Original Article

Evaluation of antifungal susceptibility and clinical characteristics in fungal keratitis in a tertiary care center in North India

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Purpose: To study the antifungal susceptibility of common corneal pathogenic fungi to antifungal agents in the North Indian population. **Methods:** Prospective study of the antifungal sensitivity testing (natamycin, amphotericin B, voriconazole, itraconazole, fluconazole, posaconazole, caspofungin, micafungin) of fungal isolates from 50 cases of culture positive fungal keratitis by using E test method. Details noted included demographic data, visual acuity, clinical details, grade of keratitis, healing time, and success in medical management. **Results:** Of 50 patients with fungal keratitis (mean age: 40.28 ± 16.77 years), 12 eyes healed within 3 weeks, 14 had a delayed healing response, and 24 had chronic keratitis. Among the 15 cases of Fusarium isolates, 93.3% were sensitive to natamycin, while 40% to amphotericin B; 66.6% to voriconazole, 13.4% to itraconazole and fluconazole each. 80% of Fusarium cases (n = 12) showed susceptibility to posaconazole. Among Aspergillus flavus isolates, 53.4% (n = 8) were sensitive to natamycin, with only 40% (n = 7) showing sensitivity to amphotericin B and good susceptibility to azoles. MIC against susceptible Fusarium spp. for natamycin was 3–16 μg/mL, amphotericin B: 1–8 μg/mL, voriconazole: 0.5–1.5 μg/mL, itraconazole: 0.5–12 μg/mL, posaconazole: 0.094–1.5 μg/mL. MIC against Aspergillus flavus was natamycin: 8–32 μg/mL, amphotericin B: 0.5–16 μg/mL, voriconazole: 0.025–4 μg/mL, itraconazole: 0.125–8 μg/mL, posaconazole: 0.047–0.25 μg/mL; against Aspergillus niger isolates, to natamycin was 6 μg/mL (n=1), amphotericin B 8–12 μg/mL (*n* = 3), voriconazole: 0.125–0.19 μg/mL (*n* = 3), itraconazole: 0.38–0.75 μg/mL, posaconazole: 0.064–0.19 μg/mL and against Aspergillus fumigatus (*n* = 1), was natamycin4 μg/mL, amphotericin B - 8 μg/mL, voriconazole 0.25 μg/mL, itraconazole 1 μg/mL, and posaconazole 0.19 μg/mL. MIC against susceptible Acremonium spp. for natamycin was 1.5–16 μg/mL, amphotericin B: 0.5–8 μg/mL, voriconazole: 0.19–3 μg/mL, itraconazole: 0.125 μg/mL, posaconazole: 0.125–0.5 μg/mL and against susceptible Curvularia was natamycin 0.75–4 μg/mL, amphotericin B 0.5–1 μg/mL, voriconazole 0.125–0.19 μg/mL, itraconazole 0.047–0.094 μg/mL, posaconazole 0.047–0.094 μg/mL. MIC against Mucor spp.+ Rhizopus spp. (*n* = 1) was natamycin: 8 μg/mL, amphotericin B: 0.75 μg/mL, posaconazole: 1.5 μg/mL. MIC against of Alternaria (*n* = 1) was voriconazole: 0.19 μg/mL, posaconazole: 0.094 μg/mL. MIC against Penicillium (n=1) was natamycin: 8 μg/mL, voriconazole: 0.25 μg/mL, itraconazole: 0.5 μg/mL, and Posaconazole: 0.125 μg/mL. **Conclusion:** Our observations highlight the variations in susceptibility to antifungal agents. Posaconazole seems to be effective with low MIC against common corneal pathogenic fungal isolates.

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Fungal keratitis is a serious suppurative and ulcerative corneal infection that can result in reduced vision or blindness. The occurrence of fungal keratitis is higher in warm, humid regions with agricultural and developing economies, where it constitutes more than 50% of corneal infections.[1] Besides a wide geographic variation in the trends of cornea pathogenic fungi, variation also exists between regions of the same country.[2] Keratomycosis due to *Aspergillus spp.* is more common in North India, while mycotic keratitis due to Fusarium species is more prevalent in South India.[3-5] The current trends in topical antifungal therapy revolve around the use of natamycin, voriconazole, and amphotericin B.[6] Recent evidence in the literature points toward changing trends in antifungal susceptibility patterns for common corneal pathogenic fungi.[7,8]

Published literature on the antifungal susceptibility of common corneal pathogenic fungi to the newer antifungal agents in the North Indian population is minimal. Thus, the current study was performed with a primary aim to evaluate the antifungal susceptibility of corneal pathogenic

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fungal isolates to the commonly used topical antifungal agents (polyenes: natamycin and amphotericin B; azoles: voriconazole, fluconazole, itraconazole, and posaconazole; and echinocandins: caspofungin and micafungin) in patients with mycotic keratitis. The correlation of clinical response of antifungal therapy to in-vitro susceptibility testing was also analyzed to observe differences in minimum inhibitory concentration (MIC) levels from that reported in the literature.

Methods

A prospective study of 50 cases of culture-positive fungal keratitis was done after obtaining clearance from the institute's ethics committee. Patients with fungal keratitis, above 12 years of age, seen in our outpatient department with positive culture on corneal scraping, and willing to participate in the study and follow-up were recruited. Informed consent was taken from all study participants, and study procedures were done in accordance with the tenets of the Declaration of Helsinki. After complete ophthalmic examination and documentation of ulcer characteristics, corneal scraping specimens were taken and sent in Sabouraud dextrose agar for microbiological evaluation (fungal culture and antifungal susceptibility testing). Fungal isolates from the fungal keratitis samples were subjected to antifungal susceptibility testing (AFST) by using the E-test method (E-test strips (Himedia Laboratories Pvt Ltd, Mumbai, India)); concentration range of antifungals used were natamycin: 0.016–256 µg/mL, amphotericin B: 0.002–32 µg/mL, voriconazole: 0.002–32 µg/mL, itraconazole: 0.002–32 µg/mL, fluconazole: 0.016–256 µg/mL, Posaconazole: 0.002–32 µg/mL, caspofungin: 0.002–32 µg/mL, and micafungin: 0.002–32 µg/mL. The AFST was done using standard procedure, and results were available 48–72 h after obtaining growth on fungal culture media.

