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# Bacterial and fungal causes of infectious keratitis among patients attending Research Institute of Ophthalmology

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## Abstract

**Background:** Corneal ulcer is a potentially sight threatening ocular condition and the leading cause of monocular blindness in developing countries. Knowing the predisposing factors and etiologic microorganism can help prompt diagnosis and treatment to prevent devastating outcomes

The aim of this study was to detect the prevalence of bacteria and fungi in infectious keratitis. And to detect the antimicrobial susceptibility pattern against these causative bacterial and fungal pathogens using antibacterial and antifungal discs.

**Results:** Out of 50 cases (= 50 eyes), fungal growth was predominant 23/50 representing 46% with *Aspergillus flavus* being the most prevalent 14/23(61%). Bacterial growth was 7/50 (14%), 4/7 was gram-positive cocci (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and 3/7 was *pseudomonas* spp. While twenty out of 50 cases (40%) showed no growth.

**Conclusion:** Ocular trauma was the major cause of infectious keratitis, more in rural population. Fungal growth; mainly *Aspergillus* spp. was the most prevalent pathogen encountered in all cases. Voriconazole proved to be the first choice in the treatment of mould keratitis with 100% susceptibility. While alarmingly, fluconazole should no longer be used for the empirical therapy as it showed resistance to all the fungal isolates.

**Keywords:** Keratitis risk factors, Ocular trauma, Antifungal therapy

## Background

Infectious keratitis is an ocular emergency that requires prompt and specific management to preserve ocular integrity. It is infection of the cornea by infective organisms like bacteria, fungi, viruses, or parasites (Sedhu et al. 2017). It affects both males and females across all age groups worldwide. It presents clinically with pain, photophobia, redness, infiltration, corneal edema, corneal ulceration, and anterior chamber reaction. If left untreated, it can lead to endophthalmitis and even corneal perforation and blindness (Suwal et al. 2016). Keratitis rarely occurs in the normal eye because of the

cornea's natural resistance to infection (Suwal et al. 2016). However, predisposing factors such as trauma, contact lens wear, dry eyes, ocular surface disorders, and immune suppression may alter the defense mechanism of the outer eye and permit bacteria to invade the cornea (Lin et al. 2019).

Knowing the predisposing factors and etiologic microorganism can help control and prevent this problem. Etiologic and epidemiologic pattern of keratitis varies with the patient population, geographic location and climate. Bacteria and fungi are frequently responsible for suppurative keratitis especially in the developing countries (Sedhu et al. 2017).

Microbial keratitis requires prompt diagnosis and treatment to prevent devastating outcomes. This is achieved by routine microbiological examination of

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patients with keratitis in order to analyze and compare the changing trends of the etiology and their susceptibility patterns (Ranjini et al. 2016).

The aim of this study was to detect the predisposing risk factors and the causative agents of infectious keratitis, i.e., bacteria, fungi, and to detect the antimicrobial susceptibility pattern against these causative bacterial and fungal pathogens using antibacterial and antifungal discs, since the bacterial sensitivity to various antimicrobial agents varies from place to place and in the same place from time to time. 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 (Tesfaye et al. 2013).

### Subjects and methods

This study was carried out on fifty patients (= 50 eyes), presented with symptoms of infectious keratitis to the Cornea Outpatient Clinic of Research Institute of Ophthalmology in the period from April 2017 to December 2017. The study was approved by the local ethical committee.

The demographic characteristics (age, sex, residence, occupation) and risk factors of the patients were recorded. After detailed ocular examinations, ophthalmologist collected a corneal sample after taking the patient's consent.

### Inclusion and exclusion criteria

#### *Inclusion criteria*

Cases diagnosed clinically as infectious keratitis before giving antibiotic therapy or 48 h after discontinuing local or systemic antibiotics and local or systemic antifungal. Including cases with mild or moderate or severe keratitis both males and females.

#### *Exclusion criteria*

**Cases with non-infectious keratitis or children.** Corneal specimens were collected from the edges of the ulcer using sterile Kimura spatula under aseptic conditions by an ophthalmologist under the magnification of a slit lamp after instillation of local anesthetic eye drops. The material obtained was directly inoculated onto blood agar, MacConkey's agar, chocolate agar, and Sabouraud's dextrose agar medium (SDA) in multiple C or linear shaped streaks. Sterile cotton swab was used to rub gently the ulcer and then directly cut into a tube of sterile brain heart infusion broth medium (BHI) that was incubated at 37 °C for 24–48 h and then on the second day subculture was done on blood agar, chocolate agar, MacConkey agar, and Sabouraud's dextrose agar. For direct smear examination, other corneal scrapings were

taken and carefully spread on a glass slide for gram stain and KOH+Calcofluor white stain (Robinson et al. 2016).

