Published in final edited form as:
Am J Ophthalmol. 2014 February ; 157(2): 318–326. doi:10.1016/j.ajo.2013.10.004.

In Vitro Susceptibility of Filamentous Fungal Isolates From a Corneal Ulcer Clinical Trial

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Abstract

Purpose—To describe the minimum inhibitory concentration (MIC) of fungal isolates to natamycin and voriconazole, and to compare these MICs to previous ocular susceptibility studies.

Design—Experimental laboratory study using isolates from a randomized clinical trial.

Methods—The Mycotic Ulcer Treatment Trial I was a randomized, double-masked, multicenter trial comparing topical natamycin and voriconazole for fungal keratitis treatment. Susceptibility testing to natamycin and voriconazole were performed according to Clinical and Laboratory Standards Institute methods. The relationship between organism and MIC was assessed. A literature review was performed to compare results to previous ocular susceptibility studies.

Results—Of the 323 patients enrolled in the trial, MICs were available for 221 (68%). *Fusarium* (*N*=126) and *Aspergillus* species (*N*=52) were the most commonly isolated organisms. MICs to natamycin and voriconazole were significantly different across all genera (*P*<0.001). The MIC median (MIC_{50}) and 90th percentile (MIC_{90}) for natamycin were equal to or higher than voriconazole for all organisms, except *Curvularia* species. Compared to other organisms, *Fusarium* species isolates had the highest MICs to voriconazole and *A. flavus* isolates had the highest MICs to natamycin. Our results were similar to previous reports except the voriconazole MIC_{90} against A*spergillus* species was 2-fold higher and the natamycin MIC_{90} against *A. fumigatus* was 4-fold higher in our study.

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B) Financial Disclosures: Dr. Nisha Acharya served as a consultant to Xoma and Santan.

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Conclusion—In this large susceptibility study, *Fusarium* isolates were least susceptible to voriconazole and *A. flavus* isolates were least susceptible to natamycin when compared to other filamentous fungi. In the future, susceptibility testing may help guide therapy if performed in a timely manner.

INTRODUCTION

Fungal keratitis is a leading cause of visual impairment worldwide. It is endemic in tropical areas, such as South India, where up to half of all infectious keratitis cases are caused by fungus.[1-3] Filamentous fungi, especially *Fusarium* species, are the predominant cause of fungal ulcers in tropical regions and are thought to be particularly virulent.[4,5] Currently, fungal keratitis treatment is largely empirical, with no consensus on the role of susceptibility testing in guiding treatment decisions. Natamycin has long been considered the standard of care for filamentous fungal keratitis and is the only topical ophthalmic antifungal approved by the US Food and Drug Administration. However, newer azoles, including voriconazole, are reported to have good in vitro activity against most isolates from fungal ulcers, though there is mixed evidence regarding activity against *Fusarium* species.[5,6]

Antifungal susceptibility studies frequently use systemic isolates or focus on yeast. There are limited reports on filamentous fungi, likely due to the absence of established minimum inhibitory concentration (MIC) clinical breakpoints, which classify isolates as susceptible, intermediate, or resistant to an antimicrobial agent. Susceptibility studies investigating natamycin are also limited, as natamycin is used primarily for treating fungal keratitis.[6-10] The ocular studies that are present often have small sample sizes[5,11-13] or focus on one particular genus or species.[8-10] Here, we report the in vitro activity of natamycin and voriconazole against filamentous fungal isolates collected as part of a large, randomized comparative trial on fungal keratitis treatment,[14] and investigate the association between organism and MIC. Our relatively large sample size of isolates provides more precision in the estimation of the MIC median (MIC_{50}) and 90th percentile (MIC_{90}) than previously available. For comparison purposes, we also performed a literature review to identify ocular susceptibility studies on filamentous fungi using similar antifungals.

METHODS

The Mycotic Ulcer Treatment Trial I (MUTT I) was a randomized, double-masked trial comparing clinical outcomes of filamentous fungal keratitis in patients receiving 5% topical natamycin (Natacyn, Alcon, Fort Worth, TX) versus 1% topical voriconazole (VFEND IV, Pfizer, New York, NY).[14] Detailed methods for MUTT I have been reported previously.[14] In brief, we enrolled 323 patients with fungal keratitis who had presenting visual acuity of 0.3 logMAR (20/40) to 1.3 logMAR (20/400) at the Aravind Eye Care System (Madurai, Pondicherry, and Coimbatore) in India. The dosing schedules were identical in both treatment arms and consisted of 1 drop to the affected eye every 1 hour while awake for 1 week, then every 2 hours while awake until 3 weeks from enrollment.[14] Continuation of the masked treatment was then at the discretion of the physician. For ethical reasons, physicians were allowed to add or change medications if deemed medically necessary. The MUTT I trial obtained informed consent from all patients, adhered to the Declaration of Helsinki, and received prospective Institutional Review Board (IRB) approval at Aravind, Dartmouth, and the University of California San Francisco (UCSF). MUTT is registered at Clinicaltrials.gov (NCT00996736).

