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Microbiological Features and Risk Factors of Microbial Keratitis in Cairo University

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Abstract:

Purpose: To identify the causative organisms and associated risk factors in cases of microbial keratitis (MK) presenting to Cairo University Hospitals.**Setting**: Tertiary care university center**Methods**: A prospective study where corneal scrapings were obtained from 85 patients with MK over 9 months were subjected to direct smear examination using Gram and potassium hydroxide stains, and culture using blood, chocolate, MacConkey, and Sabouraud's-dextrose agar. In culture-positive cases, antimicrobial sensitivity testing was performed.**Results**: From September 2020 to May 2021, 85 cases of MK were enrolled. Corneal scrapings were performed in 76 cases. A positive culture was obtained in 47 cases (61.8%). Among culture-positive cases, 36 cases (76.6%) were fungal, and 11 (23.4%) were bacterial. Among culture-negative cases (n=34), based on clinical appearance and response to antimicrobial therapy, 23 cases were considered fungal (67.6%), 6 bacterial (17.6%), and 5 due to acanthamoeba species (14.7%). Positive correlation was found between fungal etiology and previous herpetic keratitis (p=0.52), agricultural occupation (p=0.67), and ocular trauma of plant origin (p=0.11). A statistically significant correlation was found between presumed acanthamoebal etiology and contact lens wear (p<0.001).**Conclusions**: Positive culture was obtained in 61.8% of cases. Among these, 76.6% were fungal, and 23.4% were bacterial. In culture-negative cases, almost 70% were clinically presumed to be fungal, and the were considered either bacterial or acanthamoebal (approximately 15% each).

Keywords:Microbial keratitis, Corneal ulcer, Microbiological profile, Fungal, Bacterial, Antimicrobial susceptibility

1. Introduction

Microbial keratitis (MK) is a serious ocular infectious disease, and one of the leading causes of preventable monocular blindness worldwide.1 It has been estimated that 1.5-2 million cases of MK occur annually in developing countries, and the actual number is probably greater.2 Bacterial keratitis is the most common in temperate climates such as the USA, accounting for 89% to 96% of cases of MK,3,4 while fungal keratitis can cause up to 50% of MK in tropical climates.5,6 It affects both sexes and all ages.1

The fate of corneal ulcers varies dramatically from resolving without any sequelae, to healing by an opaque vascularized scar, to progressing to corneal perforation, which may end in blindness. 7 There is noticeable variation regarding risk factors, dominance of different microorganisms, and outcome. This may be attributed to differences in demographic factors, climate, and socioeconomic conditions. 9

In general, early identification of causative organisms and prompt initiation of adequate antimicrobials are essential to prevent permanent vision loss.10 Due to regional differences, updates of information from different regions of the world are always needed. The ophthalmology department at Cairo University is an important tertiary referral center for MK, serving Greater Cairo and surrounding governorates. This study aims to identify the causative organisms in cases of MK presenting to Cairo University Hospitals, and any associated risk factors.

Methods

This cross-sectional analytic study was conducted at Cairo University Hospitals, from September 2020 to May 2021. The study was approved by Kasr Al Aini Hospital Ethics Committee on 2020 with approval number (MS-427-2020).

The study was a prospective study. It included 85 eyes of 85 patients. Patients were recruited from the Ophthalmology outpatient clinics and casualty department in Cairo University Hospitals. Patients with a clinical diagnosis of MK were included, with no age or gender restriction. Exclusion criteria included a clinical picture suggestive of pure viral keratitis without superimposed secondary infection, or non-microbial keratitis, such as Mooren's ulcer, marginal keratitis, and autoimmune peripheral ulcerative keratitis.

All patients received a thorough explanation of the study and the corneal scraping procedure. Consent was taken according to the Declaration of Helsinki and parents signed informed consent for their children before enrollment of study.

Patients were subjected to comprehensive history-taking, including personal history, analysis of symptoms, ocular pain pattern and inquiry about known risk factors of MK, including ocular trauma, surgery, history of herpetic eye disease, relevant systemic conditions like diabetes, and immunosuppression.

Full ophthalmological examination, including visual acuity, Slit-lamp examination with documentation of extent of epithelial loss, stromal infiltration, and hypopyon, if present. Corneal sensation. Intraocular pressure measurement, digitally or by Goldmann applanation tonometer (Haag-Streit slit-lamp tonometer – United States) whenever possible. Fundus examination. If there was no fundus view, the posterior segment was assessed by B-scan ultrasound (Sonomed Escalon – United States). Grading of the ulcer was done according to modified Jones criteria. 12

In patients without previous treatment, sampling was done immediately. In patients already under treatment, but deemed non-responsive to this treatment, all topical antimicrobial agents were discontinued for 48 hours before sampling.

After sampling, empirical antimicrobial therapy was initiated, based on clinical evaluation. Cases of suspected bacterial keratitis were started on a fourth-generation fluoroquinolone (Vigamox – Novartis Pharma AG, Switzerland), given as hourly eyedrops while awake. If a fungal etiology was suspected, the patient was started on hourly fluconazole 0.2% (Diflucan vial – Pfizer, United States) or voriconazole 1% eyedrops (Vfend vial – Pfizer, United States) while awake. Patients with presumed acanthamoeba keratitis were started on propamidine isethionate 0.1% eyedrops (in house pharmacist preparation) 6 times per day, with or without chlorhexidine 0.02 % (in house pharmacist preparation) 4 times per day, and fluconazole 0.2% 4 times per day. In all three groups, antimicrobial therapy was modified according to response, and lab results.
Topical cycloplegic drops were given in all cases, and antiglaucoma medications were given according to need. In patients with severe corneal melting and a high risk of perforation, anti-melting medications were given (oral ascorbate and doxycycline (Vibramycin – Pfizer, United States). Systemic antimicrobial therapy was given only if there was associated scleritis. In patients with presumed underlying herpetic keratitis, oral acyclovir (Acyclovir 400 stada – Global Napi pharmaceuticals, Egypt) was given.
All scrapings were performed by the same ophthalmologist (NL). After instillation of benoxinate hydrochloride 0.4 % drops (Benox – EIPICO, Egypt) , the eyelid skin and eyelashes were prepped with povidone-iodine 10 % solution (Pharaonia Pharmaceutical, Egypt). The procedure was performed under an operating microscope or slit lamp. Under aseptic conditions, a wire eyelid speculum was placed. Corneal scrapings were obtained from the edges of the ulcer, at the junction of infiltrated and healthy corneal tissue, using a sterile Bard-Parker no. 15 blade. After collecting a good yield of tissue, and under magnification, the tissue was transferred from the tip of the blade to a sterile cotton-tipped swab moistened with sterile normal saline. The swab was then used to make two linear strokes on each of two dry glass slides, for stained smears. Scrapings were obtained again, transferred to the sterile swab, and then the swab was used to make several C- or S-shaped strokes on a blood agar plate. This step was repeated 4 more times for inoculation to one chocolate agar plate, one MacConkey plate, and two Sabouraud's dextrose agar (SDA) plates. The slides and plates were labeled and delivered to the central laboratory of the Clinical Pathology Department.
Sample processing included: Direct smears stained with Gram stain and 10 % KOH were prepared and examined microscopically. All samples cultured on blood, chocolate, and MacConkey agar were incubated aerobically at 37°C for 24-48 hrs. All samples cultured on SDA were incubated at 20° for 1-2 weeks. Identification of isolated microorganisms was done according to standard criteria, namely microscopic features in stained smears, colony morphology, and specific biochemical reactions.
Antimicrobial susceptibility was routinely done for positive yields. Antibiotic susceptibility testing was performed by the disc diffusion method (modified Kirby-Bauer technique) using Muller-Hinton agar, with aerobic incubation at 35°C for 16-18 hours. Sensitivity was done according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), 2020. 13