Blood agar, MacConkey agar, and broth were incubated aerobically at 37 °C for 24–48 h. Chocolate agar plates were placed into a candle jar for fastidious bacterial pathogens, which require CO<sub>2</sub> at 37 °C for 24–48 h. The plates were examined after 24 and 48 h. The growth of bacteria or fungus in culture is considered significant if the growth is confluent (more than 10 colonies) on the site of inoculation on solid media, or the organism was seen in the smears, or if the same organism was grown in more than one medium. SDA plates were incubated at room temperature (25 °C) and observed daily for the first 7 days and on alternate days for next 14 days for observing slow growing fungi.

Bacterial growth was identified by their colony morphology, gram staining and conventional biochemical tests. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method and was interpreted using the Clinical and Laboratory Standard Institute (CLSI) breakpoints, (CLSI, 2017).

The anti-bacterial discs used were erythromycin (10 µg), aminoglycosides as tobramycin (10 µg), amikacin (30 µg), and gentamicin (10 µg), fluoroquinolones as ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), moxifloxacin (30 µg), polymyxin B 300 unit, chloramphenicol (30 µg), teicoplanin (30 µg), trimethoprim sulphamethoxazole (1.25/23.75 µg), and ceftioxin (30 µg).

Fungal growth was grossly identified by its colony morphology, pigment production and microscopically by lacto-phenol cotton blue stain. All filamentous fungal isolates were tested for their antifungal susceptibility by disc diffusion method against voriconazole (1 µg), fluconazole (25 µg), itraconazole (50 µg), ketoconazole (10 µg), and amphotericin B (20 µg) and interpreted according to (Sabatelli et al. 2006; Espinel-Ingroff et al. 2007 and Johnson, 2008).

### Statistical analysis

Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described in the form of mean and standard deviation. Qualitative variables were described as number and percent. Qualitative variables were compared using chi-square ( $\chi^2$ ) test. *P* value < 0.05 is considered.

### Results

Demographic characteristics of the studied population are shown in Table 1.

Ocular trauma was the most common predisposing factor observed in 15/50 of the patients. Ocular trauma with organic objects as rice paddy stalks, dust and grass were reported in 9 patients, while 6 patients received

**Table 1** Demographic characteristics of the studied population

Demographics	Indicator	No. (%)
Age (in years)	< 20	1 (2%)
	21–40	7 (14%)
	41–60	29 (58%)
	> 60	13 (26%)
Sex	Male	27 (54%)
	Female	23 (46%)
Occupation	Farmers	15 (30%)
	Housewives	19 (38%)
	Other jobs as employees and industrial workers (blacksmith, mechanic, and carpenter)	16 (32%)
Residence	Urban	19 (38 %)
	Rural	31 (62 %)

ocular trauma with non-organic objects as metallic foreign body

Other risk factors observed in other patients are shown in Fig. 1.

There was a statistically significant difference between ocular trauma with organic and non-organic objects in relation to sex, occupation and residence while there was no statistical significant difference regarding other predisposing factors as shown in Table 2.

*P* value < 0.05 is considered significant

Figure 2 shows that ocular trauma with organic objects 9/15 (60%), were more in males farmers living in rural areas. While ocular trauma with non-organic objects were more in males working in other jobs as blacksmith, mechanic, and carpenter were living in urban areas.

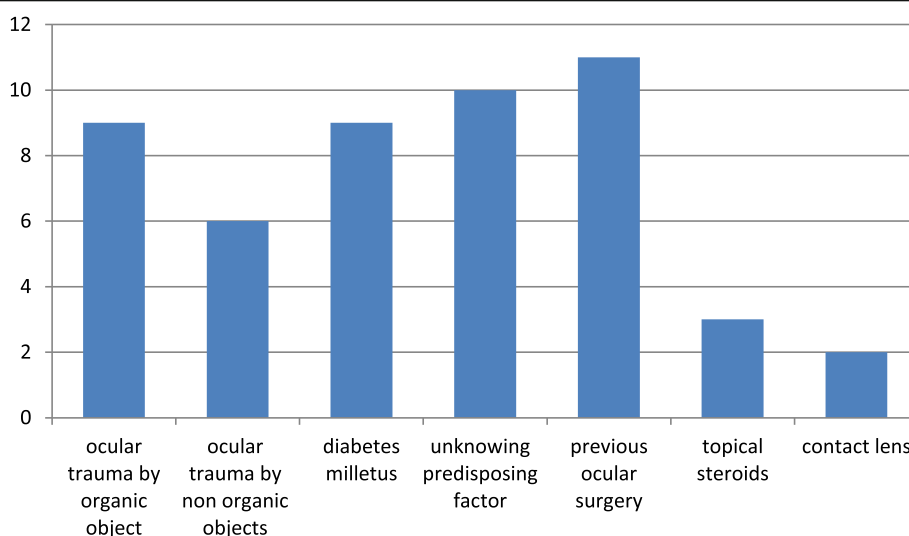
Culture positive cases of infectious keratitis were 30/50 (60%) while the remaining 20 cases (40%) showed no growth.