Microbiology

Detailed microbiological methods have been described previously.[6,7] In brief, corneal scrapings were obtained from all patients who were eligible for the trial, and Gram stains

and potassium hydroxide (KOH) wet mounts were performed. Eligible patients were required to have a KOH wet mount positive for fungus and a Gram stain negative for bacteria at enrollment. Fungal cultures were determined to be positive if there was growth on two or more media, or if there was moderate to heavy growth on one medium. Fungal identification was performed using gross and microscopic characteristics, as described previously.[7]

All samples with a positive fungal culture had susceptibility testing to natamycin and voriconazole performed according to standardized methods outlined in the Clinical and Laboratory Standards Institute (CLSI) document M38-A2.[15] Briefly, broth microdilutions were performed for susceptibility testing using Dimethyl sulfoxide (DMSO) as the drug diluent for voriconazole and natamycin.[15] *Aspergillus flavus ATCC20430* was included as a quality control isolate. MIC was defined as the lowest concentration that exhibited a 100% visual reduction in turbidity when compared with the control tube for natamycin at 48 hours, and an 80% reduction in turbidity for voriconazole.[15] Only natamycin and voriconazole were tested since these were the treatments used in the clinical trial.

Statistical Analyses

Differences in clinical characteristics between isolates with MIC values and those without were analyzed using Student's t-test for continuous variables, chi-square test or Fisher's exact test for categorical variables, and log-rank test for time to reepithelialization. Multiple comparisons were corrected for using the Holm's method in R 3.0.0 (R Foundation for Statistical Computing, www.R-project.org, Vienna, Austria). The lowest antibiotic concentration that inhibits bacterial growth is termed the MIC and concentrations that inhibit 50% (MIC_{50}) or 90% (MIC_{90}) The MIC_{50} and MIC_{90} were estimated using the PERCENTILE function in Microsoft Excel (Microsoft Inc, Redmond, Washington) and verified by hand to ensure accuracy. The MIC_{90} was estimated for organisms with at least 9 observations, the smallest number where extrapolation would not be necessary. The 95% confidence intervals (CI) for the MIC_{50} and MIC_{90} were estimated as bootstrap percentile confidence intervals in Mathematica 8 (Wolfram, Champaign, IL) for genus and species with at least 9 observations. Differences in MIC across groups of organisms were analyzed with one-way analysis of variance (ANOVA). For each genus and species, the MIC to natamycin was compared with the MIC to voriconazole using Wilcoxon signed-rank test. All statistical analyses were conducted using Stata 10.0 (StataCorp, College Station, Texas) unless otherwise specified.

Literature Search for Prior Susceptibility Studies

To identify studies reporting MIC data for ocular isolates tested against natamycin and voriconazole, searches were conducted in Web of Science (all dates up to 9/27/13) and PubMed (all dates up to 9/28/13) using the topic search terms (corneal ulcer or keratitis) and (fungal or fungus or fungi) and (susceptibility or susceptibilities). Titles and abstracts were screened to exclude ineligible studies. Eligible studies included those that used ocular fungal isolates and reported MICs, MIC_{50}, MIC_{90} or MIC range for *Aspergillus* genus, *A. flavus*, *A. fumigatus*, *Fusarium* genus or *F. solani* against natamycin, voriconazole, or amphotericin B using CLSI protocols (M38-P, M38-A, or M38-A2). Excluded studies included review articles; studies using only systemic isolates, bacterial or parasitic isolates, or animal samples; non-English language studies; and articles without an accessible web link. In addition, the bibliographies of included studies were searched to identify additional studies.

