Antimicrobial Susceptibility of *Fusarium*, *Aspergillus*, and Other Filamentous Fungi Isolated From Keratitis

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**Objective:** To characterize the susceptibility of filamentous fungi isolated from keratitis to amphotericin B, natamycin, caspofungin acetate, itraconazole, voriconazole, and posaconazole.

**Methods:** Ninety isolates from fungal keratitis cases at Aravind Eye Hospital in South India were tested using macrobroth dilution for susceptibility to amphotericin B, natamycin, caspofungin, itraconazole, voriconazole, and posaconazole. The minimum inhibitory concentration (MIC) median and 90th percentile were determined.

**Results:** The 90 isolates included 41 *Aspergillus* species, 38 *Fusarium* species, and 11 others. The triazoles and caspofungin had the lowest MICs against *Aspergillus* species; voriconazole, amphotericin B, and posaconazole had the lowest MICs against *Fusarium* species, and none of the *Fusarium* species were inhibited by itraconazole or caspofungin. Amphotericin B had significantly lower MICs compared with natamycin, but after correcting for the typical prescription dose, natamycin was superior.

**Conclusion:** No single agent was universally most effective, but voriconazole and other triazoles demonstrated the broadest spectrum. Itraconazole and caspofungin were not effective against *Fusarium* species.

**Clinical Relevance:** Fungal ulcers are commonly treated empirically; drugs are typically selected without regard to susceptibility data. The nonocular infectious disease literature suggests modern fungal susceptibility methods are clinically relevant, but ocular studies are limited. Our results suggest antifungal therapy might be tailored to individual organisms.

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OVER THE PAST 4 DECADES, there has been an increase in the percentage of infectious keratitis caused by fungal infection.1,2 Keratitis is a leading cause of monocular blindness worldwide,3,4 and it is estimated that more than half of corneal ulcers in some areas of the world are due to fungus.5 Filamentary fungal ulcers are thought to have a particularly poor prognosis.5 Only 1 antifungal agent, natamycin, is approved by the US Food and Drug Administration for topical ophthalmic use, although several others have been studied in vitro and in animal models or used clinically in compounded formulations.6-13 Despite the advent of improved laboratory diagnosis and increasing evidence that fungal susceptibility testing provides clinically relevant information, the treatment of fungal ulcers is still most commonly empirical.14,15 The absence of laboratory studies results in a gap in the ophthalmic literature regarding the potential application of emerging antifungal agents to fungal keratitis. Linares et al16 determined the susceptibility of 244 fungal isolates, but only 14 isolates were *Fusarium* species, and these were from nonocular disease. Marangon et al17 reviewed 419 cases of fungal keratitis but only tested the susceptibility of 9 *Fusarium* species and 4 *Aspergillus* species. Neither of these studies determined the susceptibility of isolates to natamycin, posaconazole, or caspofungin.

Classes of antifungal medications used for filamentary fungal keratitis include the polyenes, triazoles, and echinocandins.12 Natamycin, a polyene, has long been considered the mainstay of treatment for filamentous fungal keratitis. However, for systemic diseases such as pulmonary aspergillosis, newer triazoles such as voriconazole are the recommended therapy.20 In the past, techniques for performing antifungal susceptibility testing have been difficult to perform and standardize. Consequently, many of the new antifungal agents...
Table 1. Distribution of Fungal Isolates

<table>
<thead>
<tr>
<th>Organism Isolated</th>
<th>Isolates Collected (n = 98)</th>
<th>Isolates Processed (n = 90)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Fusarium species</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Curvularia species</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Bipolaris species</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Cladosporium species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acremonium species</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Botryodiplodia species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Exserohilum species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Trichoderma species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Unidentified hyaline fungus</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Of the 98 consecutive cases of culture-positive fungal keratitis seen at Aravind Eye Hospital between March and July 2004, 8 isolates were contaminated by bacteria and could not be processed for susceptibility testing against the 6 antifungals.

This prospective, nonrandomized interventional study was performed in the Cornea Department of Aravind Eye Hospital, Madurai, India, after approval by the institutional review boards of the Aravind Medical Research Foundation and the University of California, San Francisco.

Fungal cultures were isolated from 98 consecutive cases of culture-proven fungal keratitis seen at the Aravind Eye Hospital cornea clinic between March and July 2004. Consistent with the standard of care at Aravind, each patient with keratitis gave a clinical history and underwent a detailed clinical examination using a slitlamp biomicroscope to measure the size and depth of ulceration. Scraping of the corneal ulcer was performed under magnification using a Kimura spatula, and the material obtained was evaluated with a gram stain and 10% potassium hydroxide mount. Subsequent scrapings were plated onto blood agar and potato dextrose agar and permitted to grow at room temperature for at least 7 days prior to testing. Any positive fungal isolate was identified to the genus level, with Aspergillus species identified to the species level.