Fungal growth was the most prevalent pattern of growth among culture positive cases 23/30 (77%) with *Aspergillus* spp. being the most prevalent 21/23(91%) followed by *Fusarium* spp. 2/23 (9%). Bacterial growth among culture positive cases was 7/30 (23%); 3/7 were *S. aureus*, 3/7 was *Pseudomonas* spp. and the remaining isolate was *Streptococcal pneumoniae* as shown in Fig. 3.

The type of isolated pathogens of keratitis in relation to demographic characteristics among the studied populations was as follows:

Regarding sex: there was no difference between males and females regarding bacterial or fungal causes. Regarding residence: patients who lived in rural areas were more exposed to fungal infection than urban ones. Regarding occupation: farmers and housewives were more exposed to fungal infection than other occupations. There was no statistically significant difference between bacterial and fungal infection regarding demographic characteristics as shown in Table 3.

Regarding predisposing factors in relation to causative agent of infectious keratitis, ocular trauma was the most common predisposing factor with the positive culture cases 11/30 (37%) of the patients. Also among patients with positive culture, history of corneal injury with organic object, history of diabetes mellitus, previous ocular surgery and topical steroids represented 8/30 (27%), 7/30 (24%), 7/30 (24%), and 3/30 (10%) of cases, respectively. While in patients with no growth, unknown predisposing factor represented 8/20 (40%) as shown in Table 4.



**Fig. 1** Distribution of predisposing factors for keratitis among the studied population

**Table 2** Distribution of predisposing factors according to occupation, sex, and residence

Predisposing factors	♀	♂	P value	Urban	Rural	P value	Farmer	Other jobs	Housewives	P value
Ocular trauma with non organic objects	0	6	< 0.05	2	4	0.367	1	5	0	< 0.05
Ocular trauma with organic objects	1	8	< 0.05	0	9	< 0.05	8	0	1	< 0.05
Previous ocular surgery	7	4	0.365	6	5	0.762	2	2	7	0.103
Idiopathic	4	6	0.527	7	3	0.157	1	4	5	0.272
Diabetes mellitus	6	3	0.317	2	7	0.114	3	1	5	0.263
Contact lens	2	0	0.157	2	0	0.367	0	2	0	0.135
Topical steroids	3	0	0.083	0	3	0.223	0	2	1	0.367

The antimicrobial susceptibility testing of bacterial isolates by disk diffusion method showed that out of the 7 cases of bacterial keratitis, the antibiotic susceptibility pattern of the 3 *Pseudomonas* isolates revealed that (100%) were susceptible to tobramycin, amikacin and all quinolones (ciprofloxacin, ofloxacin, levofloxacin and gatifloxacin). Whereas two-thirds (66.6%) were susceptible to both gentamicin, and polymyxin B. while the remaining isolate showed intermediate susceptibility to both antibiotics as shown in Fig. 4.

The antibiotic susceptibility pattern of the 3 isolates of *S. aureus* revealed that all isolates (100%) were susceptible to cefoxitin, all quinolones (ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin), chloramphenicol, tobramycin and gentamicin. Teicoplanin and erythromycin 2/3 (75%) showed intermediate resistance. While one-third (33% and 25%) were resistant to trimethoprim sulfamethoxazole (SXT) and erythromycin, as respectively shown in Fig. 5.

The antibiotic susceptibility pattern of the single isolate of *S. pneumoniae* is shown in Fig. 6.

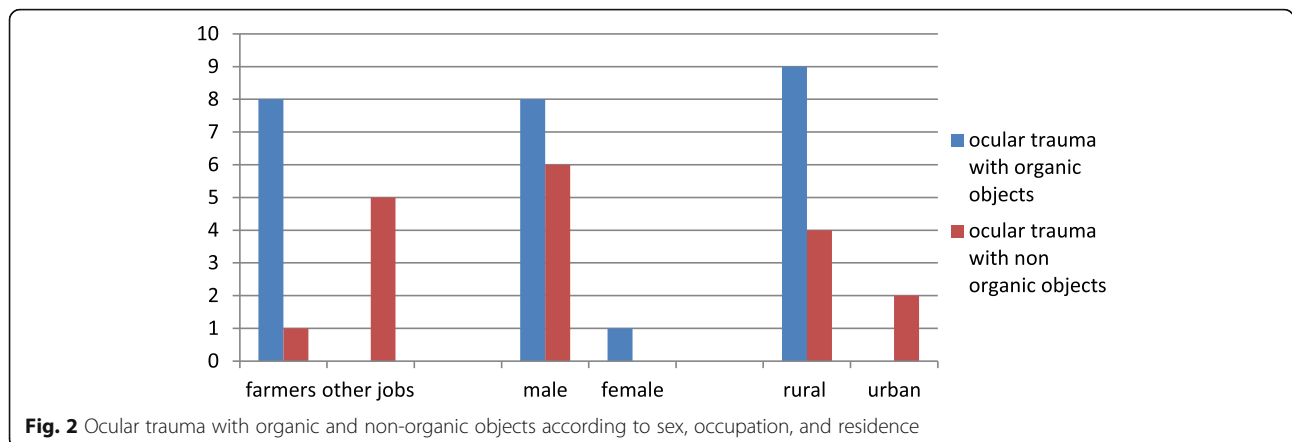
Antifungal susceptibility pattern of the 23 fungal isolates using disk diffusion method revealed that all isolates 100% were susceptible to voriconazole and ketoconazole, followed by itraconazole 74% (17/23). While 100% of isolates were resistant to fluconazole followed by amphotericin 96% (22/23). Susceptibility to