RESULTS

Of the 323 patients enrolled in the trial, 256 samples (79%) had a positive fungal culture, and 221 (68%) had MIC results available and were included in the analysis. The 35 isolates that were fungal culture positive but missing MIC values had difficult growth during susceptibility testing. Identification of these 35 isolates revealed 13 (37%) unidentified hyaline organisms, 12 (34%) unidentified dematiaceous organisms, 3 (9%) *Curvularia* species, 2 (6%) *Fusarium* species, 2 (6%) *Aspergillus* species, 1 (3%) *Alternaria* species, 1 (3%) *Exserophilum* species, and 1 (3%) *Lasodiplopia* species. Table 1 shows the clinical characteristics of isolates with MIC values compared to those without. No statistically significant differences were seen in the baseline characteristics between the two groups. Among isolates with MIC values present, the most common genus was *Fusarium* (*N*=126, 57%) followed by *Aspergillus* (*N*=52, 24%).

The MIC_{50} and MIC_{90} for natamycin were equal to or higher than those for voriconazole for all organisms except *Curvularia* species, which had a higher MIC_{90} for voriconazole (Table 2). For *Aspergillus* isolates, the MICs for natamycin were significantly higher than those for voriconazole ($P<0.001$). In particular, *A. flavus* isolates had the highest MICs to natamycin relative to other organisms (Figure, top panel). *Fusarium* isolates had the highest MICs to voriconazole compared to other organisms (Figure, bottom panel).

Based upon our search criteria, 21 studies were found to have explored in vitro antifungal susceptibility patterns to natamycin, voriconazole, or amphotericin B using ocular *Fusarium* or *Aspergillus* isolates (Table 3).[6-13,16-28] These studies used different methods to report MIC (MIC_{50}, MIC_{90}, and range) and demonstrated variable MICs. The natamycin and voriconazole MIC_{50} and MIC_{90} found in our study for *Fusarium* isolates were within the range of published values for *F. solani* and for all *Fusarium* species. Among *Aspergillus* species, our natamycin MIC values for *A. flavus* were consistent with previous study results, but the MIC_{90} for *A. fumigatus* was 4-fold higher than previous reports. Our voriconazole MIC_{90} against A*spergillus* species exceeded values reported in the ophthalmic literature by 2-fold.

DISCUSSION

In this study, we investigated the in vitro activity of natamycin and voriconazole against 221 patient isolates from a fungal keratitis clinical trial and compared our findings to prior ocular susceptibility studies. Overall, organisms in our study had lower MICs to voriconazole than natamycin, though MICs were significantly different across all genera. Relative to other organisms, *A. flavus* isolates appeared least susceptible (highest MICs) to natamycin, while *Fusarium* isolates were least susceptible to voriconazole. The current literature has few studies on susceptibility testing of filamentous fungi against natamycin and voriconazole. The relatively large sample size of this study allows precision in the estimation of the MIC_{50} and MIC_{90} for *Fusarium* and *Aspergillus* species in particular, crucial for determining if antifungal susceptibility testing can actually optimize treatment decisions.

We found that *Fusarium* isolates had the same MIC_{50} and MIC_{90} for natamycin and voriconazole, though the estimated 95% CI for the MIC_{90} was higher for voriconazole than natamycin, respectively (8 to 16 vs 4 to 8, Table 2). Relative to other genera, *Fusarium* isolates had lower MICs to natamycin and higher MICs to voriconazole. These observations suggest that *Fusarium* isolates may have increased susceptibility to natamycin and decreased susceptibility to voriconazole, compared with other filamentous fungi. Two previous studies found similar results for *Fusarium* cases.[6,7] These results may be clinically relevant since *Fusarium* ulcers have been shown to have poor clinical outcome with voriconazole

compared to natamycin.[14,29] In MUTT I, natamycin-treated cases performed significantly better than voriconazole-treated cases, largely due to improvement in *Fusarium* ulcers.[14] Subgroup analysis of *Fusarium* cases showed 4-line improvement in 3-month visual acuity with natamycin treatment compared to voriconazole treatment.[14]

Reports suggest *F. solani* isolates are more resistant to antifungals and have worse outcomes than other *Fusarium* species isolates.[8,9,18,30] In the Mycotic Ulcer Therapeutic Exploratory Trial (*N*=120) performed in preparation for MUTT, *Fusarium* speciation was performed using DNA sequencing (Lalitha P, written communication, 9/2013).[31] The vast majority of *Fusarium* isolates were *F. solani* (*N*=39/43, 91%), followed by *Giberrela fujikuroi* (*N*=3/43, 7%) and *F. dimerum* (*N*=1/43, 2%) (Lalitha P, written communication, 9/2013). The MIC_{50} to voriconazole for *F. solani* was 3 μg/mL compared to 1 μg/mL for *G. fujikuroi* and *F. dimerum* (Lalitha P, written communication, 9/2013).[8] The MIC_{50} to natamcyin was 2 μg/mL for *F. solani*, 3 μg/mL for *G. fujikuroi*, and 1 μg/mL for *F. dimerum* (Lalitha P, written communication, 9/2013). Since the majority of ulcers were due to *F. solani*, it was difficult to make substantial comparisons among *Fusarium* species regarding susceptibility. While reports have shown that *F. solani* isolates have higher levels of resistance to voriconazole than *F.* non-*solani* isolates (Table 3),[8, 9, 18] our exploratory trial results found minimal difference between the strains. Since MUTT participants came from the same population as the exploratory trial, we expected the breakdown of *Fusarium* species to be similar with *F. solani* in the majority. As such, we did not speciate *Fusarium* isolates in MUTT.