Demographic data, visual acuity, and clinical details of the keratitis (grading, epithelial defect size, size of hypopyon, infiltrate size, endothelial plaque size of keratitis, treatment details, healing time, need for surgical intervention, response to therapeutic penetrating keratoplasty (TPK), and microbiological data (fungi isolated and MIC on AFST)) were recorded at baseline and final follow-up visit after 3 months.

The clinical response of the fungal keratitis was categorized as group A: healed (<3 weeks), group B: delayed healing (3 weeks–3 months), and group C: medical treatment failure (chronic keratitis for >3 months or required therapeutic keratoplasty) and correlated with the antifungal susceptibility. Study parameters were recorded on a predesigned proforma, and statistical analysis was done using statistical software STATA 12.1; $P < 0.05$ were considered statistically significant.

Results

Of the 50 patients with fungal keratitis (mean age: 40.28 ± 16.77 years (range: 14–80 years); males: 31, females: 19), 12 eyes healed within 3 weeks (group A: 41.09 ± 15.19 years (range: 1–65 years)), 14 had a delayed healing response (group B: 31.93 ± 12.47 years (range: 14–67 years)), and 24 had chronic keratitis (group C: 44.75 ± 18.37 years (range: 15–80 years)). Fungi isolated included Fusarium spp.: 15 eyes (30%), *Aspergillus spp*.: 19 eyes (38%) (*Aspergillus flavus*: 15, *Aspergillus niger*: 3, *Aspergillus fumigates*: 1), *Acremonium spp*.: 10 eyes (20%), *Curvularia*: 3 eyes (6%), *Mucor spp*. + *Rhizopus spp*.: 1 eye (2%), *Alternaria*: 1 eye (2%), and *Penicillium*: 1 eye (2%). None of the patients in groups A and B had hypopyon or endothelial plaque. Hypopyon was present in 9 eyes in group C. Corneal ulcers progressed to perforation in 11 eyes (group A: 2 eyes, group B: 1 eye, and group C: 8 eyes) with a mean time of occurrence of perforation from the time of presentation being 28 ± 16.03 (13–61) days. Hypopyon was present in 6/8 eyes that had perforation. The details of the total healing time and healing of epithelial and stromal infiltrate are elaborated in Table 1. In the 24 patients who had chronic keratitis (group C) necessitating TPK (mean time to surgery from the time of presentation: 27.34 ± 15.13 days (range 13–61) days), 17 eyes healed with TPK (59 ± 26.09 (15–91) days), while 7 eyes had reinfection, of which 4 eyes required repeat therapeutic keratoplasty. Graft reinfection was noted at a mean time of 56.72 ± 23.31 days (range: 23–77 days) in the 7 eyes that had reinfection. Fungi isolated on reinfection included *Fusarium spp.* (2), *Aspergillus niger* (1), *Aspergillus flavus* (1), *Curvularia* (1), and *Acremonium spp.* (2). The fungi isolated in accordance with the healing response are detailed in Table 2.

The MIC values of the eight antifungal agents in the 50 cases of fungal keratitis isolates are detailed in Tables 3 and 4 (for susceptible fungal isolates). The analysis of the sensitivity of the fungal keratitis isolates to the antifungal agents is elaborated in Table 5**.** Analysis of sensitivity to the antifungal agents to the healing response and correlation of antifungal susceptibility to healing time is tabulated in Tables 6 and 7, respectively. There was no statistically significant correlation between the MIC of the antifungal agents and healing time of mycotic cases of the three fungi – *Fusarium spp*., *Aspergillus spp*., and *Acremonium spp*., (with >10 isolates) [Table 8]**.**

Discussion

Fungal keratitis is recognized as a significant cause of ocular morbidity and blindness, especially in developing countries.[9] Plant fungal pathogens are an emerging cause of keratitis in humans, especially dematiaceous fungi.[10] Rapid identification of the causative fungal pathogens and initiation of appropriate antifungal agents remain the key factors in the successful management of fungal keratitis. An ideal treatment protocol should include antifungal agents chosen by in-vitro susceptibility of the fungus. Despite the wide reporting of the usefulness of incorporating AFST as a guiding tool in the management protocol of mycotic keratitis, treatment continues to be largely empirical in several cornea practice setups.[11,12] This is because AFST testing is time-consuming, requiring culture growth for the testing, which precludes its adoption into routine cornea practice. The in-vitro E-test has been described as a simple and practical method for assessing AFST in aiding clinical consideration of choosing the optimal antifungal therapy.[13] Natamycin still prevails as the standard of care for filamentous fungal keratitis, with susceptibility studies on natamycin being limited.[2,14-17] Voriconazole has been shown to possess good in-vitro activity against most isolates from fungal ulcers.[18-20] There is mixed evidence regarding activity against *Fusarium spp*.[14,19] Our study analyzed the in-vitro activity of the commonly used topical antifungal agents in North India – natamycin, itraconazole, voriconazole, fluconazole (commercially available antifungals), osaconazole, amphotericin B, and echinocandins (caspofungin and micafungin) (which are

Table 1: Details of healing response

	Mean Healing Time (range) days		
	Epithelial	Stromal	Total Healing time
Group A (*n*=12 eyes)	13.5±2.28 (10-16)	16.09±2.24 (12-18)	17.17±2.37 (13-20)
Group B (*n*=14 eyes)	19.58±1.61 (16-22)	24.65±3.98 (20-34)	26.36±4.15 (22-35)
*P	0.001	0.001	0.001

*Two sample *t*-test

used as reconstituted formulations) against the 50-culture positive filamentous fungi isolates obtained from corneal scraping specimens obtained from patients with fungal keratitis. We found that natamycin is a good choice as a first-line treatment for fungal keratitis caused by *Fusarium spp., Aspergillus fumigatus, Acremonium, Curvularia, Penicillium*, and *Rhizopus-Mucor spp*. Amphotericin B is effective against *Aspergillus fumigatus, Aspergillus niger, Curvularia*, and *Rhizopus-Mucor spp*. Voriconazole is most efficacious against *Aspergillus spp., Alternaria, Penicillium*, and to a lesser extent against *Fusarium spp., Acremonium*, and *Curvularia*. AFST noted posaconazole to be very efficacious against *Fusarium spp., Aspergillus spp., Mucor-Rhizopus, Alternaria*, and *Penicillium* isolates and comparable to voriconazole against *Curvularia* and *Acremonium spp.* We also noted a good susceptibility of all fungal isolates to posaconazole. We noted the variations in susceptibility to current antifungal agents [Table 9] and observed the usefulness of AFST in customizing antifungal treatment strategies and the efficacious role of posaconazole in the management of mycotic keratitis cases in North India.