itraconazole showed different results with different fungal species as shown in Fig. 7.

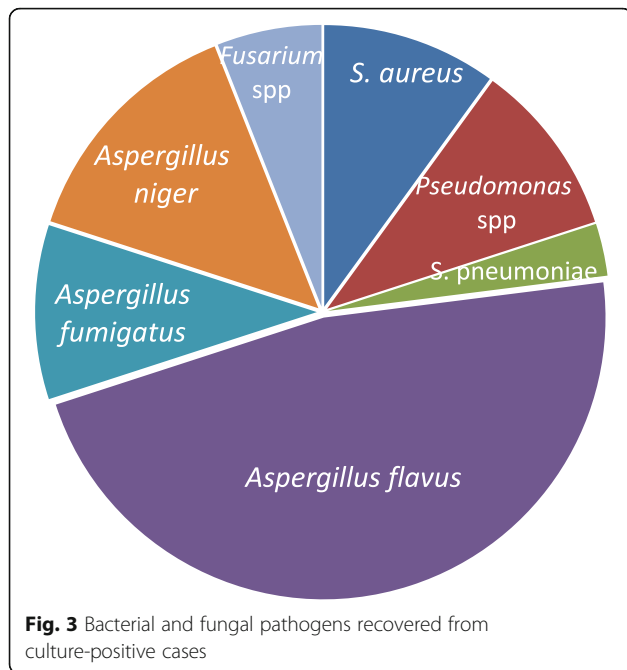
**Discussion**

In this study, the majority of patients 29/50 (58%) were between 4th and 6th decade. The incidence of infectious keratitis is higher in males 27/50 (54%) than in females 23/50 (46%). Thirty-one out of 50 (62%) of cases live in rural areas. Housewives represented 38%, farmers 30%, and other jobs represented 32%. In similar findings, (Ravinder et al. 2016) found that infectious keratitis was more common in males (62%) than females (38%) more commonly observed in rural populations (65%), with higher prevalence in agricultural workers (47.5%) followed by industrial workers (23.75%), housewives (12.5%), and students (8.75%). Sedhu et al. 2017 showed that 70% of patients with infectious keratitis lived in urban areas mainly housewives (21%) followed by farmers (16.9%), laborers (13.6%), and carpenters (12.3%)

In this study, trauma was the most common predisposing factor observed in 15/50 (30%) of infectious keratitis; ocular trauma with organic material represents 9/15 (60%) and is more in male farmers living in rural areas. This is in agreement with (Ravinder et al. 2016) and (Choudhury et al. 2017) where ocular trauma was the most common predisposing factor with 46% and 69%, respectively. Also according to Basak et al. 2005



**Fig. 2** Ocular trauma with organic and non-organic objects according to sex, occupation, and residence



and Senthil Vadivu 2013, corneal injury with vegetable matter was the most common factor causing infectious keratitis with 59.6% and 47.817%, respectively. Ravinder et al. 2016 reported that farmers were more prone for ocular trauma with organic material. El Shabrawy et al. 2013 found history of ocular trauma to be the most important predisposing factor for fungal keratitis (63.6%). The above observations clearly show that in developing countries, agricultural workers are more prone to vegetative matter-induced ocular trauma as a major cause of infectious keratitis in rural populations more than urban populations.

While in developed countries wearing contact lense constitutes a major risk factor for infectious keratitis, according to Keay L et al. 2006, Jeng B. H. et al. 2010, and Mun et al. 2019, in our study, history of wearinf contact lens and using topical corticosteroids represented only 2/50 (4%) and 3/50 (6%), respectively.

In the present study, positive culture samples of infectious keratitis cases are 60% (30/50). Similar to our results, Marasini et al. 2016, found a positive culture rate of 57.8%. However, lower positive culture results were reported by Shoja and Manaviat 2004 and Amescua et al. 2012 with 40% and 38%, respectively. While Stefan and Nenciu 2006 and Al-Shakarchi et al. 2015 demonstrated higher results of positive corneal scraping culture samples with 86.7% and 70%, respectively.