For *A. flavus* and *A. fumigatus*, natamycin MICs were significantly higher than voriconazole MICs. Interestingly, *A. flavus* had the highest MIC to natamycin compared to other genera and species, including *A. fumigatus.* Four studies have reported similar findings regarding *A. flavus*.[7,10,12,32] From our results, it is likely that *A. flavus* isolates have decreased susceptibility to natamycin. It is not known if this finding of decreased susceptibility is inherent to the species or an emerging resistance. To the best of our knowledge, no report of *A. flavus* resistance to natamycin has been described to date. In MUTT I, *Aspergillus* cases had better clinical outcomes with voriconazole treatment than natamycin treatment, though this was not significant (Sun CQ, written communication, 4/2013). Other studies have also shown that voriconazole treatment is efficacious against *Aspergillus* ulcers, while natamycin treatment has poor efficacy.[12,33, 34] Prior in vitro susceptibility studies have demonstrated that *A. flavus* and *A. fumigatus* have MICs that are highest for natamycin, followed by amphotericin B, and then voriconazole, confirming that voriconazole may be most effective against *Aspergillus* species (Table 3).[6,7,10-12,18,19,21,27,28]

The absence of established MIC clinical breakpoints for antifungals against all filamentous fungi makes the interpretation of MIC data challenging.[35] Breakpoints are the MIC values used to classify isolates as susceptible, intermediate, or resistant to antimicrobials. The CLSI has not established breakpoints for filamentous fungi, but the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has for voriconazole against *A. fumigatus*.[36,37] In the absence of breakpoints, systemic epidemiological cut-off values (ECVs) have been proposed for voriconazole and *Aspergillus* species using CLSI methodology.[38-40] The upper limit of the MIC distribution for the wild-type population (strains that exhibit no acquired resistance to the drug in question) is defined as the ECV.[41] The ECV should encompass at least 95% of isolates in the wild-type distribution.[41] Non-wild-type isolates have MICs greater than the ECV and may have acquired resistance mechanisms.[41] Using the proposed CLSI ECV for voriconazole against *A. flavus* (1μg/ml) and *A. fumigatus* (1μg/ml),[38,39] we found that the percentage of non-wild-type isolates in our study were 12.5% (*N*=4/32) and 10% (*N*=1/10), respectively, more than had been noted in previous systemic reports (Figure 1B).[38,41,42] Since the proposed ECVs were determined

and tested using systemic isolates, it is not known if they are different for ocular isolates. Further studies using ocular isolates are necessary to confirm these findings.

Conclusions about treatments for fungal keratitis using in vitro susceptibility results have certain limitations. As discussed, standardized systemic or topical breakpoints using CLSI methodology are not yet available for antifungals against filamentous fungi. This limitation makes it challenging to correlate studies with clinical outcome, as can be done in bacterial infections.[43] In addition, while organisms overall had lower MICs to voriconazole than natamycin, the concentration of natamycin used clinically (5%) is typically 5-fold higher than that of voriconazole (1%). More information about the actual therapeutic corneal concentration and bioavailability of the antifungals would aid in the interpretation of MIC results.

This is a large filamentous fungal susceptibility study using ocular isolates from keratitis cases in South India. We describe the in vitro activity of natamycin and voriconazole against filamentous fungal isolates and compare our findings to prior ocular susceptibility studies. Overall, organisms had lower MICs to voriconazole than natamycin, though MICs were significantly different across genera. Specifically, *Fusarium* isolates were less susceptible to voriconazole, relative to other organisms. *A. flavus* isolates appeared to have lower susceptibility to natamycin compared to other organisms. In vitro susceptibility testing may help guide treatment decisions when performed in a timely manner. The establishment of MIC clinical breakpoints for filamentous fungi will likely help facilitate this practice.