In antifungal susceptibility testing, microdilution methods were performed according to guidelines of The European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI). 14,15

Statistical methods

Data were analyzed using IBM© SPSS© Statistics version 26 (IBM© Corp., Armonk, NY). Numerical variables were presented as mean ± SD and range or median and interquartile range.

Categorical variables were presented as numbers and valid percentages and differences were compared using Fisher's exact test. Ordinal data were compared using the chi-squared test for trend. Time-to-event analysis was done using the Kaplan-Meier method. The log-rank test was used to compare Kaplan-Meier curves. P-values <0.05 were considered statistically significant.

Results

A total of 85 eyes of 85 patients clinically diagnosed with MK were enrolled in this study.

Of the 85 patients, 52 (61.2%) were males and 33 (38.8%) were females. The mean age of patients was 38.7 ± 20.5 SD. 18 patients (21.2%) were between 0-19 years of age, 26 (30.6%) were 20-39 years of age, 24 (28.2%) were 40-59, and 17 (20%) were >60 years of age.

The mean time between onset and presentation was 46.7 days ± 57.3 SD. Time between onset and presentation were variable. Only 16 patients (18.8%) presented <1 week of onset of symptoms, 20 (23.5%) patients presented between 1-4 weeks of onset, while the majority of our study population 35 cases (41.2%) presented 4 to 12 weeks of onset, and 14 patients (16.5%) presented after more than 12 weeks of onset. As per modified Jones guidelines, the severity was mild in 12 patients (14.1%), moderate in 20 patients (23.5%), and severe in 53 patients (62.4%).

Hypopyon was present in 36 patients (42.4%), scleritis in 2 patients (2.4%), and kerato-neuritis in one patient (1.2%).

Visual acuity was PL to HM in 66 patients (78.8%), 1/60-6/60 in 14 patients (16.5%), and >6/60 in 3 patients (3.5%). Vision could not be assessed in 1 patient (1.2%) (a newborn baby).

The most common local risk factor was ocular trauma, recorded in 30 patients (35.3%), of which 18 cases (21.2%) were of plant origin. This was followed by post-surgery in 12 patients (14.1%), herpetic keratitis in 11 patients (12.9%), contact lens wear in 9 patients (10.6%), exposure keratopathy secondary to facial palsy in 4 patients (5%), and agricultural occupation in 4 patients (5%).

Diabetes Mellitus was present in 6 patients (7.1%). There was no identifiable risk factor in 15 patients (17.6%). Table 3 summarizes the risk factors.

Table 1. Prevalence of risk factors in the study population.

Variable		Count
Local risk factors	Ocular trauma	30
	Trauma of plant origin	18
	Herpetic keratitis	11
	Contact lens wear	9
	Post-surgery	12
	Agricultural occupation	4
	Exposure keratopathy	4
	No identifiable risk factors	15
Type of Trauma	Trauma of non-plant origin	12/30
	Trauma of plant origin	18/30

Laboratory Results:

Corneal scraping was done on 76 eyes (89.4%). In 8 patients (10.5%), scraping was performed before receiving treatment. In 56 patients (73.7%), topical treatment was discontinued 48 hours before scraping. In 12 patients on medication (15.8%), scraping was performed immediately, because the patient could not return after 48 hours for scraping, or because the ulceration was so aggressive, that it was too risky to defer treatment.

In 9 cases, the patient (or the patient's guardian), refused to consent to the scraping procedure. Empirical treatment was given based on clinical picture.

Direct smear was done in 52 patients. Positive smears were found in 12 cases (23.1%) of Gram-stained smears: gram-positive cocci in 9 cases, gram-negative bacilli in 2 cases, and gram-negative cocci- bacilli in 1 case.

Fungal smears were all negative.

Culture was done in all 76 patients who underwent corneal scraping. Culture-positive results were found in 47 cases (61.8%), and no growth was seen in 29 cases (38.2%).

In the 8 cases that had not received any medication before presentation, a positive culture was obtained in all 8 cases (100%). In the 56 cases where treatment was discontinued for 48 hours, a positive culture was obtained in 33 cases (59%). In the 12 cases that underwent immediate scraping despite previous therapy, a positive culture was obtained in 6 cases (50%).

Among positive cultures, pure fungal growth was present in 36 samples (47.4%), and pure bacterial growth in 11 samples (14.5%). In patients with a high clinical suspicion of acanthamoeba keratitis, acanthamoeba infection could not be confirmed by laboratory methods due to the unavailability of the requisite culture medium (non-nutrient Agar with an overlay of E. coli).

Among fungal cultures, Aspergillus spp. was commonest isolated organism, constituting 23 scrapings (48.9%), followed by Fusarium spp. in 9 scrapings (19.1%) and Candida spp. in 4 scrapings (8.5%). Among bacterial cultures, staphylococcus aureus was detected in 4 scrapings (8.5%), streptococcus spp. in 4 scrapings (8.5%), and Pseudomonas aeruginosa in 3 scrapings (6.4%) as shown in Figure 1.