Our study showed that fungal growth is the most prevalent pattern of growth 23/30 (77%), while bacterial growth is 7/30 (23%). This is in accordance with a study by EL Shabrawy et al. (2013) conducted in Egypt. Joshi et al. (2017) and Manikandan et al. (2019) who reported that 55%, 65%, and 98%, respectively, of culture positive cases were identified to be due to fungal causes while the remaining cases were due to bacterial causes. However, a study by Tewari et al. 2012 and Ghosh et al. 2016 reported that out of the positive isolates, 65% and 61% belonged to the bacteria, while 35% and 39% belonged to fungi, respectively.

In this study, *Aspergillus* spp. are being the most prevalent 21/23(91%), followed by *Fusarium* spp. 2/23(8%). This finding is in accordance with Al-Shakarchi 2007 and Tewari et al. 2012 who demonstrated that *Aspergillus* spp. was the most common isolate with 57% and 35% followed by *Fusarium* spp. with 27% and 22% among fungal pathogens, respectively. However, a study conducted in Egypt by EL-Shabrawy et al. 2013 revealed that the most frequent fungal pathogens were *Penicillium* spp. (24.2%) followed by *Aspergillus fumigatus* (21.2%) then *Fusarium* spp. (9%).

The incidence of fungal keratitis is on the rise in the densely populated continents of Asia and Africa (Ravinder et al. 2016). This can be explained by the difference in climatic conditions. A study done in the National Research Centre in Egypt to detect the association between fungal keratitis and the climatic changes concluded that the climatic conditions directly affect the frequency of fungal keratitis and that the incidence of this disease will

**Table 3** Distribution of demographic characteristics according to causative agent of keratitis among the studied populations

Demographic	Indicator	total	Bacterial keratitis N (%)	Fungal keratitis N (%)	No growth N (%)	P value
Sex	Male	27	3(11%)	12(44.5%)	12(44.5%)	0.715
	Female	23	4(17%)	11(48%)	8(35%)	
Residence	Urban	19	3(16%)	5(26%)	11(58%)	0.07
	Rural	31	4(13%)	18(58%)	9(29%)	
Occupation	Farmers	15	1(6%)	10(67%)	4(27%)	0.124
	Workers	16	2(12%)	4(25%)	10(63%)	
	Housewives	19	4(21%)	9(47%)	6(32%)	

P value < 0.05 is considered significant

**Table 4** Distribution of predisposing factors according to causative agents of keratitis

Predisposing factors	Total no.	Bacterial Keratitis	Fungal keratitis	No growth(20)	P value	
Ocular trauma	With non organic objects	6	2	1	3	0.606
	With organic objects	9	0	8	1	< 0.05
Diabetes mellitus	9	0	7	2	< 0.05	
Previous ocular surgery	11	4	3	4	0.913	
Idiopathic	10	0	2	8	< 0.05	
Contact lens	2	0	0	2	0.135	
Topical steroids	3	1	2	0	0.367	

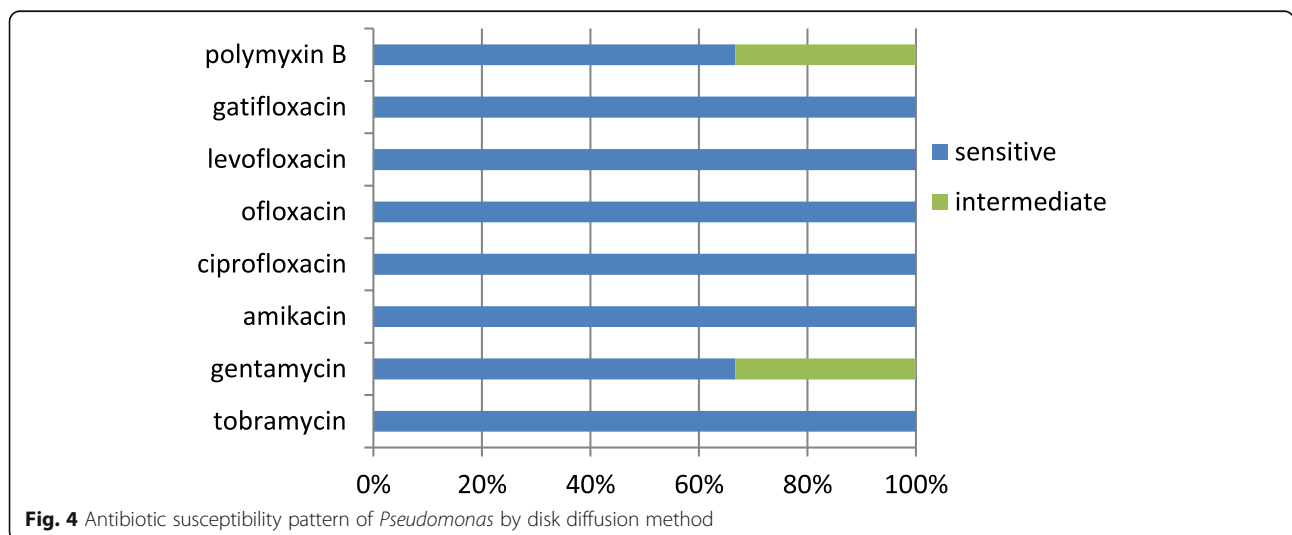
P value < 0.05 is considered significant