With the delay in the availability of fungal culture reports, antifungal therapy is commonly initiated with topical natamycin, amphotericin B, and voriconazole for mycotic keratitis in most clinical setups. Thus, it is understandable that the cases with an early healing response were the ones that showed good susceptibility to these three commonly used antifungal agents (group A: susceptibility of 91.7% to natamycin, 50% to amphotericin B, and 58.4% to voriconazole). It was interesting to note that among fungal ulcers that had delayed healing (group B: 71.3% susceptible to natamycin, 50% susceptible to amphotericin B, and 85.8% susceptible to voriconazole), there was lower susceptibility to natamycin but higher susceptibility to voriconazole. This suggests that despite a favorable susceptibility to voriconazole, controlling infection and healing took more time with voriconazole therapy in these cases (moderate to severe grade of infection). Though susceptibility to natamycin seems to favor a rapid healing response, no statistical significance was seen in the correlation of susceptibility with healing response [Table 7], perhaps due to the small sample size.

The two most common filamentous fungi reported to cause mycotic keratitis are *Aspergillus spp. and Fusarium spp. Fusarium species* has been noted to be among the most common causative agents of fungal keratitis in tropical countries such as India.[31] Different genotypes isolated from *Fusarium spp.* keratitis showed varying susceptibility to voriconazole, terbinafine, natamycin, and miconazole.[29] The inhibitory effects of amphotericin B against *Fusarium spp.* have also been reported to vary for different isolates.[40] A recent study from North India

Table 2: Details of fungi isolated in accordance with the healing response

Fungi isolated	Healing response		
	A	B	C
Fusarium	2	5	9
Aspergillus spp			
Aspergillus flavus	3	5	6
Aspergillus niger	0	2	1
Aspergillus fumigatus	1	0	0
Acremonium spp	2	2	6
Curvularia	2	0	1
Mucor spp. + Rhizopus spp.	1	0	0
Alternaria	0	0	1
Penicillium	1	0	0

that isolated *Fusarium spp.* from 33 clinical specimens of varied infections (endophthalmitis, sinusitis, pulmonary involvement, onychomycosis, and keratitis) reported a lower MIC against amphotericin B as compared to other antifungal agents.[36] Varying susceptibility trends have been reported with *Fusarium* strains, with some authors observing multidrug resistance to azoles and caspofungin and better MIC to natamycin and amphotericin B, while others noted high MICs to amphotericin B, natamycin, and echinocandins.[16,37]

In the current study, *Fusarium* isolates were found to be the least susceptible to voriconazole, and *Aspergillus flavus* isolates were the least susceptible to natamycin when compared to other filamentous fungi. AFST for the common corneal pathogenic fungal isolates in 50 cases of mycotic keratitis from our tertiary care center observed a higher susceptibility of *Fusarium spp.* to natamycin (93.3%) as compared to amphotericin B (40%) [Table 4]. *Aspergillus flavus* was highly susceptible to voriconazole (93.4%) as compared to natamycin (53.4%). Prajna *et al.*[41] have also reported lower MIC of voriconazole against *Aspergillus spp.* than natamycin. They noted the absolute MICs for natamycin to be higher for all organisms than the MICs for voriconazole, except for that of *Fusarium spp.*, which had a higher MIC for voriconazole, similar to our observations. The Mycotic Ulcer Treatment Trial I (MUTT I) study also observed *Fusarium spp.* exhibiting the highest MICs to voriconazole and *Aspergillus flavus* isolates exhibiting the highest MICs to natamycin.[42] Our observations also concur with these results. The reported MIC of natamycin against *Fusarium* isolates is similar to our study,[15,16,20,26,43] while a higher MIC range with more resistant isolates shas been noted by Homa *et al.*[44] [Table 10]. Though the reported MIC

Table 3: MIC values obtained on antifungal sensitivity testing in the study participants