Of the 98 isolates, 8 were not processed because of bacterial contamination. The remaining 90 isolates were tested for susceptibility to 6 antifungal agents: amphotericin B, natamycin, caspofungin, atiroconazole, voriconazole, and posaconazole. Susceptibility was determined according to methods outlined in Clinical and Laboratory Standards Institute documents M38-A and M27-A.²⁰,²⁸ The inoculum was prepared by overlaying mature slants with sterile distilled water and gently scraping the surface with a wooden applicator stick. The suspension was then adjusted spectrophotometrically to the correct optical density for each species as outlined in M38-A, providing an inoculum concentration of 2.5–5 × 10⁶ conidia/mL, which was verified by colony count. Broth macrodilutions were created by adding 0.1 mL of 10× concentrated drug to 0.9 mL of inoculum. The test medium was RPMI-1640 for the azoles and antibiotic medium 3 for the polyenes and caspofungin.²⁹-³³ Tests were incubated at 35°C until growth was visible in the drug-free control tube.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration that exhibited a 100% visual reduction in turbidity when compared with the control tube for the polyenes at 48 hours.³⁰ An 80% reduction in turbidity was considered the end point for the azoles.³⁰ The minimum effective concentration, or the lowest concentration that induces aberrant growth, was used to determine the cutoff for caspofungin.³⁴ The MIC median (MIC₅₀) and 90th percentile (MIC₉₀) were determined for Fusarium species, Aspergillus species, and all 90 isolates together (PERCENTILE function in Microsoft Excel; Microsoft Inc, Redmond, Wash). The MICs of antifungals were compared using a Wilcoxon signed rank test (Stata 7.0; StataCorp, College Station, Tex).

The relative MIC₅₀ is included alongside the absolute MIC₅₀. The relative MIC₅₀ normalizes the discrepancies between the typical prescription doses of the 6 antifungals, which range considerably from 0.15% to 5%. The relative MIC₅₀ (no units) is calculated by dividing the absolute MIC₅₀ (micrograms per milliliter) by the typical prescription dose (weight per volume percentage or grams per 100 mL). Although the relative MIC₅₀ does not account for the bioavailability or penetration of topical drugs, it does take into account that, for example, each drop of natamycin administered has 30-fold the drug concentration compared with amphotericin B, and this approach may therefore provide some additional information.

The 90 processed isolates included 41 Aspergillus species, 38 Fusarium species, and 11 others (Table 1), allowing for MIC₅₀ and MIC₉₀ estimation of these 2 principle organisms (Table 2). The MICs ranged markedly among antifungal agents and organisms. The triazoles performed best against Aspergillus species (Figure 1A), with posaconazole having significantly lower MICs than the second most effective agent, voriconazole (P<.001). Voriconazole and amphotericin B had the lowest MICs vs Fusarium species, and they were not significantly different from each other (P = .21) (Figure 1B). None of the 38 Fusarium species were inhibited at even the highest tested concentration of itraconazole (8 µg/mL) or caspofungin acetate (16 µg/mL). Compared with natamycin, amphotericin B had significantly lower MICs vs Aspergillus species (P < .001) and vs Fusarium species (P < .001).

Each antifungal has a typical prescription dose that can differ markedly from other antifungals. For example, amphotericin B is regularly given at 0.15%, whereas natamycin is usually given at 5%. Therefore, 1 drop of natamycin has 30 times the amount of drug than 1 drop of amphotericin B. Figure 2 normalizes the MIC₅₀ relative to the typical prescription dose. Compared with amphotericin B, natamycin had significantly lower relative MICs against Aspergillus species (P = .006) and against Fusarium species (P < .001). While amphotericin B had nearly the same absolute MICs as voriconazole vs Fusarium spe-
cies (P = .21), voriconazole had significantly lower MICs after adjusting for the higher typical prescription dose (P < .001). When including all fungal isolates, voriconazole and posaconazole offered the lowest relative MICs.

### COMMENT

In this study, we investigated the in vitro efficacy of traditional and newer antifungals against isolates from fungal keratitis cases using macrodilution assays that represent the current gold standard. As is the case for antibiotics and bacteria, no single antifungal was most effective for all fungi. Posaconazole had the lowest MIC90 against *Aspergillus* species (n=41), closely followed by itraconazole, caspofungin, and voriconazole. Amphotericin B and voriconazole had the lowest MIC90 vs *Fusarium* species (n=38). The results for *Aspergillus* species are consistent with the nonophthalmic literature, where the newer triazoles are considered the treatment of choice for systemic aspergillosis. Both caspofungin and itraconazole were reasonably effective against *Aspergillus* species but were completely ineffective against *Fusarium* species. For itraconazole, which is available commercially as a topical agent in India, these data are consistent with clinical studies that reported itraconazole was effective against *Aspergillus* species but not *Fusarium* species. These data are also consistent with in vitro data reporting that *Fusarium* is not susceptible to either itraconazole or caspofungin.