continue to rise as long as the global warming is increasing and the greenhouse gases will continue to rise (EL Shabrawy et al. 2013). Species of *Fusarium* and *Aspergillus* are widespread in nature being important pathogens in fungal keratitis (Manikandan et al. 2019).

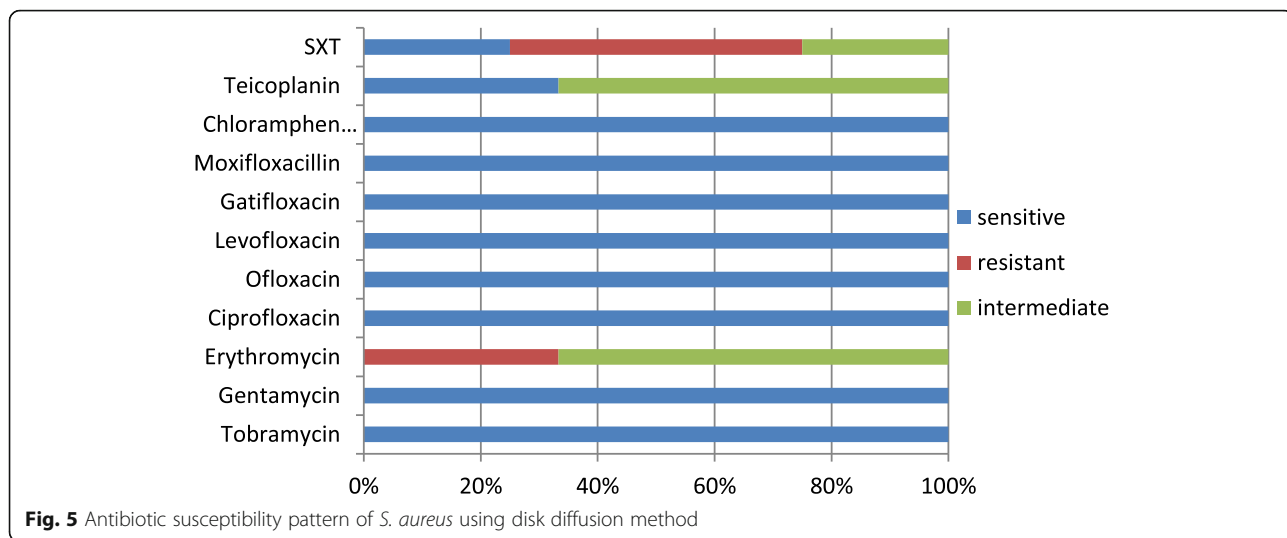
In the present study, the bacterial growth among positive culture cases is 7/30 (23%); 3/7 were *S. aureus*, 3/7 was *Pseudomonas* spp., and the remaining isolate was *S. pneumoniae*. A study conducted by AL-Yousuf, 2009 in Bahrain reported that *Pseudomonas aeruginosa*, *Staphylococcus*, and *Streptococcus* were the most frequent pathogens. This is near to a study by Tewari et al. 2012 and Mun et al. 2019 who reported that the most frequent bacterial isolates were *S. aureus* followed by *Pseudomonas aeruginosa*.

Proper diagnosis and treatment of bacterial keratitis are essential to achieve resolution of infection and minimize damage to the cornea. In our study, the antibiotic susceptibility pattern of the 3 isolates of *S. aureus* revealed (100%) susceptibility to ceftazidime (methicillin sensitive) and all quinolones (ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin). This is in

accordance with Senthil Vadivu 2013 who founded 7/9 (77%) methicillin-sensitive *S. aureus*, with 77% sensitivity to ciprofloxacin and Mun et al. 2019 who reported 11/13(85%) methicillin-sensitive *S. aureus* with higher sensitivity to levofloxacin and moxifloxacin; 95.8% and 93.8%, respectively. Treatment of *P. aeruginosa* eye infections often becomes a challenge due to the ability of this bacterium to be resistant to antibiotics via intrinsic and acquired mechanisms (Subedi et al. 2018). In the present study, the 3 *Pseudomonas* isolates (100%) were susceptible to tobramycin, amikacin, ciprofloxacin, ofloxacin, levofloxacin, and gatifloxacin followed by gentamicin and polymyxin B 2/3(66.6%). This is similar to a study conducted by Marasini et al. 2016 where all *Pseudomonas aeruginosa* isolates were sensitive to gentamicin and ciprofloxacin. Mun et al. 2019 also found that all *Pseudomonas* species isolates were sensitive to amikacin and ciprofloxacin. While Cho and Lee, 2018 reported less than 10% resistance in *P. aeruginosa* to ciprofloxacin and gentamicin. According to SenthilVadivu, 2013, *P. aeruginosa* isolates showed lower sensitivity to ciprofloxacin (66%) and ofloxacin (83%).