Case No	MIC Antifungal Drug (µg/ml)								Rx	Healing	Severity of keratitis	Ulcer size (mm)
	N	A	V	I	F	P	C	M				
Fusarium (*n*=15)												
2	≥256	1	≥32	0.5	2	≥32	1	≥32	N, V	C	Severe	6.4×3.5
5	16	≥32	1.5	≥32	≥256	0.19	≥32	0.002	N, A	C	Moderate	3.2×2
6	16	≥32	3	≥32	≥256	0.02	≥32	≥32	N, A	C	Moderate	4.2×3
8	3	1	0.5	≥32	≥256	1.5	≥32	≥32	N, A	B	Moderate	4.5×3
11	6	1.5	≥32	≥32	≥256	≥32	≥32	≥32	V, I	C	Mild	1.2×1
13	6	8	≥32	≥32	≥256	≥32	≥32	≥32	N, A	A	Mild	1.2×1
26	12	12	1	≥32	≥256	0.75	≥32	≥32	N, A	B	Moderate	3.5×2
28	4	≥32	≥32	≥32	≥256	0.25	≥32	≥32	N, A	A	Moderate	3×2
30	6	6	1.5	≥32	≥256	0.25	≥32	≥32	N, A	B	Mild	1.8×1
32	6	≥32	0.5	≥32	≥256	0.25	≥32	≥32	N, V	B	Mild	1.5×1.2
35	4	≥32	0.75	≥32	≥256	0.125	≥32	≥32	A, I	C	Severe	5.3×3
37	6	≥32	2	≥32	≥256	0.25	≥32	≥32	A, I	C	Severe	5.2×3
47	4	≥32	1	12	16	0.094	≥32	≥32	A	C	Severe	5.6×4.5
48	12	≥32	≥32	≥32	≥256	1.5	≥32	≥32	A, V, I	C	Moderate	4.8×4
49	6	≥32	1.5	≥32	≥256	0.25	≥32	≥32	N, A	B	Moderate	3.5×3
Aspergillus spp. (*n*=19)												
Aspergillus flavus (*n*=15)												
3	≥256	≥32	0.125	0.38	4	0.94	≥32	≥32	A, N	C	Severe	5.2×3.5
4	≥256	≥32	0.38	≥32	≥256	0.19	≥32	≥32	N, V, I	B	Severe	6.2×4
7	≥256	0.5	0.38	0.125	≥256	0.047	≥32	≥32	N, V	B	Moderate	3.5×3
12	16	4	0.19	≥32	≥256	0.125	≥32	≥32	N, I	C	Moderate	4.2×2.5
14	8	1.5	0.38	0.25	≥256	0.125	≥32	≥32	N	C	Mild	1.6×1.5
15	12	6	0.025	0.75	≥256	0.19	≥32	≥32	N, A	B	Moderate	3.2×1.5
16	≥256	6	0.25	0.25	≥256	0.125	≥32	≥32	N	C	Moderate	4×2
17	8	≥32	0.19	0.125	≥256	≥32	≥32	≥32	N, V	A	Moderate	3.5×2
23	16	≥32	0.25	0.38	≥256	0.125	0.125	0.125	N, A	B	Mild	1.8×1
24	16	≥32	0.38	0.75	≥256	0.19	0.125	0.5	N, A	C	Moderate	4.5×3
31	≥256	≥32	0.5	8	≥256	0.25	≥32	≥32	N	C	Severe	5.3×4
36	16	≥32	0.19	0.25	≥256	0.125	≥32	≥32	N, V, I	A	Mild	1.4×0.5
43	32	≥32	0.38	0.5	≥256	0.19	≥32	≥32	V, I	A	Severe	5.1×3
45	≥256	16	4	0.19	75	≥32	≥32	≥32	A, V	B	Moderate	4.5×3
50	≥256	≥32	≥32	2	≥256	0.125	≥32	≥32	N, I	B	Severe	5.1×2
Aspergillus niger (*n*=3)												
18	6	12	0.125	0.75	≥256	0.125	≥32	≥32	N, I	B	Mild	1.8×1
19	≥256	8	0.19	0.75	≥256	0.19	≥32	≥32	N, A	C	Moderate	4.5×3
33	≥256	12	0.125	0.38	≥256	0.064	≥32	≥32	N, V	B	Moderate	3.5×3
Aspergillus fumigatus (*n*=1)												
44	4	8	0.25	1	≥256	0.19	≥32	≥32	N, V, I	A	Moderate	3.5×2
Acremonium spp. (*n*=10)												
1	4	≥32	0.25	≥32	≥256	0.125	≥32	0.002	N, A, I	C	Severe	6.4×3.5
20	8	8	3	≥32	≥256	≥32	≥32	≥32	N	C	Severe	5.5×3.5
22	4	≥32	≥32	≥32	≥256	≥32	≥22	≥32	N, A	C	Moderate	3.5×3
25	8	≥32	0.5	≥32	≥256	0.38	≥32	≥32	N, V, I	A	Mild	1.2×0.8
38	12	≥32	≥32	≥32	≥256	0.5	≥32	≥32	N, V, I	B	Moderate	4×2
39	16	≥32	≥32	≥32	≥256	0.125	≥32	≥32	V, A	C	Severe	5.1×3
40	8	≥32	1.5	≥32	≥256	0.125	≥32	≥32	N, V	B	Severe	5.1×2

Contd...

Table 3: Contd...

Case No	MIC Antifungal Drug (µg/ml)								Rx	Healing	Severity of keratitis	Ulcer size (mm)
	N	A	V	I	F	P	C	M				
41	6	0.5	≥32	≥32	≥256	≥32	≥32	≥32	N, V, I	C	Moderate	4.5×3.5
42	≥256	≥32	≥32	0.125	24	0.125	≥32	≥32	N, V, I	A	Moderate	3.5×2
46	1.5	1	0.19	≥32	≥256	0.19	≥32	≥32	N, I	C	Severe	5.5×4
						Curvularia (*n*=3)						
10	3	1	0.19	0.094	1.5	0.047	≥32	≥32	N, V	A	Severe	5.1×3
29	4	1	0.125	0.047	0.125	0.094	≥32	≥32	N, V, A	A	Mild	1.6×0.5
34	0.75	0.5	≥32	≥32	≥256	≥32	≥32	≥32	N, V, I	B	Severe	5.5×3.5
						Mucor spp+Rhizopus spp. (*n*=1)						
9	8	0.75	≥32	≥32	≥256	1.5	≥32	≥32	N, A	A	Moderate	2.5×1.5
						Alternaria (*n*=1)						
21	≥256	≥32	0.19	≥32	≥256	0.094	0.064	0.125	V, I	C	Severe	5.4×3
						Pencillium (*n*=1)						
27	8	≥32	0.25	0.5	≥256	0.125	≥32	≥32	N, V, I	A	Mild	1.5×1

Anitfungals: A-Amphotericin-B, N-Natamycin, V-Voriconazole, I-itraconazole, F-fluconazole, P-posaconazole, C-caspofungin M-micafungin. Healing: A: group A-Healed; B: group B-Delayed Healing; C: group C-Chronic keratitis group

Table 4: MIC values of antifungal agents for susceptible fungal isolates

FUNGI	MIC-50 of the Antifungal agents (µg/ml)				
	N	A	V	I	P
Fusarium spp. (*n*=15)	3-16 (*n*=14)	1-12 (*n*=6)	0.5-2 (*n*=10)	0.5-12 (*n*=2)	0.094-1.5 (*n*=12)
Aspergillus spp.					
Aspergillus flavus (*n*=15)	8-32 (*n*=8)	0.5-16 (*n*=7)	0.025-4 (*n*=14)	0.125-8 (*n*=13)	0.047-0.25 (*n*=13)
Aspergillus niger (*n*=3)	6 (*n*=1)	8-12 (*n*=3)	0.125-0.19 (*n*=3)	0.38-0.75 (*n*=3)	0.064-0.19 (*n*=3)
Aspergillus fumigates (*n*=1)	4 (*n*=1)	8 (*n*=1)	0.25 (*n*=1)	1 (*n*=1)	0.19 (*n*=1)
Acremonium spp. (*n*=10)	1.5-16 (*n*=9)	0.5-8 (*n*=3)	0.19-3 (*n*=5)	0.125 (*n*=1)	0.125-0.5 (*n*=7)
Curvularia (*n*=3)	0.75-4 (*n*=3)	0.5-1 (*n*=3)	0.125-0.19 (*n*=2)	0.047-0.094 (*n*=2)	0.047-0.094 (*n*=2)
Mucor spp + Rhizopus spp. (*n*=1)	8 (*n*=1)	0.75 (*n*=1)	-	-	1.5 (*n*=1)
Alternaria (*n*=1)	-	-	0.19 (*n*=1)	-	0.094 (*n*=1)
Penicillium (*n*=1)	8 (*n*=1)	-	0.25 (*n*=1)	0.5 (*n*=1)	0.125 (*n*=1)