Acknowledgments

A) Funding/Support: This work was supported by the following grants: National Eye Institute U10EY018573 (TML) and K23 EY017897 (NRA); That Man May See (NRA, SDM, TML); the Harper/Inglis Trust (TML); The South Asia Research Foundation (TML); Research to Prevent Blindness (NRA, TML); and the UCSF Academic Senate Committee on Research (Sun). Natamycin and voriconazole were donated by Alcon and Pfizer, respectively. The sponsors did not have any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript.

D) Other Acknowledgements: none

Appendix: Mycotic Ulcer Treatment Trial Group

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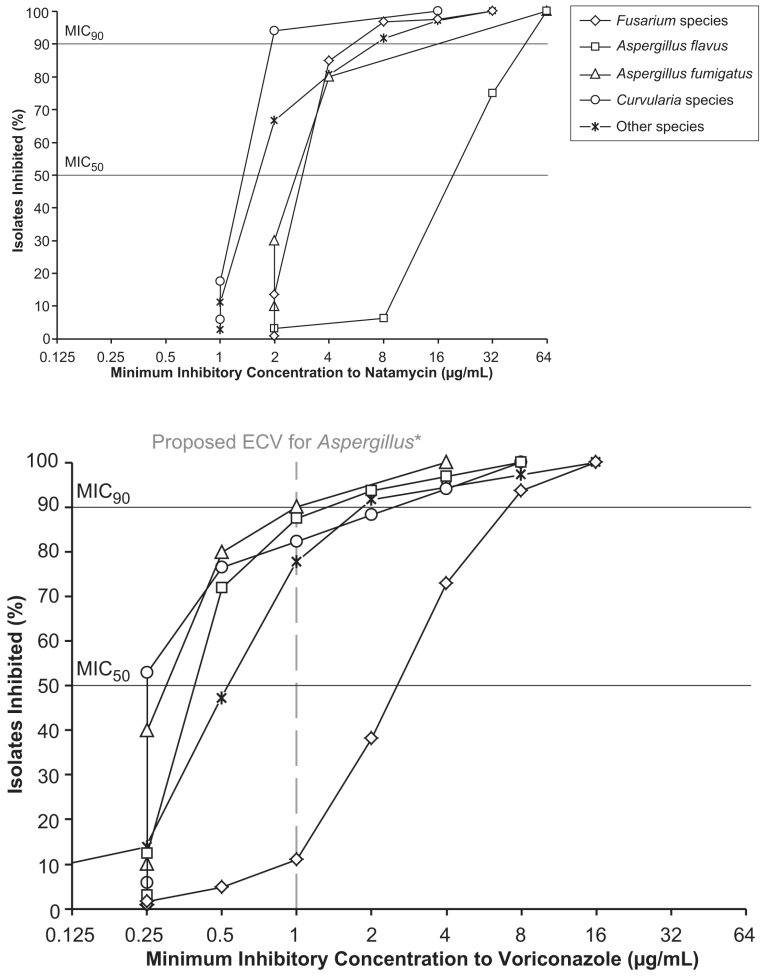

FIGURE. Percentage of Fungal Isolates Inhibited at Different Drug Concentrations in a Fungal Keratitis Clinical Trial

Percentage of fungal isolates inhibited at increasing concentrations of natamycin (Top) or voriconazole (Bottom). Organisms include *Fusarium* species (*N*=126), *Aspergillus flavus* (*N*=32), *Aspergillus fumigatus* (*N*=10), *Curvularia* species (*N*=17), and all other fungal species (*N*=36). Horizontal black lines represent the threshold for the minimum inhibitory concentration median (MIC_{50}) and 90th percentile (MIC_{90}). *Vertical grey dashed line (Bottom) represents the proposed epidemiological cut-off value (ECV) of 1μg/mL using Clinical and Laboratory Standards Institute methodology for *A. flavus* and *A. fumigatus* against voriconazole.[38, 39] The ECV distinguishes wild-type strains (exhibit no acquired resistance to the drug in question) from non-wild-type strains.[41] Non-wild-type strains have MICs greater than the ECV and may have acquired resistance mechanisms.[41]

Table 1

Clinical Characteristics of Fungal Keratitis Isolates With and Without Minimum Inhibitory Concentration Values