**Fig. 4** Antibiotic susceptibility pattern of *Pseudomonas* by disk diffusion method

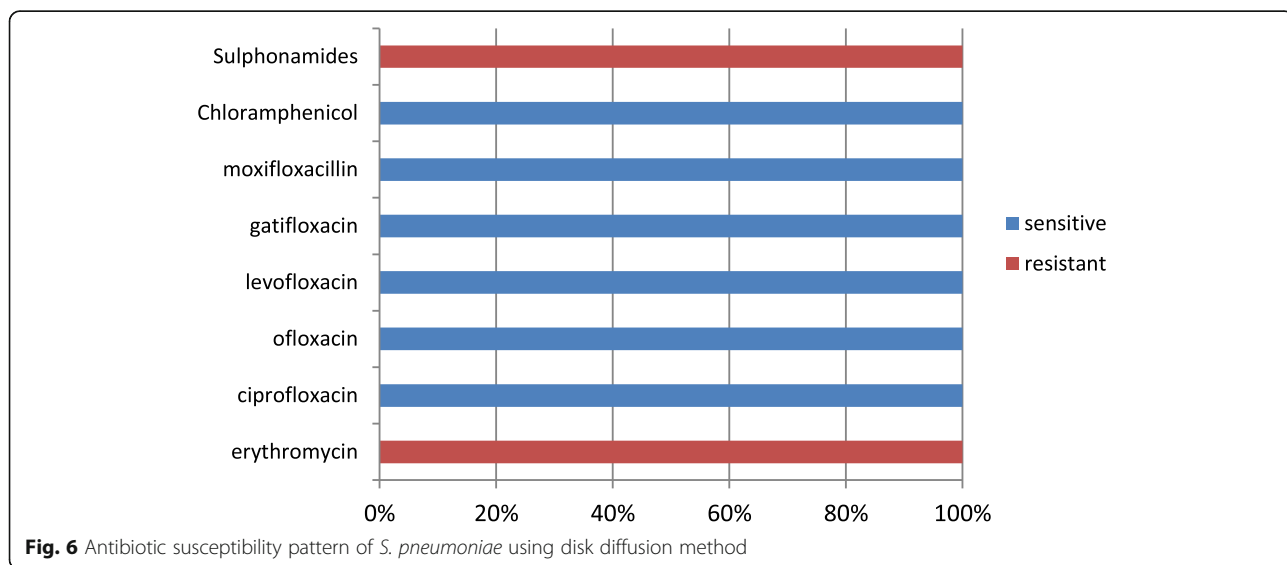


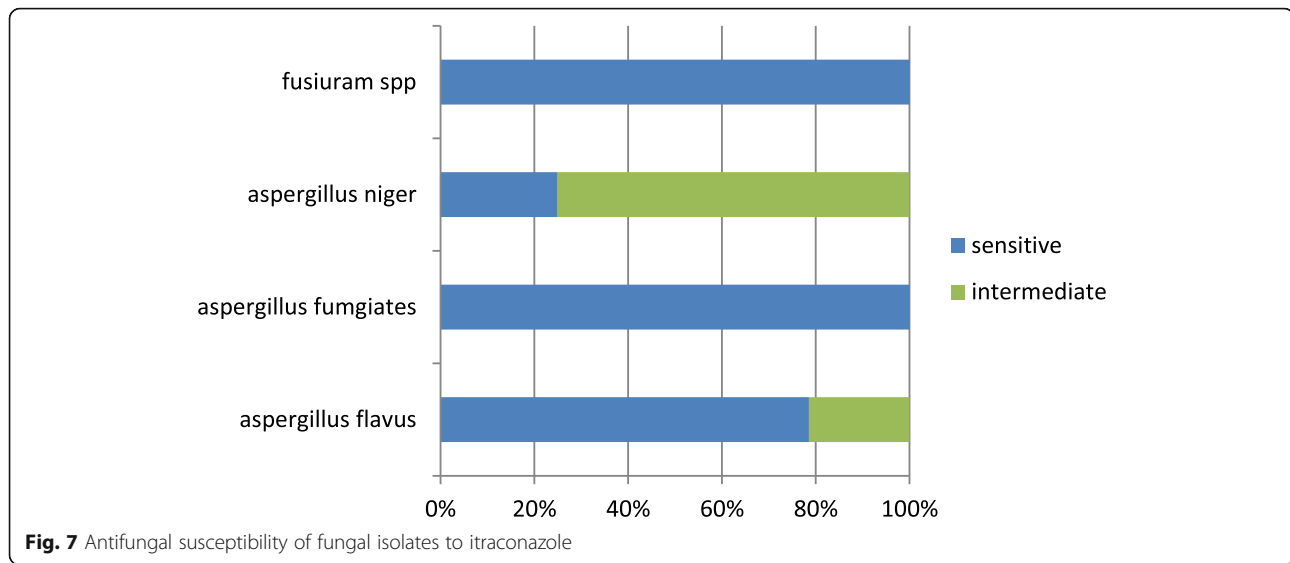
Treatment of fungal keratitis is one of the most difficult problems encountered by ophthalmologists due to poor response to the therapy as well as the limited availability of antifungal agents. Although voriconazole and other triazoles have broad-spectrum activity against causative fungal isolates, clinically no single drug was found to be effective against fungal keratitis (Manikandan et al. 2019). In this study, antifungal susceptibility pattern of the 23 fungal isolates revealed that all isolates are 100% susceptible to ketoconazole and voriconazole. In accordance with our findings, a study conducted in Upper Egypt by Gharamah et al. 2014 showed that ketoconazole at 0.5% and 1% concentrations was effective against all fungal isolates, except for three *Fusarium* species tested. However, Sirisha et al. 2015 reported lower