A-Amphotericin-B, N-Natamycin, V-Voriconazole, I-itraconazole, F-fluconazole, P-posaconazole,

Table 5: Analysis of sensitivity to the antifungal agents on AFST

Fungal isolate	Polyenes		Azoles				Echinocandins	
	N	A	V	P	I	F	C	M
Fusarium spp.	93.3%	40%	66.6%	80%	13.4%	13.4%	6.7%	6.7%
Aspergillus flavus	53.4%	40%	93.4%	86.7%	86.7%	13.4%	13.4%	13.4%
Aspergillus niger	33.4%	100%	100%	100%	100%	0%	0%	0%
Aspergillus fumigatus	100%	100%	100%	100%	100%	0%	0%	0%
Acremonium spp.	90%	30%	50%	70%	10%	10%	0%	10%
Curvularia spp.	100%	100%	66.6%	66.6%	66.6%	66.6%	0%	0%
Mucor&Rhizopus spp.	100%	100%	0%	100%	0%	0%	0%	0%
Alternaria spp.	0%	0%	100%	100%	0%	0%	100%	100%
Penicillium spp.	100%	0%	100%	100%	100%	0%	0%	0%

A-amphotericin-B, N-Natamycin, V-Voriconazole, I-itraconazole, F-fluconazole, P-posaconazole, C-caspofungin, M-micafungin

range of amphotericin B against *Fusarium*[16] concurs with our observation, some studies report much lesser values,[43,45,46] while others have obtained a higher MIC range[15,26,43] [Table 10]. The reported MIC range of voriconazole against *Fusarium spp.*

Table 6: Antifungal susceptibility in accordance with the healing response

Healing response	Antifungal Sensitivity	Antifungal agents (eyes%)							
		N	A	V	P	I	F	C	M
GROUP -A (*n*=12)	Susceptible	91.7%	50%	58.4%	66.7%	58.4%	33.4%	0%	0%
GROUP-B (*n*=14)	Susceptible	71.3%	50%	85.8%	100%	42.9%	0%	7.2%	7.2%
GROUP-C (*n*=24)	Susceptible	70.9%	45.9%	70.9%	70.9%	37.5%	16.7%	12.5%	16.7%

A-amphotericin-B, N-natamycin, V-voriconazole, I-itraconazole, F-fluconazole, P-posaconazole, C-caspofungin, M-micafungin

Table 7: Correlation of antifungal sensitivity to healing response

Antifungal agent	*P
Natamycin	0.36
Amphotericin B	0.94
Voriconazole	0.58
Itraconazole	0.32
Fluconazole	0.19
Posaconazole	0.09
Caspofungin	0.80
Micafungin	0.50

* Fishers exact test

Table 8: Correlation of antifungal susceptibility MIC to healing time

Antifungal agent	Correlation Coefficient (*P**)		
	Aspergillus spp.	*Fusarium spp.*	*Acremonium spp.*
Natamycin	0.28 (0.32)	0.27 (0.34)	−0.38 (0.29)
Amphotericin B	−0.26 (0.36)	0.28 (0.32)	−0.28 (0.44)
Voriconazole	0.22 (0.44)	−0.15 (0.61)	−0.19 (0.61)
Itraconazole	−0.04 (0.89)	0 (1.0)	−0.41 (0.25)
Fluconazole	−0.14 (0.64)	0 (1.0)	0.41 (0.25)
Posaconazole	−0.11 (0.71)	−0.3 (0.29)	−0.41 (0.71)
Caspofungin	−0.12 (0.69)	−0.22 (0.44)	-
Micafungin	−0.1 (0.74)	−0.13 (0.66)	−0.41 (0.25)

*Spearman correlation coefficient

is higher than that observed in our study.[15,16,26,44,45,47,48] MIC for posaconazole reported by Leung *et al*.[45] against *Fusarium spp*. is higher as compared to that noted in our study [Table 10].

MIC values have been noted to differ among the groups of organisms, with higher MIC values being associated with an increased likelihood of perforation.[46] In vitro, voriconazole was found to have a lower MIC against *Aspergillus spp*. than natamycin.[42] Another derivative analysis from the MUTT study also noted that among natamycin-treated organisms, *Aspergillus flavus* had the highest MICs, and among voriconazole-treated organisms, Fusarium species had the highest MICs.[42] This study noted a significant association of a twofold increase in MIC with a larger 3-month infiltrate/scar size along with increased odds of perforation. However, there was no significant association between MIC and 3-month visual acuity. MIC-90 of voriconazole against *Aspergillus spp*. was noted to be twofold higher, and natamycin MIC-90 against *Aspergillus fumigatus* was fourfold higher in comparison to the published literature.[20] Prajna *et al*.[42] in MUTT I study measured the MICs in baseline cultures to determine changing trends in antifungal resistance during the trial. Evaluation of MIC data in 221 fungal isolates noted a significant 2.14-fold increase per year in voriconazole MICs after controlling for the infectious organism; however, this association was not seen with the natamycin MICs of baseline cultures. This prompted them to conclude that susceptibility to voriconazole decreased during the enrolment period of the clinical trial due to the increased resistance of environmental fungi. Our range of MIC levels of natamycin against *Aspergillus flavus* is less in comparison to those reported.[17,50,52] The reported MIC of amphotericin B against *Aspergillus flavus* varies with some reporting lesser MIC levels,[51,53] while our study noted a higher MIC similar to that of Manikandan *et al*.[17] [Table 10]. However, an earlier study, with a larger sample size from our center, noted a wider and higher range of MIC of amphotericin B against *Aspergillus flavus*.[24] The MIC levels of voriconazole against *Aspergillus flavus* observed in our study are comparable to those reported in the literature.[17,51,53] Reported posaconazole MIC levels against *Aspergillus flavus* are similar to the range obtained in our study[51,53] [Table 10].

Our results confirm that the polyene antifungal agent, natamycin, is still a good choice as a first-line antimycotic drug for the management of fungal keratitis caused by *Fusarium spp., Aspergillus fumigatus, Acremonium, Curvularia, Penicillium*, and *Rhizopus-Mucor spp*. Amphotericin B is effective against *Aspergillus fumigatus, Aspergillus niger, Curvularia*, and *Rhizopus-Mucor spp*. Voriconazole is the most efficacious against *Aspergillus spp., Alternaria, Penicillium*, and to a lesser extent against *Fusarium spp., Acremonium*, and *Curvularia*. Posaconazole was noted to be very efficacious against *Fusarium spp., Aspergillus spp., Mucor-Rhizopus, Alternaria*, and *Penicillium* and comparable to voriconazole against *Curvularia* and *Acremonium spp*.

