AAC Journals.ASM.org



# *In Vitro* Studies of *Exserohilum rostratum* with Antifungal Drugs and Methylprednisolone

**S. G. Revankar,[a] V. Moudgal,[b] P. Chandrasekar,[a] J. D. Sobel[a]**

Division of Infectious Diseases, Department of Medicine, Wayne State University, Detroit, Michigan, USA[a]; Division of Infectious Diseases, Department of Medicine, St. Joseph Mercy Hospital, Ann Arbor, Michigan, USA[b]

*Exserohilum rostratum* was the primary fungal species implicated in the recent nationwide outbreak of meningitis due to contaminated steroid injections (1). This species is an extremely rare cause of human infection, and optimal therapy is not known (2). Despite prolonged administration of voriconazole with or without amphotericin B, clinical failures have been seen. In addition, the steroid component of the injection may have stimulated growth, leading to enhanced virulence.

We studied 6 clinical isolates of *E. rostratum* from 6 patients from the outbreak with respect to their *in vitro* susceptibility to antifungals, including antifungal combinations, as well as the effect of methylprednisolone on growth. The isolates were all obtained from patients at a single institution who received contaminated steroid injections from the same lot, suggesting that the isolates may be clonal. Isolates were grown on V-8 juice agar incubated at 35°C for 10 to 14 days. CLSI broth microdilution method M38-A2 was used (3), with the inoculum prepared by hemacytometer counting. Check plates were used to confirm the inoculum concentrations. MICs were read at 48 h. We tested the susceptibilities of all isolates to voriconazole, itraconazole, and posaconazole; 3 isolates were tested with combinations of voriconazole and amphotericin B, caspofungin, flucytosine, or terbinafine. Concentration ranges were 0.01 to 16 μg/ml for voriconazole; 0.25 to 16 μg/ml for amphotericin B, caspofungin, itraconazole, and posaconazole; 1 to 64 μg/ml for flucytosine; and 0.01 to 1 μg/ml for terbinafine. The fractional inhibitory concentration (FIC) index (based on 100% growth inhibition) was calculated for each combination; an FIC of <0.5 indicated synergy, 0.5 to 4 indicated additive/indifference, and >4 indicated antagonism. The effects of methylprednisolone on growth were studied with 2 methods. (i) Three isolates of *E. rostratum* were grown on agar plates containing 0, 1, 10, 100, and 1,000 μg/ml methylprednisolone, and colony diameter was assessed at 48 h. (ii) Broth microdilution testing was performed with voriconazole (0.03 to 16 μg/ml) and the addition of methylprednisolone at 0, 1, 10, 100, and 1,000 μg/ml; results were read at 48 h.

The *in vitro* susceptibility results are summarized in Table 1. The MICs of flucytosine and terbinafine were remarkably high for all isolates. Those of the other drugs, however, were lower but did not suggest a very potent *in vitro* activity. The newer triazole antifungals had similar MICs, which were generally lower than those of the other agents tested. This is in contrast to a prior study (4), in which triazoles were highly active. However, our results are generally consistent with testing performed by the CDC on a large collection of isolates from the national outbreak, including those from the same lot as our isolates (5). The choice of combinations tested was based on previous studies of other fungi (6). Combination antifungal testing did not reveal any synergistic activity, though there was no antagonism observed. Methylprednisolone did not affect the MIC of voriconazole in broth microdilution at any concentration at 48 h. However, when voriconazole was added to agar media, only the 1,000-μg/ml plate showed a 25% mean reduction of colony diameter (from 32 mm to 24 mm) at 48 h.

**TABLE 1** *In vitro* susceptibility testing of *Exserohilum* sp.

| Drug or combination (no. of isolates) | MIC range (μg/ml) | Mean FIC index |
|---|---|---|
| Voriconazole (6) | 2 | |
| Itraconazole (6) | 1–2 | |
| Posaconazole (6) | 0.5–1 | |
| Amphotericin B (3) | 2–4 | |
| Caspofungin (3) | 4 | |
| Flucytosine (3) | >64 | |
| Terbinafine (3) | >1 | |
| Voriconazole + amphotericin B (3) | | 1.0 |
| Voriconazole + caspofungin (3) | | 1.0 |
| Voriconazole + flucytosine (3) | | 2.0 |
| Voriconazole + terbinafine (3) | | 0.8 |

The interpretation of *in vitro* susceptibility testing for *E. rostratum* is not established, and the clinical significance is unclear. In the present study, this species does not appear to be highly susceptible to the agents tested; however, conclusions are limited due to the possibility that these isolates may be highly related or identical. Alternative strategies to treat this fungal infection should be explored.

## ACKNOWLEDGMENT

Isolates were kindly provided by the St. Joseph Mercy Hospital Laboratory, Ann Arbor, MI.

## REFERENCES

1. **Kainer MA, Reagan DR, Nguyen DB, Wiese AD, Wise ME, Ward J, Park BJ, Kanago ML, Baumblatt J, Schaefer MK, Berger BE, Marder EP, Min JY, Dunn JR, Smith RM, Dreyzehner J, Jones TF, Tennessee Fungal Meningitis Investigation Team.** 2012. Fungal infections associated with contaminated methylprednisolone in Tennessee. N. Engl. J. Med. **367:** 2194–2203. http://dx.doi.org/10.1056/NEJMoa1212972.
2. **Revankar SG, Sutton DA.** 2010. Melanized fungi in human disease. Clin. Microbiol. Rev. **23:**884–928. http://dx.doi.org/10.1128/CMR.00019-10.
3. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38–A2. CLSI, Wayne, PA.

Published ahead of print 24 March 2014

Address correspondence to S. G. Revankar, srevankar@med.wayne.edu.



doi:10.1128/AAC.02357-13

4. **da Cunha KC, Sutton DA, Gené J, Capilla J, Cano J, Guarro J.** 2012. Molecular identification and in vitro response to antifungal drugs of clinical isolates of *Exserohilum*. Antimicrob. Agents Chemother. **56:**4951–4954. http://dx.doi.org/10.1128/AAC.00488-12.
5. **Lockhart SR, Pham CD, Gade L, Iqbal N, Scheel CM, Cleveland AA, Whitney AM, Noble-Wang J, Chiller TM, Park BJ, Litvintseva AP, Brandt ME.** 2013. Preliminary laboratory report of fungal infections associated with contaminated methylprednisolone injections. J. Clin. Microbiol. **51:**2654–2661. http://dx.doi.org/10.1128/JCM.01000-13.
6. **Cuenca-Estrella M.** 2004. Combinations of antifungal agents in therapy—what value are they? J. Antimicrob. Chemother. **54:**854–869. http://dx.doi.org/10.1093/jac/dkh434.