percentages of sensitivity to ketoconazole for *Aspergillus fumigatus* (85%), *Fusarium* spp. (83%), *Aspergillus flavus* (73%), and *Aspergillus niger* (50%).

A study conducted by (Saha et al. 2014) showed that voriconazole had the lowest minimal inhibitory concentration (MIC) against *Aspergillus* spp. and *Fusarium* spp., followed by amphotericin B, ketoconazole, itraconazole, and that it is still the first choice in the treatment of mould keratitis.

In the present study, susceptibility to itraconazole gave different results. Both *A. fumigatus* and *Fusarium* spp. show 100% susceptibility, while *A. flavus* and *A. niger* showed intermediate susceptibility with 21% and 75%, respectively. This finding is in partial agreement with Senthil vadivu 2013 where *A. fumigatus* and *A. niger*





showed 100% susceptibility while *A. flavus* and *Fusarium* spp. showed 78% and 83%, respectively. Also a study conducted by Sirisha et al. 2015 showed that all 29 fungal isolates (*Fusarium* spp, *A. flavus*, and *A.niger*) were 100% susceptible to itraconazole except for *A. fumigatus* (85%).

In our study, 96% (22/23) are resistant to amphotericin B. However, Senthilvadiu 2013 reported in his study that *A. fumigatus*, *Fusarium* spp, *A. flavus*, and *A. niger* isolates were sensitive to Amphotericin B (70%), (66%), (64%), and (58%), respectively. Also, a study by Gharamah et al. (2014) showed that the (MIC) of amphotericin B was at 0.1% or 0.5% concentrations for most fungal species tested while there was no effect on the 3 *Fusarium* species. Amphotericin B was quite sensitive to genus *Aspergillus* and *Fusarium* but due to poor penetration in cornea and the requiring of high dosage, it was not used in such keratitis (Saha et al. 2014). In the present study, all isolates were resistant to fluconazole. This is in agreement with Senthilvadiu 2013, Sirisha et al. 2015, and Senthilvadiu and Stalin, 2018.

## Conclusion

Ocular trauma was the major cause of infectious keratitis. It was more in the rural population. Fungal growth, mainly *Aspergillus* spp. was the most prevalent pathogen encountered in all cases. The incidence of fungal keratitis is on the rise due to increased global warming. Voriconazole is the first choice in the treatment of mould keratitis with 100% susceptibility. While alarmingly, fluconazole no longer can be used for the empirical therapy as it showed resistance to all fungal isolates

## Recommendation

Further studies are recommended on a wider scale of population to provide more data about epidemiology and causative agents of infectious keratitis in Egypt. The practice of routine microbiological analysis and sensitivity testing for all infectious keratitis is recommended in order to have enough epidemiological information for empirical therapy.

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## Authors' contributions

AI had carried out the performance of lab work, the collection of data of cases, and participated in writing the manuscript. SA had made the approval of work design and research plan, supervising the steps of the work. AM supervised work steps and participated in writing the manuscript. AE participated in writing and approving the article for submission to the journal as a corresponding author. All authors read and approved the final manuscript.

## Funding

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## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Ethics approval and consent to participate

The work is ethically approved by the Scientific Research Committee of the Research Institute of Ophthalmology RIO, Egypt, prior to the beginning of the study. Corneal scrapping samples used in the study were obtained after patients' consent.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.



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