Our study noted a good susceptibility of all fungal isolates to posaconazole suggesting that topical posaconazole therapy may be considered an effective alternative therapy in cases of keratitis secondary to infection with common corneal pathogenic fungi. Echinocandins do not seem to be effective against the common corneal pathogenic fungal organisms other than *Alternaria*. Posaconazole is considered an extended-spectrum antifungal agent due to its unique spectrum of activity against various fungi, including most yeasts, filamentous fungi, and azole-resistant *Candida spp*.[8] and has been reported to be effective against *Fusarium* keratitis as well.[19,54] Although in-vitro studies indicate variable activity against *Fusarium spp*., posaconazole has been observed to show activity against isolates resistant to voriconazole and to have greater clinical efficacy

Table 9: Published literature on antifungal susceptibility studies

Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
Qiu Wy *et al.*, 2005[13]	China	E test	61 fungal keratitis isolates	AMB IZ FZ	Fusarium - 60.6% susceptible to AMB & all resistant to IZ, FZ Aspergillus - all sensitive to IZ, 44.4% - sensitive to AMB, 22.2% sensitive to FZ Other fungi: AMB - 89.5%, IZ- 68.4%, FZ - 52.6%	Fungal keratitis is most frequently caused by Fusarium and Aspergillus. In vivo E test is a simple test to assess antifungal drug susceptibility and aids in choosing an antifungal agent in the treatment of fungal keratitis.
Therese K L *et al.*, 2006[21]	Chennai, South India	Agar dilution	180 ocular fungal isolates (130 filamentous fungi and 50 yeasts)	AMB FZ KZ	Yeast: 100% sensitive to AMB 4% resistant to FZ 10% resistant to KZ Filamentous fungi 4.6% resistant to AMB 37.7% resistant to FZ 7.6% resistant KZ	MIC detection by in-vitro antifungal susceptibility testing by agar dilution method is a reliable, cost-effective, and easy test to determine the minimum inhibitory concentration (MIC) of amphotericin B, fluconazole, and ketoconazole on ocular fungal isolates.
Prajna L *et al.*, 2007[14]	Madurai, South India	Broth macrodilution	90 fungal keratitis isolates	AMB N C IZ VZ PZ	Triazoles & Caspofungin - lowest MICs against Aspergillus species; VZ, AMB & PZ - lowest MICs against Fusarium species none of the Fusarium species were inhibited by IZ/C. AMB had significantly lower MICs as compared to N	No single agent was universally most effective, VZ & other triazoles - broadest spectrum. IZ/C - not effective against Fusarium species Antifungal therapy may be tailored according to individual organisms and case to case basis.
L Xie *et al.*, 2008[22]	Shandong, China	Disk diffusion method	674 fungal keratitis isolates	AMB KZ, MZ IZ FZ F TR	Aspergillus (10.8%) was the most sensitive to natamycin, next to icrotiter, and then to amphotericin B. Relatively, both Fusarium (77.6%) and Aspergillus (10.8%) were insensitive to ketoconazole, miconazole, itraconazole, fluconazole, and fluorocytosine.	Fusarium, which was the most common pathogen, and Aspergillus were sensitive to N, AMB, and TR. Natamycin is the first choice in the treatment of hypomycetic keratitis.
Iqbal NJ *et al.*, 2008[16]	Multiple US states, Singapore and Hong Kong	Broth microdilution and E test	85 Fusarium spp. Isolates from keratitis	8 antifungal Agents	Fusarium solani species complex - higher MICs to the triazole drugs IZ, VZ & PZ as compared to other species complexes	High MICs to amphotericin B, natamycin, and echinocandins were consistently obtained with no discrimination based on species

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Table 9: Contd...

Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
					High MICs to AMB, N & echinocandins were consistently obtained with no discrimination based on species or method	or method. Further work is required to determine any potential correlation between MIC and clinical outcomes in keratitis.
Perdomo *et al*., 2011[23]	United States	RPMI medium icrotiter wells	75 Acremonium species	AMB N IZ PZ VZ FZ AN C MZ F TR	MICs for PZ and VZ were high compared to those found for other hyaline molds such as *Aspergillus fumigatus*, where MICs were 0.006-2.0 µg/mL for PZ and 0.12-4.0 µg/mL for VZ, and associated epidemiological cutoff values (ECVs) were reported as 1.0 µg/mL for VZ and 0.25 µg/mL for PZ.	Acremonium isolates are difficult to identify using morphological or molecular methods. Antifungal susceptibility testing demonstrated high MICs for all agents tested, except for terbinafine.
Nayak N *et al*., 2013[24]	New Delhi, North India	Broth microdilution method	160 Aspergillus keratitis isolates	AMB	Amphotericin B-sensitive 52.5% - low MIC (≤1.56 µg/mL) Amphotericin B-resistant. 47.5% -high MICs (≥3.12 µg/mL)	A. niger and A. flavus isolates had higher MICs compared to A. fumigatus, suggesting a high index of suspicion for amphotericin B resistance.
Oechsler R A *et al*., 2013[2]	Miami, Florida, USA	Broth microdilution methods	58 isolates of Fusarium	VZ	MIC90- Fusarium solani isolates: 16 µg/mL Non-Fusarium solani isolated: 4 µg/mL	F.solani isolates exhibited higher MIC90 with worse prognosis with longer healing time, poorer BCVA and higher surgical intervention need
Gajjar D U *et al*., 2013[25]	Ahmedabad, West India	Broth microdilution method	74 fungal Keratitis isolates	N IZ FZ AMB	Fusarium spp. (26.6%)- FZ >32 µ g/mL, N 4-8 µ g/mL, AMB 0.5-1 µ g/mL, IZ >32 µ g/mL, Aspergillus (21.6%)- Dematiaceous fungi (11.6%)- Curvularia spp. was highly resistant	Curvularia spp. was highly resistant to all antifungal agents. N & AMB - most effective Healing response with better VA was seen in the Dematiaceous group as compared to Fusarium & Aspergillus
Manikandan P *et al*., 2013[17]	Coimbatore, South India	Broth microdilution method	200 Aspergillus isolates	AMB N, CL EZ, FZ KZ, VZ IZ	EZ, CZ, and KZ were effective against A. flavus	Aspergillus keratitis is a significant problem in patients with ocular lesions in South-Indian states, warranting early diagnosis and initiation of specific antifungal therapy to improve the outcome.
Homa M *et al*., 2013[26]	Coimbatore, South India	Broth microdilution method	70 Fusarium isolates	AMB N, TR	TR, N, AMB - most effective drugs,	TR, N, and AMB are the proposed