Characteristics	Isolates with MIC values (N=221)	Isolates without MIC values (N=102)	P-value[a]
Treatment arm, *N* (%)			0.50
Natamycin	108 (49)	54 (53)	
Voriconazole	113 (51)	48 (47)	
At Presentation:			
Age (years), median (IQR)	46 (38-55)	48 (40-60)	0.21
Gender, *N* (%)			0.06
Male	133 (60)	50 (49)	
Female	88 (40)	52 (51)	
Duration of symptoms (days), median (IQR)	5 (3-9)	7 (3-10)	0.29
Trauma, *N* (%)	111 (50)	50 (50)	0.87
Ocular surface disease, *N* (%)	3 (1)	0 (0)	0.55
Systemic inflammatory disease, *N* (%)	14 (6)	8 (8)	0.64
Fungal culture positive, *N* (%)	221 (100)	35 (34)	ND
Visual acuity (logMAR), mean (SD)	0.71 (0.39)	0.66 (0.38)	0.29
Infiltrate/Scar size (mm), mean (SD)	3.35 (1.15)	3.26 (1.26)	0.56
Clinical Outcomes:			
3-month visual acuity (logMAR), mean (SD)	0.53 (0.64)	0.37 (0.52)	0.03
3-month scar size (mm), mean (SD)	3.71 (1.66)	3.33 (1.80)	0.08
Corneal perforations, *N* (%)	41 (19)	11 (11)	0.08
Epithelial defect healed, *N* (%)[b]	136 (62)	71 (70)	0.16
Time to reepithelialization (days), median (IQR)[b]	15.5 (7-21)	9.5 (2.5-21)	0.15

Abbreviations: *N*, number; ND, not determined; MIC, minimum inhibitory concentration; SD, standard deviation; IQR, interquartile range; logMAR, logarithm of the minimum angle of resolution.

[a] Used Holm method to correct for multiple comparisons. The adjusted significance level threshold started at $P \leq 0.004$.

[b] Right censored at 21 days

Table 2

Natamycin and Voriconazole Minimum Inhibitory Concentration Values From a Fungal Keratitis Clinical Trial

Organism	*N*	*Natamycin (μg/mL)*			*Voriconazole (μg/mL)*		
		MIC_{50} (95% CI)[b]	**MIC_{90}[a] (95% CI)[b]**	**MIC Range**	**MIC_{50} (95% CI)[b]**	**MIC_{90}[a] (95% CI)[b]**	**MIC Range**
Identified hyaline	178	4	32	1 to 64	2	8	0.25 to 16
Fusarium species	126	4 (4 to 4)	8 (4 to 8)	2 to 32	4 (4 to 4)	8 (8 to 16)	0.25 to 16
Aspergillus species	52	32 (16 to 32)	64 (32 to 64)	1 to 64	0.5 (0.5 to 0.5)	2 (1-4)	0.25 to 8
A. flavus	32	32 (32 to 32)	64 (32 to 64)	2 to 64	0.5 (0.5 to 0.5)	2 (1-4)	0.25 to 8
A. fumigatus	10	4 (2 to 4)	64 (4 to 64)	2 to 64	0.5 (0.25 to 0.5)	1.3 (0.5 to 4)	0.25 to 4
A. niger	2	5	ND	2 to 8	3	ND	2 to 4
A. terreus	3	16	ND	8 to 16	0.5	ND	0.5 to 0.5
Other	5	4	ND	1 to 32	0.5	ND	0.5 to 2
Unidentified hyaline	4	4	ND	2 to 8	0.75	ND	0.03 to 8
Identified dematiaceous	34	2	2	1 to 16	0.5	2	0.25 to 8
Curvularia species	17	2 (2 to 2)	2 (2 to 16)	1 to 16	0.25 (0.25 to 0.5)	4 (0.5 to 8)	0.25 to 8
Exserohilum species	8	2	ND	1 to 2	1	ND	0.5 to 2
Alternaria species	2	2	ND	2 to 2	1	ND	1 to 1
Bipolaris species	4	2	ND	2 to 2	0.25	ND	0.25 to 1
Lasiodiplodia species	3	2	ND	2 to 2	0.5	ND	0.5 to 2
Unidentified dematiaceous	3	4	ND	2 to 4	1	ND	0.25 to 2
Other species	2	2	ND	2 to 2	8.25	ND	0.5 to 16
Total	**221**	**4**	**32**	**1 to 64**	**2**	**8**	**0.03 to 16**
P-Value			<0.001[c]			<0.001[c]	

Abbreviations: *N*, number; ND, not determined; MIC, minimum inhibitory concentration; CI, confidence interval; MIC_{50}, minimum inhibitory concentration median; MIC_{90}, minimum inhibitory concentration 90th percentile.

[a] MIC_{90} estimated for genus or species with at least 9 isolates, the smallest number where extrapolation would not be necessary.