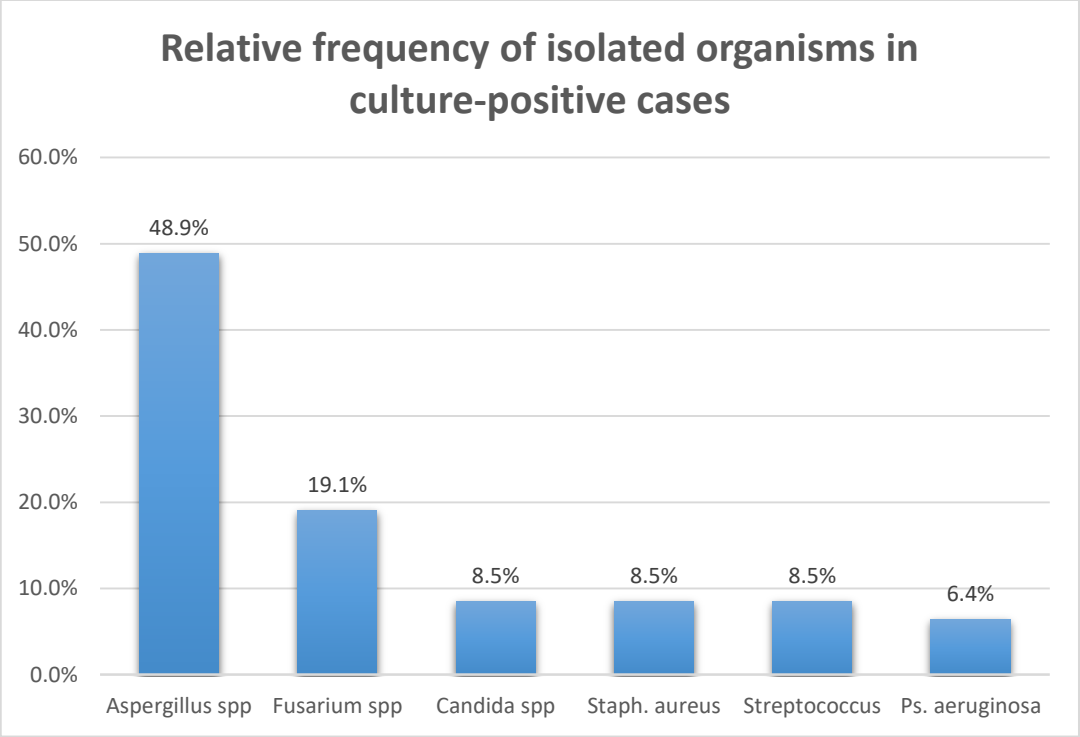

Figure 1: Isolated Organisms.

Regarding the antimicrobial susceptibility, table 1 shows the detailed outcomes of the antimicrobial susceptibility of the isolated organisms of culture positive cases to the different antimicrobials available.

Table 2: Antimicrobial susceptibility

Antimicrobial agent	Sensitivity	Count	Valid %
Fluconazole	Resistant	12	33.3%
	Intermediately sensitivity	3	8.3%
	Sensitive	21 (11 Aspergillus spp, 6 Fusarium spp, 4 Candida spp.)	58.3%
Voriconazole	Resistant	1	3.4%
	Intermediately sensitivity	0	0.0%
	Sensitive	28 (17 Aspergillus spp, 7 Fusarium spp, 4 Candida spp.)	96.6%
Ketoconazole	Resistant	12	33.3%
	Intermediately sensitivity	2	5.6%
	Sensitive	22 (11 Aspergillus spp, 8 Fusarium spp, 3 Candida spp.)	61.1%
Itraconazole	Resistant	4	13.8%
	Intermediately sensitivity	3	10.3%
	Sensitive	22 (13 Aspergillus spp.,	75.9%

		6 Fusarium spp, 3 Candida spp.)	
Natamycin	Resistant	3	42.9%
	Intermediately sensitivity	0	0.0%
	Sensitive	4 (1 Aspergillus spp, 3 Fusarium spp.)	57.1%
Vancomycin	Resistant	0	0.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	8 (4 Staphylococci, 4 Streptococci)	100.0%
Amikacin	Resistant	2	25.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	6 (4 Staphylococci, 3 Pseudomonas)	75.0%
Gentamicin	Resistant	4	40.0%
	Intermediately sensitivity	1	10.0%
	Sensitive	5 (2 Staphylococci, 2 Pseudomonas, 1 Streptococci)	50.0%
Tobramycin	Resistant	0	0.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	1 (Pseudomonas aeruginosa)	100.0%
Ofloxacin	Resistant	1	14.3%
	Intermediately sensitivity	0	0.0%
	Sensitive	6 (3 Staphylococci, 1 Pseudomonas, 2 Streptococci)	85.7%
Ceftazidime	Resistant	1	33.3%
	Intermediately sensitivity	0	0.0%
	Sensitive	2 (2 Pseudomonas aeruginosa)	66.7%
Linezolid	Resistant	0	0.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	5 (4 Staphylococci, 1 Streptococci)	100.0%
	Sensitive	4	100.0%
Polymyxin B	Resistant	0	0.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	1 (1 Pseudomonas aeruoginosa)	100.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	0	0.0%
Levofloxacin	Resistant	0	0.0%
	Intermediately sensitivity	0	0.0%
	Sensitive	2 (1 Pseudomonas, 1 Streptococci)	100.0%

For further data analysis, in fungal culture-positive cases (n=36), figure 2 shows the relative frequency of sensitive isolates for each antifungal.