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Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
				VZ, EZ CL, IZ	followed by VZ, MIC of CZ, EZ, & IZ - high (≥64 µg mL/l)	antifungal agents for the treatment of fusarium keratomycosis. The observed synergistic interactions between N and TR suggest that this combination could be a promising base for effective therapy in the treatment of Fusarium keratitis after in vivo experiments.
Prajna L *et al.*, 2014[1]	South India	Broth microdilution method	221 fungal isolates of MUTT I trial	N VZ	MIC of N ≥ VZ for all organisms except Curvularia species. Fusarium spp. had the highest MICs to VZ Aspergillus flavus had the highest MICs to N VZ MIC90 against Aspergillus species was 2-fold higher & N MIC90 against Aspergillus fumigatus was 4-fold higher than reported	Fusarium isolates were least susceptible to VZ & A. flavus isolates were least susceptible to natamycin
Shobana C S *et al.*, 2015[27]	Coimbatore South India	RPMI 1640 medium	60 filamentous fungi isolate from keratitis	IZ VZ KZ EZ CZ	MICs : KZ: 16 µg/mL-0.03 µg/mL CZ:: 4 µg/mL-0.015 µg/mL VZ : 8 µg/mL-0.015 µg/mL EZ: 8 µg/mL-0.015 µg/mL IZ: 32 µg/mL-0.06 µg/mL CZ followed by VZ, EZ had lower MICs	The present study observed a variation in the overall activity of the azole drugs depending on the type of the fungal species and the drug concentration.
O Spierer *et al.*, 2015[28]	Miami, Florida, USA	Microtitration method	68 Candida keratitis isolates	AMB N VZ FZ	Sensitivity: 100% to AMB, N 85% to VZ 77% to C albicans 93% to C non-albicans	AMB, N - equally effective F- not drug of choice in C. albicans & non-albicans VZ- stronger concentration needed for higher effectiveness
Sun S *et al.*, 2015[29]	Central China	Broth microdilution method	758 Fusarium isolates	VZ KZ, T N, F FZ, AMB EZ, CZ MZ, IZ	Sensitive to N, VZ, AMB>50% of strains were sensitive to EZ, KZ, IZ, T Resistant to FC, FZ, Nys, CZ	Different genotypes of Fusarium from keratitis had different susceptibility to vorionazole, terbinafine, natamycin and miconazole
Wang L *et al.*, 2015[30]	Central China	Disk diffusion method	535 fungal keratitis isolates	AMB FZ N	The size of the inhibition zones of Aspergillus spp., Fusarium spp., and	The predominant organisms were Aspergillus and Fusarium. Aspergillus

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Table 9: Contd...

Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
					other fungal genera differed significantly in response to voriconazole, terbinafine, and natamycin. The inhibition zone associated with natamycin correlated significantly with the clinical outcome of fungal keratitis, but no other such correlations were found for the other drugs tested.	was associated with the worst outcomes. The inhibition zones of fungal isolates in response to natamycin significantly correlated with the treatment outcomes of keratitis. Specifically, the smaller the natamycin inhibition zone, the lower the probability that the fungal keratitis had been eliminated.
Hassan *et al*., 2016[31]	Coimbatore, South India	Broth microdilution method	65 Fusarium keratitis isolates	AMB VZ, CZ EZ, KZ MZ, IZ	Among six isolates of *F. delphinoides*, one had low MIC (0.5 µg mL^{-1}) values for EZ, KZ, IZ, and MZ.	AMB, VZ & CZ - most effective followed by EZ
Sunada *et al*., 2016[32]	Japan	Broth microdilution method	72 isolates from fungal keratitis	M AMB F, FZ IZ, VZ MZ, PM	Yeast-like fungi: susceptibility rates>80% for VZ, PM, FC, MF, AMB, FZ Filamentous fungi: susceptibility rate <50% [except for PMR (90%)] Fusarium spp.: susceptible to AMB and PM, with lower susceptibility rates as compared to other genera	Different fungi have different susceptibility, and the treatment should be based on case to case basis.
Prajna NV *et al*., 2016[33]	South India	Broth microdilution method	221 fungal keratitis isolates (MUTT I trial)	VZ N	2.14-fold increase per year in VZ MICs This association was not found in N MICs	Decrease in susceptibility to VZ Probably due to increased resistance of environmental fungi
Tupaki-Sreepurna A *et al*., 2018[34]	South India	Broth microdilution method	12 Fusarium spp. (FFSC) isolates	AMB VZ PZ N C	Natamycin (GM MIC 0.80 g/mL) and voriconazole (GM MIC 2.67 lg/mL) were found to be the most active agents against all the FFSC isolates, closely followed by amphotericin B (GM MIC 3.18 lg/mL). Posaconazole (GM MIC 11.31 lg/mL) and caspofungin (GM MEC 25.39 lg/mL) MIC/MECs were found to be much higher. Among the FFSC isolates, F. verticillioides presented the most favorable susceptibility	N & VZ - most effective followed by amphotericin B.