[b] Estimation of 95% CI only performed for genus or species with at least 9 isolates

[c] Analysis of variance test (ANOVA) comparing MIC values among genera

Table 3

Prior Ocular Susceptibility Studies With Minimum Inhibitory Concentrations to Natamycin, Voriconazole or Amphotericin B against *Fusarium* and *Aspergillus* species

Study[a]	Species (Number of Isolates)	Natamycin (μg/mL)			Voriconazole (μg/mL)			Amphotericin B (μg/mL)		
		MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range
Debourgogne et al., 2012[22]	*F. solani* (48)	–	–	–	–	8	1 to 16	–	2	0.13 to 2
Edelstein et al., 2012[23]	*F. solani* (1)	–	–	16	–	–	8	–	–	4
Giaconi et al., 2006[16]	*Fusarium* spp. (1)	–	–	–	–	–	8	–	–	–
Homa et al., 2013[17]	*Fusarium* spp. (60)	8[c]	>64[c]	2 to >64	64[c]	>64[c]	0.13 to >64	16[c]	>64[c]	0.13 to >64
	F. solani (43)	8[c]	>64[c]	2 to >64	64[c]	>64[c]	0.13 to >64	8[c]	64[c]	0.13 to >64
	F. non-*solani* (17)	8[c]	>64[c]	2 to >64	64[c]	>64[c]	16 to >64	16[c]	>64[c]	4 to >64
Iqbal et al., 2008[9]	*Fusarium* spp. (85)	–	–	4 to 16	–	–	0.5 to >8	–	–	0.5 to >8
	F. solani (57)	8	–	4 to 16	>8	–	8 to >8	2	–	0.5 to >8
	F. non-*solani* (35)	–	–	4 to 8	–	–	0.5 to >8	–	–	1 to 4
Kondori et al., 2011[18]	*Fusarium* spp. (5)	–	–	–	–	–	0.25 to >8	–	–	–
	F. solani (1)	–	–	–	–	–	−>8	–	–	–
	F. non-*solani* (4)	–	–	–	–	–	0.25 to 1	–	–	–
Lalitha et al., 2007[6]	*Fusarium* spp. (38)	8	16	–	2	4	–	4	4	–
Lalitha et al., 2008[19]	*Fusarium* spp. (41)	4	4	2 to 8	–	–	–	–	–	–
Lalitha et al., 2012[7]	*Fusarium* spp. (44)	4	8	2 to 16	4	16	2 to 16	–	–	–
Li et al., 2008[20]	*Fusarium* spp. (38)	4	16	2 to 16	–	–	–	4	4	2 to 16
Oechsler et al., 2013[8]	*F. solani* (NAT 44, VRC 15, AMB 43)	4.8	4.8	2.4 to 9.6	16	16	4 to ≥16	2	2	1 to ≥16
	F. non-*solani* (NAT 14, VRC 12, AMB 14)	2.4	4.8	2.4 to 4.8	4	4	2 to ≥16	2	2	1 to 2

Study[a]	Species (Number of Isolates)	Natamycin (μg/mL)			Voriconazole (μg/mL)			Amphotericin B (μg/mL)		
		MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range
Ozdemir et al., 2012[13]	*Fusarium* spp. (9)	–	–	–	4[c]	8[c]	1 to 8	1[c]	2[c]	0.5 to 2
	F. solani (2)	–	–	–	4[c]	–	4 to 8	1[c]	–	1 to 2
	F. non-*solani* (7)	–	–	–	4[c]	–	1 to 4	0.5[c]	–	0.5 to 2
Pradhan et al., 2011[12], *d*	*Fusarium* spp. (15)	–	–	4 to 8	–	–	–	–	–	–
Shapiro et al., 2010[11]	*Fusarium* spp. (23)	8	16	4 to 16	–	–	–	–	–	–
Sponsel et al., 2002[24], *b*	*F. solani* (1)	–	–	32	–	–	–	–	–	2
Taylan et al., 2012[25]	*F. solani* (1)	–	–	–	–	–	8	–	–	0.5
Tu et al., 2007[26]	*F. solani* (1)	–	–	–	–	–	8	–	–	4
Wang et al., 2009[21]	*Fusarium* spp. (71)	–	–	–	–	–	–	0.5	1	0.06 to 1
Xie et al., 2008[27]	*F. solani* (34)	–	–	–	–	–	–	1	2	–
Total Range	***Fusarium* spp.**	**4 to 8**	**4 to >64**	**2 to >64**	**2 to 64**	**4 to >64**	**0.13 to >64**	**0.5 to 16**	**1 to >64**	**0.06 to >64**
	F. solani	**4.8 to 8**	**4.8 to >64**	**2 to >64**	**4 to 64**	**8 to >64**	**0.13 to >64**	**1 to 8**	**2 to 64**	**0.13 to >64**
	F.* non-*solani	**2.4 to 8**	**4.8 to >64**	**2 to >64**	**4 to 64**	**4 to >64**	**0.25 to >64**	**0.5 to 16**	**2 to >64**	**0.5 to >64**
Kondori et al., 2011[18]	*Aspergillus* spp. (29)	–	–	–	–	–	0.13 to 8	–	–	–
	A. fumigatus (23)	–	–	–	–	–	0.13 to 8	–	–	–
Lalitha et al., 2007[6]	*Aspergillus* spp. (41)	32	>32	–	0.25	0.5	–	2	4	–
Lalitha et al., 2008[19]	*Aspergillus* spp. (59)	–	–	1 to 64	–	–	–	–	–	–
	A. flavus (32)	32	64	8 to 64	–	–	–	–	–	–
	A. fumigatus (18)	4	4	1 to 4	–	–	–	–	–	–