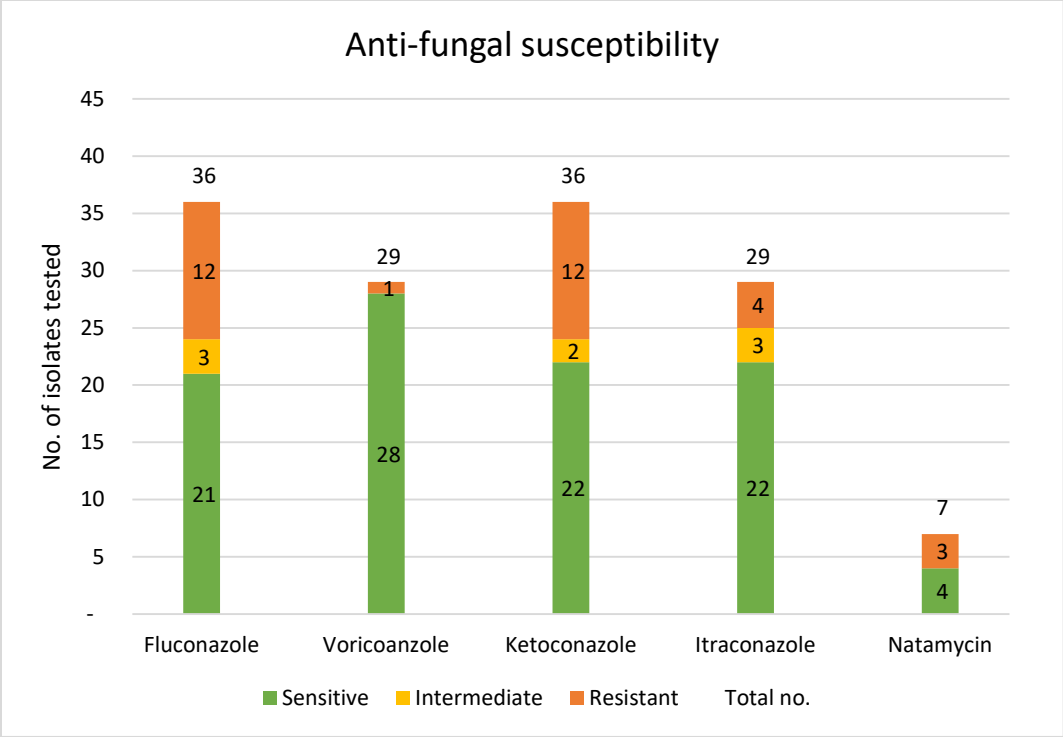

Figure (2) Sensitivity of fungal isolates to the tested antifungal agents.

In bacterial culture-positive cases (n=11), susceptibility to different antibiotics was tested according to antibiotic disc availability. Figure (3) shows the relative frequency of sensitive isolates for each antibiotic.

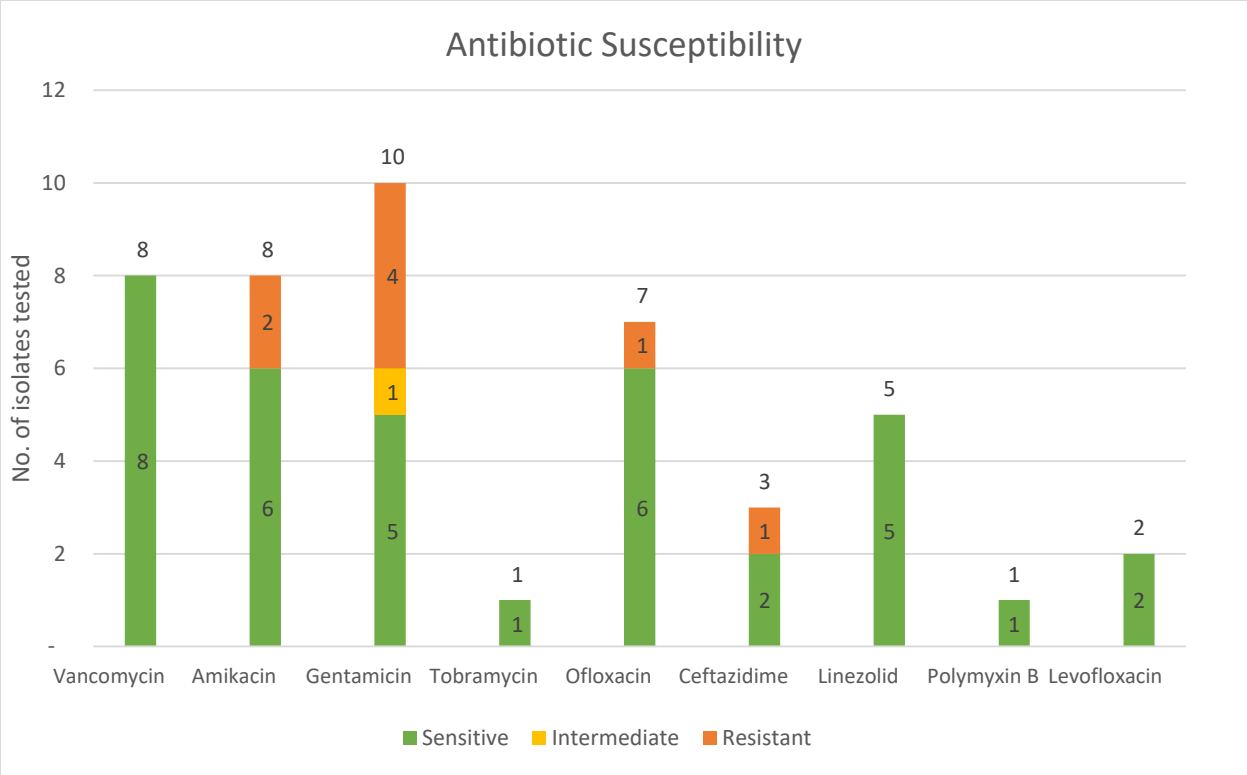

Figure 3: Sensitivity of bacterial isolates to the common antibiotic agents used in ophthalmic practice.

Table (3) shows a correlation between the different local risk factors for MK identified in our study and the causative microbial etiology. For this analysis, culture-negative cases (n=29) plus 5 out of 9 cases who refused scraping but continued follow-up till resolution, were considered bacterial, fungal or acanthamoebal based on clinical picture and resolution of keratitis in response to empirical antibiotic, antifungal, or anti-acanthamoebal therapy respectively. A statistically significant correlation was found between contact lens wear and acanthamoebal etiology. There was a positive correlation between fungal etiology and ocular trauma of plant origin, agricultural occupation, and previous herpetic keratitis, but this was not statistically significant, most probably due to sample size.

Table (3): Relation between local or systemic risk factors and type of infection

		Ultimate diagnosis						
		Fungal (n=59)		Bacterial (n=17)		Acanthamoeba (n=5)		
Variable		n	Row %	n	Row %	n	Row %	P-value*
Ocular trauma	-	35	67.3%	12	23.1%	5	9.6%	0.180
	+	24	82.8%	5	17.2%	0	0.0%	
Herpetic keratitis	-	49	70.0%	16	22.9%	5	7.1%	0.515
	+	10	90.9%	1	9.1%	0	0.0%	

Contact lens wear	-	57	79.2%	15	20.8%	0	0.0%	<0.001
	+	2	22.2%	2	22.2%	5	55.6%	
Post-surgery	-	52	75.4%	12	17.4%	5	7.2%	0.150
	+	7	58.3%	5	41.7%	0	0.0%	
Agricultural occupation	-	55	71.4%	17	22.1%	5	6.5%	0.668
	+	4	100.0 %	0	0.0%	0	0.0%	
Exposure keratopathy	-	58	74.4%	15	19.2%	5	6.4%	0.178
	+	1	33.3%	2	66.7%	0	0.0%	

*. Fisher's excat test.