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Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
					profile with GM MIC of 0.5 lg/mL for natamycin, 1.41 lg/mL for amphotericin B, and 1.68 lg/mL for voriconazole, respectively. Fusarium sacchari, on the other hand, displayed a more resistant profile with GM MIC of 1.26 lg/mL for natamycin, 6.35 lg/mL for amphotericin B, and 2 lg/mL for voriconazole, respectively.	
Zhang Y *et al.* 2018[35]	Northern China	ROSCO disc diffusion method	5654 fungal keratitis isolates	N TR IZ FZ AMB VZ	Fusarium, Aspergillus & Alternaria sensitive rate to: N - 92.3% T - 78.5% VZ - 41.0% AMB - 40.7%	Natamycin recommended as the preferred drug for empirical therapy
Bansal Y *et al.*, 2019[36]	North India	Broth microdilution method	33 Fusarium isolates from various clinical specimens	AMB VZ IZ FZ C Anidulafungin	The species identified within FSSC, FFSC, and FIESC included *F. keratoplasticum* (*n*=6)/F. *falciforme* (*n*=6)/*F. solani* (*n*=1), *F. proliferatum* (*n*=7)/*F. sacchari* (*n*=5)/*F. anthophilum* (*n*=1), and *F. incarnatum* SC species (*n*=6)/*F. equiseti* SC species (*n*=1), respectively.	Lower MIC against AMB as compared to other antifungal agents
Todokoro D *et al.*, 2019[37]	Japan	Broth microdilution method	25 fungal keratitis isolates (18 Fusarium & 7 others)	MZ C AMB N VZ PZ Micafungin Luliconazole Lanoconazole Efinaconazole Ravuconazole	MIC90 against Fusarium species: LLCZ - 0.06 µg/mL, N 4 µg/mL VZ 8 µg/mL	Luliconazole strongest in-vitro antifungal agent against broad-range filamentous
Ghosh A *et al.*, 2020[10]	Chandigarh, North India	Broth microdilution method	Dermataceous & Hyaline fungi	AMB N IZ VZ Echinocandins	AMB MIC: ≤1 µg/mL (dematiaceous fungi) MIC ≥1 µg/mL (hyaline fungi). N: variable MIC, IZ & VZ effective (except against Fusarium sp).	Identification and antifungal susceptibility testing are important for epidemiology and to optimize therapy and improve the patient outcome.
Hassan AS *et al.*, 2020[38]	Coimbatore, South India	Broth microdilution method	73 Aspergillus keratitis	AMB MZ, KZ	A. flavus isolates were susceptible	Order of azoles efficacious against

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Table 9: Contd...

Study	Place	AFST method	Samples	Agent	Result	Clinical Implications
			strains	FZ, IZ N, IZ VZ, EZ CL	to AMB (98%; MIC: 0.25–4 µg mL^{-1}), ITZ (98%; MIC: 0.25–1 µg mL^{-1}), CLZ (91%; MIC: 0.125–1 µg mL^{-1}), KTZ (89%; MIC: 0.125^{-1} µg mL^{-1}), VCZ (79%; MIC: 0.25–0.5 µg mL^{-1}) and MCZ (93%; MIC: 0.5–1 µg mL^{-1})	Aspergillus sp. - VZ, N, IZ, CZ, EZ, KZ
Ulfik *et al.*, 2020[39]	Poland	Broth microdilution method	47 fungal keratitis isolates	FZ VZ IZ AMB	Sensitivity to AMB 100%, VZ - 82.81% FZ- 56.25%	Voriconazole remains a first-line antifungal drug.

AMB- amphotericin B, N- natamycin, IZ- itraconazole, VZ- voriconazole, FZ- fluconazole, C- caspofungin acetate, PZ- posaconazole, MZ-miconazole, F-5-fluorocytosine, TR-terbinafine, CL-clotrimazole, EZ-econazole, KZ-ketoconazole, M-micafungin, PM-pimaricin, AN- anidulafungin

Table 10: Comparison of antifungal susceptibility with prior studies

Study	Antifungal agent MIC-50 (µg/mL)			
	N	V	A	P
Comparison of MIC values in Fusarium spp. Isolates				
Iqbal *et al.*,[16] 2008 (*n*=92)	4-16	0.5-8	0.5-8	-
Li *et al.*,[43] 2008 (*n*=38)	2-16	-	2-16	-
Prajna L *et al.*,[1] 2012 (*n*=44)	2-16	2-16	-	-
Ozdemir *et al.*,[47] 2012 (*n*=9)	-	1-8	0.5-2	-
Homa *et al.*,[26] 2013 (*n*=60)	2-64	0.13-64	0.13-64	-
S Leung *et al.*,[45] 2015 (*n*=134)	-	1-16	1-16	1-32
Y Bansal *et al.*,[36] 2019 (*n*=7)	-	0.5-8	0.5-2	-
Our study (*n*=15)	3-16	0.5-3	1-8	0.094-1.5
Comparison of MIC values in Aspergillus flavus isolates				
Prajna L *et al.*,[14] 2007 (*n*=32)	8-64	-	-	-
Sevtap Arikan *et al.*,[49] 2008 (*n*=29)	-	0.5-2	2-4	0.03-0.125
Shapiro *et al.*[50] 2010 (*n*=18)	8-64	-	-	-
Nayak *et al.*[24] 2011 (*n*=64)	-	-	0.03-25	-
Gonçalves *et al.*,[51] 2013 (*n*=20)	-	0.25-2	1-4	0.25-1
Manikandan *et al.*,[17] 2013 (*n*=74)	4-128	0.25-1	0.5-16	-
Our study (*n*=15)	8-32	0.025-4	0.5-16	0.047-0.94
Comparison of MIC values in Acremonium isolates				
Perdomo *et al.*,[23] 2011 (*n*=47)	2-8	1-4	2 for all	1-4
Our study (*n*=10)	1.5-16	0.5-8	0.19-3	0.125-0.5

than voriconazole.[49,55] The increased response to posaconazole has been attributed to the higher lipophilicity, which enhances its ability to penetrate ocular tissues easily.[56] Different fungi have different susceptibility, and the treatment should be based on case to case basis.[32] MIC detection by in-vitro antifungal susceptibility testing by agar dilution method is a reliable, cost-effective, and easy test to determine the minimum inhibitory concentration (MIC) of amphotericin B, fluconazole, and ketoconazole on ocular fungal isolates.[21,22,25,27,38] Infections due to Aspergillus spp. are usually associated with the worst outcomes.[30,34] Approximately 6% of the isolates from our fungal keratitis cases in our center are *Candida spp.* However, cases included in the current study were of filamentous fungi and only pathogenic molds. *Candida* has been reported to be effectively treated by amphotericin B and natamycin, whereas fluconazole is not the drug of choice.[28] *Acremonium* isolates are difficult to identify using morphological or molecular methods, and antifungal susceptibility testing demonstrated high MICs for all agents tested, except for terbinafine.[23] Our observations highlight the variations in susceptibility to current antifungal agents and indicate the potential usefulness of AFST in customizing antifungal treatment strategies and the

efficacious role of posaconazole in the management of mycotic keratitis cases in North India which are mostly due to *Aspergillus spp*. AFST may also be useful to determine the development of potential drug resistance in previously treated cases of fungal keratitis.[33] Natamycin still stays the treatment of choice for most fungal keratitis.[35]

Conclusion

Considering the variations in susceptibility to the commonly used antimycotic agents, adoption of AFST may be considered in the workup protocol of severe and refractory keratitis. Posaconazole can be useful in the management of mycotic keratitis cases in North India.

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Conflicts of interest

There are no conflicts of interest.

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