Study[a]	Species (Number of Isolates)	Natamycin (μg/mL)			Voriconazole (μg/mL)			Amphotericin B (μg/mL)		
		MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}	MIC Range
Lalitha et al., 2012[7]	*Aspergillus* spp. (17)	32	32	2 to 32	1	1	0.13 to 2	–	–	–
	A. flavus (11)	32	32	–	1	1	–	–	–	–
	A. fumigatus (5)	4	8	–	0.5	0.5	–	–	–	–
Manikandan et al., 2013[10]	*A. flavus* (74)	128	128	4 to 128	0.5	1	0.25 to 1	2	8	0.5 to 16
	A. fumigatus (14)	4	16	4 to 64	0.5	0.5	0.1 to 1	0.5	1	0.25 to 1
Nayak et al., 2011[28]	*A. flavus* (64)	–	–	–	–	–	–	3.12	12.5	0.03 to 25
	A. fumigatus (43)	–	–	–	–	–	–	3.12	3.12	0.03 to 12.5
Pradhan et al., 2011[12], *d*	*Aspergillus* spp. (24)	–	–	2 to 32	–	–	–	–	–	–
	A. flavus (13)	–	–	8 to 32	–	–	–	–	–	–
	A. fumigatus (9)	–	–	2 to 8	–	–	–	–	–	–
Shapiro et al., 2010[11]	*Aspergillus* spp. (24)	32	64	8 to 64	–	–	–	–	–	–
	A. flavus (18)	32	64	16 to 64	–	–	–	–	–	–
	A. fumigatus (1)	–	–	8	–	–	–	–	–	–
Wang et al., 2009[21]	*Aspergillus* spp. (15)	–	–	–	–	–	–	1	1	0.25 to 1
Xie et al., 2008[27]	*A. flavus* (9)	–	–	–	–	–	–	2	4	–
	A. fumigatus (9)	–	–	–	–	–	–	1	2	–
Total Range	***Aspergillus spp.***	**32**	**32 to 64**	**1 to 64**	**0.25 to 1**	**0.5 to 1**	**0.13 to 8**	**1 to 2**	**1 to 4**	**0.25 to 1**
	A. flavus	**32 to 128**	**32 to 128**	**4 to 128**	**0.5 to 1**	**1**	**0.25 to 1**	**2 to 3.12**	**4 to 12.5**	**0.03 to 25**
	A. fumigatus	**4**	**4 to 16**	**1 to 64**	**0.5**	**0.5**	**0.1 to 8**	**0.5 to 3.12**	**1 to 3.12**	**0.03 to 12.5**

Abbreviations: *N*, number; MIC, minimum inhibitory concentration; MIC_{50}, minimum inhibitory concentration median; MIC_{90}, minimum inhibitory concentration 90th percentile; spp., species; NAT, natamycin; VRC, voriconazole; AMB, amphotericin B.

[a]Susceptibility testing was performed using Clinical and Laboratory Standards Institute (CLSI) documents M38-P, M38-A, or M38-A2, except as noted.

[b] Susceptibility testing method was not specified.

[c] Estimated MIC_{50} or MIC_{90} using raw data provided in the article.

[d] Used natamycin eye drop (Sun Pharmaceutical Ind., Ltd, Mumbai, India) for susceptibility testing instead of standard natamycin pharmaceutical-grade powder.