These results are consistent with a prior study on in vitro susceptibilities of fungal isolates from keratitis and endophthalmitis, which found that voriconazole had the lowest MICs for *Aspergillus* species isolated from keratitis and endophthalmitis (n=4) and that voriconazole and amphotericin B had the lowest MICs vs *Fusarium* species (n=9). This study used E-tests, which have approximately 80% agreement with the gold standard macrodilution assays performed in our study, and natamycin, caspofungin, and posaconazole were not tested. In larger studies of isolates from systemic disease, voriconazole and amphotericin B have been shown to be more effective than itraconazole against *Fusarium* species, and voriconazole has been shown to be very effective against *Aspergillus* species.

### Table 2. MIC50 and MIC90 of Fungal Isolates

<table>
<thead>
<tr>
<th></th>
<th>Amphotericin B</th>
<th>Natamycin</th>
<th>Caspofungin Acetate</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC50</strong></td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td><strong>MIC90</strong></td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Abbreviations: MIC50, minimum inhibitory concentration median; MIC90, minimum inhibitory concentration 90th percentile.

### Figure 1

A, *Aspergillus* species (n=41). B, *Fusarium* species (n=38). Gray lines represent the threshold for the minimum inhibitory concentration median (MIC50) and 90th percentile (MIC90). Natamycin (NAT) inhibited only 76% of *Aspergillus* at the highest concentration tested (32 µg/mL), and therefore, the MIC90 is indeterminate. Posaconazole (POS) inhibited only 89.5% of *Fusarium* species at the highest concentration tested (8 µg/mL) and therefore also has an indeterminate MIC90, although the MIC50 can be extrapolated to be slightly greater than 8 µg/mL. Itraconazole (ITRA) and caspofungin acetate (CAS) both failed to inhibit growth of *Fusarium* species at every concentration tested and are represented as horizontal lines that span the respective concentrations tested. AMB indicates amphotericin B; VOR, voriconazole. AMB and NAT are polyenes, CAS is an echinocandin, and ITRA, VOR, and POS are triazoles.
Results may be reasonably predictive of the therapeutic outcome. Studies have shown that antibiotic susceptibility testing is gaining credibility in the literature. Antibacterial susceptibility testing may be valuable. The relatively poor performance of traditional treatment regimens indicates that potentially more effective agents such as voriconazole should be subjected to closer examination in rigorous clinical trials and tested against the current Food and Drug Administration–approved natamycin. Also, studies that investigate the correlation between in vitro susceptibilities and in vivo clinical results will be important in the future management of fungal keratitis.

The effectiveness of the antifungal agent is greatly affected by the substantially different concentrations of drug achieved locally. In practice, the topical antifungal medications are given in different concentrations and this is not taken into account when comparing absolute MIC values. For example, natamycin is commercially available in a 5% concentration, while amphotericin B is typically prescribed at a concentration of only 0.15% because of toxicity. After correction for the available dose, natamycin has lower relative MICs than amphotericin B for both Fusarium species and Aspergillus species. The adjusted MIC reveals that if penetration is not an issue, natamycin may be as effective as voriconazole for Fusarium species. However, neither amphotericin B nor natamycin penetrate well through an intact epithelium.

Although susceptibility testing is used far more frequently for bacterial than fungal disease, fungal susceptibility testing is gaining credibility in the literature. Antibacterial studies have shown that antibiotic susceptibility results may be reasonably predictive of the therapeutic response rate in cases of bacterial keratitis. One study found that 91% of susceptible isolates from bacterial keratitis cases showed clinical improvement compared with 73% of resistant isolates. In the nonophthalmic literature, antifungal susceptibility testing has been found to be feasible and predictive of clinical outcomes. From a wide range of studies, infections due to susceptible isolates respond to therapy approximately 90% of the time, while infections due to resistant isolates respond 60% of the time. In the ophthalmic literature, little has been done to investigate this question for fungal infections.

Results of treatment of filamentous fungal keratitis are frequently poor. Although susceptibility testing and new antifungal agents are available, clinical experience with these agents is sparse and treatment is typically empirical. This report reveals that in vitro, certain antifungals perform significantly better against specific organisms, suggesting that tailored treatment based on susceptibility testing may be valuable. The relatively poor performance of traditional treatment regimens indicates that potentially more effective agents such as voriconazole should be subjected to closer examination in rigorous clinical trials and tested against the current Food and Drug Administration–approved natamycin. Also, studies that investigate the correlation between in vitro susceptibilities and in vivo clinical results will be important in the future management of fungal keratitis.

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