In vitro studies of *Exserohilum rostratum* with antifungal drugs and methylprednisolone

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Exserohilum (E.) 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 in vitro antifungal susceptibility, 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 an identical lot, suggesting they may be clonal. Isolates were grown on V-8 juice agar incubated at 35°C for 10-14 days. CLSI microbroth dilution methodology M38-A2 was used (4), with the inoculum prepared by hemacytometer count. Check plates were used to confirm the inoculum concentrations. MICs were read at 48 hours. Susceptibility of all isolates was tested to voriconazole, itraconazole and posaconazole; 3 isolates were tested with combinations of voriconazole with amphotericin B, caspofungin, flucytosine or terbinafine. Concentration ranges were 0.01-16 µg/ml for voriconazole; 0.25-16 µg/ml for amphotericin B, caspofungin, itraconazole and posaconazole; 1-64 µg/ml for flucytosine and 0.01-1 µg/ml for terbinafine. The fractional inhibitory concentration (FIC) index (based on 100% growth inhibition) was calculated for each combination; FIC < 0.5 = synergy, 0.5-4 = additive/indifference, >4 = antagonism. The effects of methylprednisolone on growth were studied with 2 methods: 1) 3 isolates of E. rostratum were grown on agar plates containing 0, 1, 10, 100, and 1000 µg/ml.
methylprednisolone and colony diameter was assessed at 48 hours; 2) microbroth
dilution testing was performed with voriconazole (0.03-16 µg/ml) and the addition of
methylprednisolone at 0, 1, 10, 100, and 1000 µg/ml; results were read at 48 hr.
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 the other agents
tested. This is in contrast to a prior study (3), 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, and an additive effect could not be
excluded. MTP did not affect the MIC of voriconazole in microbroth dilution at any
concentration at 48 hr. However, when voriconazole was added to agar media, only the
1000 µg/ml plate showed a 25% mean reduction of colony diameter (from 32mm to
24mm) at 48 hours.
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 these isolates may be highly related or identical. Alternative
strategies to treat this fungal infection should be explored.

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Lab, Ann Arbor, MI
References


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

<table>
<thead>
<tr>
<th>Drug / combination (isolates)</th>
<th>MIC range (µg/mL)</th>
<th>Mean FIC index</th>
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<tbody>
<tr>
<td>Voriconazole (6)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Itraconazole (6)</td>
<td>1-2</td>
<td></td>
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<tr>
<td>Posaconazole (6)</td>
<td>0.5-1</td>
<td></td>
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<tr>
<td>Amphotericin B (3)</td>
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<td>Caspofungin (3)</td>
<td>4</td>
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<tr>
<td>Flucytosine (3)</td>
<td>&gt;64</td>
<td></td>
</tr>
<tr>
<td>Terbinafine (3)</td>
<td>&gt;1</td>
<td></td>
</tr>
<tr>
<td>Vori + AmB (3)</td>
<td>1.0</td>
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</tr>
<tr>
<td>Vori + Caspo (3)</td>
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<tr>
<td>Vori + 5-FC (3)</td>
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<tr>
<td>Vori + Terb (3)</td>
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