The final outcome was complete healing in 44 patients (51.8%), improving patients with ongoing follow ups in 28 patients (32.9%), worsening occurred in 8 patients (9.4%), and 5 patients were lost to follow up (5.9%).

Among healed patients, 34 (77.3%) patients healed on medical treatment only, while 10 patients (22.7%) healed with medical and surgical management.

Regarding complications, only 14 cases (16.5%) advanced to complications with central perforation recording the highest incidence in 9 out of the 14 cases (64.3%), 4 patients (28.6%) progressed to corneal thinning and descemetocele, and one patient (7.1%) worsened to corneal melting; denoting globe preservation rate of 93% in our study.

Among all cases, surgical intervention was needed in 16 patients (18.8%). 8 cases (50%) underwent amniotic membrane transplantation, 3 cases (18.8%) needed tectonic grafting, 2 cases (12.5%) had tissue glue to seal small corneal perforation and 1 patient (6.3%) had conjunctival flap performed. Two patients needed 2 surgeries; one case (6.3%) underwent AMT + tissue glue while the other case (6.3%) underwent AMT then tectonic grafting.

Visual outcome in 41 of the patients were 9 cases (22%) with a vision of PL to HM mainly due to a central corneal scar, 16 patients (39%) with a vision of 1/60 to 6/60 and 16 patients (39%) with vision > 6/60.

Rate of healing is significantly higher in bacterial keratitis compared with Acanthamoeba keratitis (incidence rate ratio = 7.1, 95% CI = 1.8 to 27.4) while differences between bacterial and fungal keratitis, and between fungal and Acanthamoeba keratitis was found to be not statistically significant.

Time taken to achieve complete healing differed with the type of keratitis. Median time to healing was 60 days for fungal keratitis, 30 days for bacterial keratitis and 120 days for acanthamoeba keratitis. The differences among them as shown in figure 4 was statistically significant.

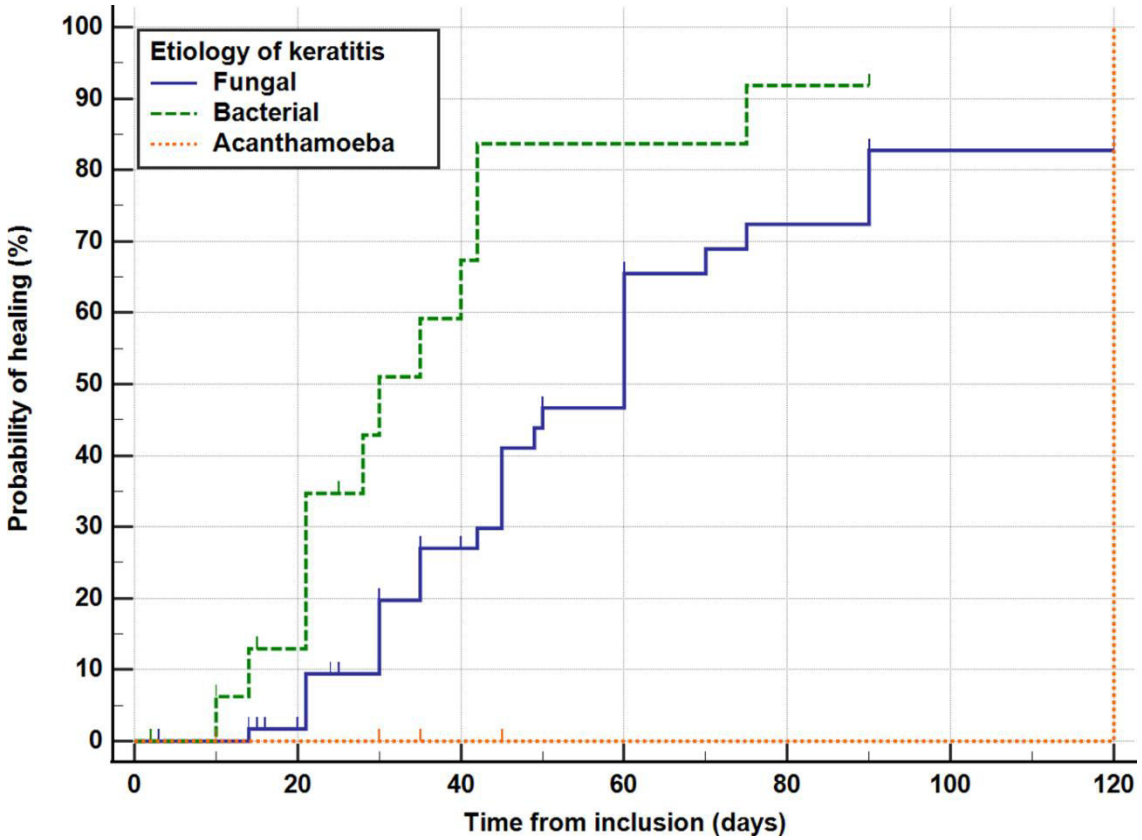

Figure (4): Kaplan-Meier (KM) curves for time to healing in fungal, bacterial or Acanthamoeba keratitis. Median time to healing = 60 (95% CI, 45 to 70) days, 30 (95% CI = 21 to 42) days and 120 days for fungal, bacterial or acanthamoeba keratitis, respectively. The differences among the three KM curves is statistically significant (Log-rank test chi-squared = 10.514, df = 2, P-value = 0.005).

Clinical representative case examples of cases in our results are shown in Figure 5. (1) was a case of pseudomonas aeruginosa that responded to fortified gentamicin. (2) represents a case of Aspergillus keratitis that responded to topical voriconazole. (3) A refractory case that showed Fusarium Solani complex on culture and responded to topical voriconazole. (4) Acanthamoeba keratitis case that showed healing after one year on topical anti-protozoal treatment.

