly active against most bacterial isolates except *Streptococcus pneumoniae* and *Staphylococcus epidermidis*. This was also reported by Akter *et al*. [2]. This poor performance of Chloramphenicol may be due to its enthusiastic use seen in common practice leading to drug resistance. Mandal *et al*. [52] had also reported that Chloramphenicol was effective only against most of the Gram- positive organisms.

In our study, *Staphylococcus aureus* sensitivity was found to be 82.8% to Vancomycin and Gatifloxacin and to a lesser extent to Amikacin 77%. On the other hand, *Staphylococcus epidermidis* had the same sensitivity to Vancomycin, Amikacin and Gatifloxacin 82.5%. *Staphylococcus aureus* and *Staphylococcus epidermid*is were moderately resistant to Ceftazidime 62.9% and 72.5% respectively, followed by Gentamicin 42.9% and 37.5% respectively. *Staphylococcus epidermidis* was highly sensitive to Chloramphenicol 87.5%. *Pseudomonas* isolates were highly sensitive to Amikacin 100%, Gatifloxacin 93.7% and Gentamicin 81.2% where as it was highly resistant to Erythromycin and Vancomycin 93.7% and 87.5% respectively.

Rahim *et al*. [53], reported *Staphylococcus aureus* sensitivity was found to be 54.6% to Amoxycillin, 27.3% to Cephradine, 100% to Neomycin, 100% to Ciprofloxacin, 81.1% to chloramphenicol and 100% to Imipenem. Resistance to Chloramphenicol among Gram positive organisms was 10%. The ocular isolates of *Staphylococcus epidermidis* showed a highly variable pattern of antibiotic sensitivity 82.9% and 75.6% of the isolates were sensitive to Amoxycillin and Cephradine respectively, whereas isolates sensitive to Neomycin, Ciprofloxacin and Chloramphenicol were found to be 95%, 92% and 87.8% respectively. Antibiotic susceptibility of *Pseudomonas aeruginosa* was found to be 2.6% to Amoxycillin, 7.7% to Cephradine, 69.2 % to Neomycin, 82% to Ciprofloxacin, 84.4% to Imipenem and 28.3% to Chloramphenicol, respectively.

Another study done by Briscoe *et al*. [54], reported that *Pseudomonas* isolates were 100% sensitive to Ceftazidime, 86% sensitive to Ciprofloxacin, with only 20% being sensitive to Ampicillin and 14% to Cephalexin.

Most of the organisms isolated during this study were resistant to Ceftazidime and Erythromycin. This study suggested that Gatifloxacin and Amikacin are the best choice for treating microbial keratitis. Monotherapy with Fluoroquinolones eye drops is a better choice than combined antibiotic therapy, due the quicker clinical response and less toxicity. In addition Fluoroquinolones have good sensitivity to common ocular isolates. It is common practice that a patient comes to the ophthalmologist when an infection becomes severe that

the ophthalmologist cannot wait for the results of the sensitivity tests and has to prescribe a broad-spectrum antibiotic but treatment modification can be made after getting the results of sensitivity test. This study showed the development of resistance, it is advisable to perform sensitivity test and modify the chemotherapy according to the results of the sensitivity test.

In our study, Voriconazole was highly effective against all the fungal species isolated with the exception of *Candida species* which was found to be sensitive to ketoconazole and metronidazole. Amphotericin B was effective for the single *Mucor* isolated, but had a moderate efficiency for *Fusauium* and did not show any significant results with *Aspergillus species*.

Riddell *et al*. [55] reported that systemic voriconazole, with or without intravitreal injection, seems to lead to more rapid response than other antifungal agents. One added advantage of voriconazole over fluconazole is that it has activity against *Aspergillus species* and fluconazole resistant *Candida species*.

In this present study, *Fusarium species* showed the highest resistance to Fluconazole followed by *Aspergillus flavus*, also the filamentous fungi which showed resistance to amphotericin B were *Aspergillus flavus*, followed by *Aspergillus niger*, these findings correlated with a study conducted by Therese *et al*. [56], in which resistance to Fluconazole was observed and also reported that *Aspergillus niger* and *Aspergillus terreus* exhibited higher percentage of resistance to Amphotericin B, Fluconazole and ketoconazole.

The emergence of antifungal drug resistance has made susceptibility testing an important tool to support therapy.

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