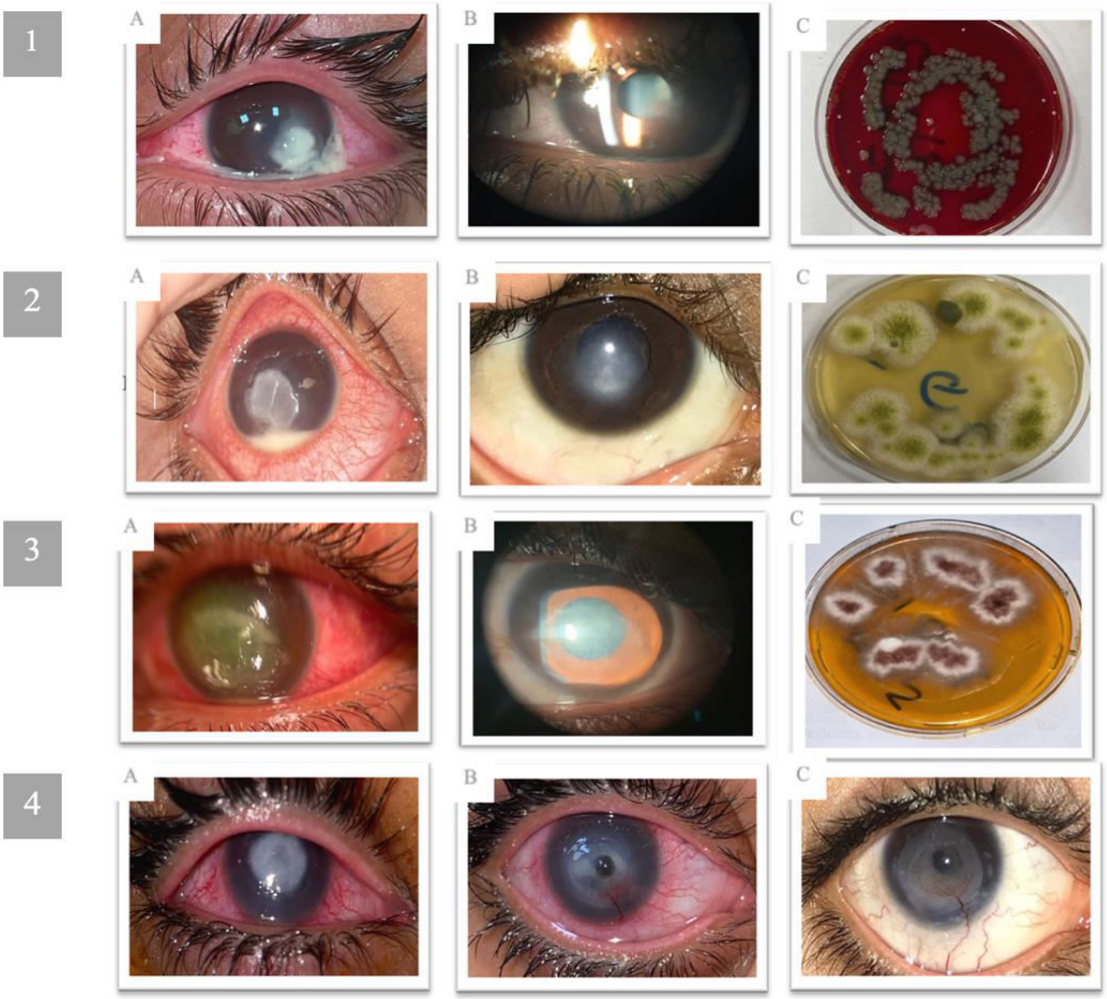

Figure 5: Representative case examples:

1. A) Pseudomonas aeruginosa keratitis on presentation. B) Outcome on fortified gentamicin.
C) Pseudomonas growth on blood agar.
2. A) Aspergillus keratitis on presentation B) Outcome on topical voriconazole C) Aspergillus species growth on Sabouraud's dextrose agar
3. A) Fusarium keratitis on presentation B) Outcome on topical voriconazole C) Fusarium Solani Complex growth on Sabouraud's dextrose agar
4. A) Acanthamoeba keratitis on presentation. B) Response to topical therapy C) Quiet eye after a year of treatment.

Discussion

We found the predominance of infectious keratitis higher in the middle age group (20-59 years old) and among males (61.2%), which could be attributed to their greater involvement in outdoor activities, different manual and agricultural occupations, making them more prone to ocular trauma, especially corneal injuries with external agents. Similar observations were reported by other studies reporting a male preponderance of 61 to 71 %.16 The majority of studies did not observe any gender dominance in MK but when gender difference exists, it is commonly related to the underlying risk factors in different regions. For instance, In CL wearers, MK exhibit a

female predominance of 57–69%, 17 which goes along with our study since 8 out of the 9 CL wearers were females. Whereas ocular trauma related MK as mentioned earlier, is associated with a male predominance of 74–78%.17,18

There were 16 patients (18.8%) with MK in the age group between 0-19 years. In previous studies, MK showed a similar incidence in pediatric patients, where 13% of all cases were in this age group. 19,20

In the current study, risk factors for microbial keratitis were identified in the majority of patients. Ocular/corneal trauma was the most common risk factor, it was present in 30 patients (35.3%). Similar common incidence is reported in the literature, as ocular trauma was reported as a common risk factor in rural areas and low income countries where it accounts for up to 77.5% of cases. 21 Eighteen cases (60%) of the ocular trauma were of plant origin, and this have been associated more with fungal keratitis.22,23 As illustrated in table 4 below, ocular trauma is the commonest risk factor in studies in India, Malaysia, Nepal, Nigeria and Egypt.

Table 4: Summary of some of the most common risk factors in microbial keratitis in the literature in different regions published between 2010 and 2020.

Authors	Study period	Region	No. of cases	Common risk factors
Cariello et al. 24	1975-2007	Brazil	16742	Post-ocular surgery (22.4%) Contact lens (12.8%) Ocular trauma (16.4%) Topical steroid (6.6%)
Oladigbolu et al. 25	1995-2005	Nigeria	228	Ocular trauma (51.3%) Traditional eye medicine (17.1%) Topical steroid (5.7%)
Sagerfors et al. 26	2004-2014	Sweden	398	Contact lens (45.5%) Ocular surface disease (9.8%) Corneal transplant (9.5%)
Dethorey et al. 27	2005-2011	France	398	Contact lens (48.1%) Ocular surface disease (33.7%) Post-ocular surgery (17.5%)
Kaliamurthy et al. 28	2005-2012	India	2170	Ocular trauma (64%) Traditional eye medicine (16.9%)
Ferreira et al. 29	2007-2015	Portugal	235	Contact lens (28.9%) Ocular trauma (28.9%) Diabetes Melliitus (13%)
Truong et al. 30	2009-2014	US	318	Contact lens (41%) Ocular surface disease (28%) Ocular trauma (17%) Topical steroid (4%)

Mandour et al. 8	2010-2013	Egypt	340	Ocular trauma (50%) Post-ocular surgery (14.7%) Topical steroids (11.8%)
Khor et al. 31	2010-2016	Malaysia	221	Ocular trauma (49.3%) Contact lens (23.1%) Ocular surface disases (5.9%)
Zbiba et al. 32	2011-2016	Tunisia	230	Ocular surface disease (58.7%) Ocular trauma (51.3%) Diabetes mellitus (16%)
Khoo et al.33	2012-2016	Australia	979	Contact lens (63%) Topical steroid (24%) Ocular surface disease (18%)
Badawi et al. 34	2013-2015	Egypt	247	Ocular trauma (51.4%) Diabetes mellitus (15.1%) Impact foreign body (5.7%)
Gautam et al. 35	2016	Nepal	259	Ocular trauma of plant origin (48%) Topical steroid (9%)
Current study	Sep. 2020-May 2021	Egypt	85	Ocular trauma (35.3%) Post-ocular surgery (14.1%) Herpetic keratitis (12.9%) Contact lens (10.6%)

Post-surgery MK came second to ocular trauma as a risk factor in 12 patients (14.1%), including, cases after cataract surgery, keratoplasty, vitrectomy and glaucoma. Similar incidence was found in studies in France, Brazil, and Egypt. 8,24,27

A history of pre-existing herpetic keratitis was found in 11 patients (13.75%), 10 of whom presented with superimposed fungal keratitis. Herpetic keratitis can predispose to secondary microbial infection through decreased sensation, geographic or neurotrophic ulceration, dry eye, and prolonged use of topical steroids. This high incidence requires further studies , as the need for systemic anti-viral therapy was essential for the treatment of all 11 cases and had satisfying results on follow up.

Contact lens (CL) wear was noted in 9 patients (10.6%), with the majority as females (8 out of 9), and more predominant (7 out of 9) with occasional wear of colored cosmetic contact lens, bought from hair salons and not medically prescribed nor adequately delivered. CL wear is reportedly one of the major risk factors for MK, due to contact lens-induced hypoxia and hypercapnia in the cornea.36 Similar incidence in our study could also be explained in view of the lack of hygiene education and improper CL wear care. A case control study has established that cosmetic CL wearers are at a 16.5 fold increased risk of infection compared with CL wear used for refractive correction. Cosmetic CL wearers made up 12.5% of microbial keratitis cases presenting to 12 university hospitals in France and was also overrepresented in a South Korean study, comprising 42.1% of cases among 22 institutions and clinics.37,38 CL sale through unlicensed vendors such as flea markets and street-side stalls, video stores, hair salons and gas stations, in addition to internet retailers has been documented and is considered one of the solid reasons for the overall increased incidence of CL related infective keratitis. 37

The main systemic association in our study was diabetes mellitus. Diabetic keratopathy is one of the major ocular complications of diabetes mellitus especially following corneal trauma or keratoplasty. 28 31 Diabetes is extensively reported to be the most common systemic risk factor for MK in several studies, for example in Portugal and El-Mansoura in Egypt.29,34

A positive culture was seen in 47 samples (61.8%). Previous studies reported a positive culture rate ranging from 35.1 % to 74.6 % as illustrated in table 5.

Regarding the microbiological features in the current study, fungal etiology was found in the majority of cases (76.5%). Since our institution is a tertiary referral center, most of the cases referred to us have usually received therapy elsewhere. Since fungal keratitis typically has a longer course with a slower response to therapy compared to bacterial keratitis, it follows that most of the cases that will seek tertiary care will tend to be fungal in origin.

The spectrum of microorganisms accounting for MK differs depending on geographic location, climate, and etiology.9 For example, gram positive bacteria are predominant in temperate climate regions, whereas Gram negative bacteria are prevalent in tropical regions.39 Pseudomonas spp. and acanthamoeba are associated with contact lens-related keratitis,40 whereas fungi are commonly linked to ocular trauma of plant origin22,23 In the literature, the incidence of fungal keratitis has been estimated to account for 20-60% of all culture- positive MK in tropical and subtropical climates.41 A hot, humid climate and agriculture-based occupations tend to make the incidence of fungal keratitis high among the Egyptian population. In our study, fungal growth was present in 36 cases. Aspergillus spp. was the commonest, followed by Fusarium spp., then Candida spp. This is similar to previous studies in Egypt, India, and China, and further microbiological data is illustrated below in table 5. The low incidence of Candida spp. could be attributed to the mild nature of Candida- related keratitis that would probably resolve from medical treatment without the need for referral to a tertiary care hospital.

Table 4 : Summary of the most common microbiological profiles of microbial keratitis in different regions in the literature published between 2010 and 2020.

Authors	Study period	Region	No. of cases	Positive culture %	Bacterial	Fungal	Acanthamoeba	Microbiological profile
Cariello et al. 24	1975-2007	Brazil	6804	48.6%	78.9%	3.6%	11%.	CoNS (41.2%) Staph. aureus (33.1%) Pseudomonas (18.5%)
Keshav et al. 7	2000-2006	Oman	188	43.18%	88.2%	11.8%		Pseudomonas (53.84%) Staphylococci (20%) S. pneumonia (18.46%)
Tam et al. 39	2000-2015	Canada	2330	57.3%	86%	2.2%	4.9%.	CoNS (37%) Pseudomonas (10%) Streptococcus spp. (15%)
Hernandez-Camarena et	2002-2011	Mexico	1638	38%	88%	0%	12%.	S. epidermidis (27.4%)

al. 42						Pseudomonas (12.1%) Staph. aureus (9%)
Lalitha et al. 43	2002-2012	India	23,897	59%	24.7% 2.2%	34.3% Fusarium spp. (14.5%) Aspergillus spp. (8.8%) S. pneumoniae (7%)
Tan et al.44	2004-2015	UK	4229	32.6%	90.6% 2.3%	7.1% CoNS (26.3%) Enterobacter (15.3%) Streptococci (13.9%)
Rautaraya et al.45	2006-2009	India	997	74.6%	23.4% 1.4%	26.4%. Aspergillus spp. (23.1%) Fusarium spp. (19.2%) Staphylococci (5.4%)
Tavassoli et al. 46	2006-2017	UK	2614	38.1%	91.6% 1.4%	6.9%. CoNS (36%) Pseudomonas)15.8%) Streptococci (7%)
Dhakhwa et al.47	Jan-Dec 2007	Nepal	414	72.5%	29.2%	33.3% Fusarium spp. (30.73%) S. epidermidis (29.57%) Pseudomonas (13.98%)
Ting et al. 48	2007-2019	UK	1333	37.7%	92.8% 4.2%	3%. Pseudomonas (23.6%) Staph. aureus (15.9%) Streptococci (13.5%)
Asbell et al.49	2009-2018	US	6091	100%	100% 0%	0 % Staph. aureus (35.9%) CoNS (29%) H. influenza (13%)
Cláudia et al.50	2009-2018	Portugal	1360	35.1%	76.78% 12.13%	8.16%. Corynebacterium macginleyi (18.41%) Staph.aureus (17.78%) S. pneumoniae (9.41%)
Lin et al.51	2010-2018	China	7229	42.8%	52.7% 0%	57.6%. CoNS (28.6%) Fusarium spp. (23.5%) Aspergillus spp. (12.2%)
Badawi et al.34	2013-2015	Egypt	247	44.5%	40% 4.5%	45.5%. Aspergillus spp. (19%) Staph. aureus (16.3%) Fusarium spp (11.8%)

Current study	Sep. 2020-May 2021	Egypt	85	61.8%	76.5%	23.5%	Aspergillus spp. (48.9%) Fusarium spp. (19.1%) Staphylococci (8.5%) Streptococci (8.5%)

Among the 11 positive bacterial cultures, 8 samples (72.7%) grew Gram-positive bacteria (Staphylococcus aureus in 4 samples, and Streptococcus spp. in 4). Gram-negative bacteria (namely Pseudomonas aeruginosa) were identified in 3 samples (27.2%). Among the S. aureus, 2 were coagulase negative staph aureus (CONS) and 2 were methicillin resistant staph aureus (MRSA), and among the streptococcus family, 3 were streptococcus pneumoniae and 1 was alpha-hemolytic streptococci which is similar to the commonly recovered bacteria from cultures in MK in different studies as shown in Table 10.

Only 5 cases of Acanthamoeba keratitis (AK) (5.8%) were reported in our study, which is consistent with other studies of MK. 52 AK diagnosis depended solely on clinical diagnosis and a positive response to anti-protozoal therapy.

Regarding antibiotic susceptibility, the bacterial isolates in our study were found to be sensitive to vancomycin in 7/7 patients, to Linezolid in 5/5 cases, to levofloxacin in 2/2 cases and polymyxin B in 1 /1 case (100% each), to ofloxacin in 6/7 cases (85.7%), to amikacin in 6/8 cases (75%), to ceftazidime in 2/3 cases (66.7%), and to gentamicin in 5/10 cases (50 %). We preferred to use fourth- generation quinolones in our study, because in addition to being equally effective to fortified antibiotics, they have the advantage of accessibility, less toxicity, and shorter duration of treatment. 53

On the other hand, gentamicin was effective in only 50% of cases. Among the 3 cases of Pseudomonas aeruginosa, one was found resistant to gentamicin and sensitive to polymyxin-neomycin. After shifting to the latter, healing occurred, emphasizing the importance of antibiotic susceptibility testing, even when the causative pathogen is recognized clinically before culture.

Regarding antifungal susceptibility testing in our study, the fungal isolates were found to be sensitive to voriconazole in 28/29 patients (96.6%), to itraconazole in 22/29 patients (75.9%), to ketoconazole in 22/36 patients (61.1%), to fluconazole in 21/36 patients (58.3%) and to natamycin in 4/7 patients (57.1%).

The profile of antifungal susceptibilities reported in previous studies is quite variable. This may be explained by regional variations in fungal pathogens, and the variability in the antifungal agents available in different locations. In our study, voriconazole was the most effective antifungal agent in vitro. Several studies were done but there is still no strong evidence in the literature regarding antifungal susceptibility in cases of MK. Wang et al. in a study done in China, on 535 eyes with fungal keratitis found that in vitro drug sensitivity tests pointed out to Aspergillus spp. being the most sensitive to terbinafine, and then voriconazole and to Fusarium spp. being similarly sensitive to both voriconazole and natamycin.54 Regarding all the tested fungi, the sizes of the inhibition zones in response to voriconazole were either larger or equal to that of natamycin which supports voriconazole as we found in our study population. Also, they concluded that the clinical results of Fusarium keratitis and other fungi were better than that of

Aspergillus which is consistent with the results of Lalitha et al.55 and Shapiro et al 56, who both found Aspergillus-induced keratitis more difficult to eliminate on comparison with Fusarium-induced keratitis. 54

The mean time from symptom-onset to presentation at our department was 46.7 days ± 57.3, ranging from 0 to more than 240 days. This is considerably longer than reports in previous studies, where the mean time from symptom-onset to presentation ranged from 8.9 to 35 days. For example, Wong et al reported the mean time from first symptoms or signs and presentation to hospital to be 8.9 (SD 15.5) days 57 , Arunga et al reported Median presentation time was 17 days from onset (IQR 8–32) 58. This delay in presentation in our study might be because most of our patients had already received therapy, and only presented when there was no response.

Fate and visual outcome: The final outcome was complete healing in 44 patients (51.8%), improving patients with ongoing follow ups in 28 patients (32.9%), worsening occurred in 8 patients (9.4%), and 5 patients were lost to follow up (5.9%)

Among healed patients, 34 (77.3%) patients healed on medical treatment only, while 10 patients (22.7%) healed with medical and surgical management.

Regarding complications, only 14 cases (16.5%) advanced to complications with central perforation recording the highest incidence in 9 out of the 14 cases (64.3%), 4 patients (28.6%) progressed to corneal thinning and descemetocele, and one patient (7.1%) worsened to corneal melting. A special pattern here was detected, we noticed the same sequence of central corneal perforation occurring in cases of aspergillus spp. keratitis while the infiltration was localizing in the central thinned cornea during the process of healing. This definitely needs further studies on more cases and a plan of prophylaxis could be themed to prevent such a drastic outcome. Also, the low incidence of complications could be attributed to proper diagnosis, the choice of treatment and correlation between lab work and clinical impression.

There are limitations to this study, we had an inconsistent supply of antifungal discs, so sensitivity testing was not done consistently in all cases. Also, since we had no access to non-nutrient agar with E.coli, which is essential for the growth and diagnosis of acanthamoeba, we had to depend solely on clinical clues for presuming a diagnosis of acanthamoeba keratitis. The relatively small study size, and the dominance of resistant corneal ulcers of fungal origin, may have also confounded our results.

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