**Fluconazole susceptibility testing of Candida inconspicua clinical isolates: comparison of four methods**

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Sir,

During the past 4 years, isolation of Candida inconspicua from clinical samples has increased in our laboratory.1 Fluconazole MIC values for C. inconspicua isolates are relatively high, as determined by standard broth microdilution (BMD), Etest or Fungitest,2–4 and according to the standard BMD method they fall mainly into the susceptible-dose dependent (S-DD) or resistant (R) categories, with few susceptible (S) isolates.1–3 Since fluconazole is the most widely used drug in the clinical setting, and in the case of rare Candida species susceptibility test results may precede definitive identification (24 or 48 h versus 48 or 72 h), the method used for susceptibility testing should be suitable to correctly determine the fluconazole susceptibility not only of the four most frequent species, but also of relatively frequently isolated rare species.

The aim of our study was to evaluate Etest, Fungitest and modified BMD methods as alternatives for determination of fluconazole susceptibility of our C. inconspicua clinical isolates and to compare their agreement with the standard method.

The isolates studied included 57 C. inconspicua strains isolated from 48 patients during a 3 year period (2001–2003). Twenty-two of the 42 hospitalized patients were immunocompromised and 15 were hospitalized in seven different intensive care units. Eleven patients received fluconazole previously. The majority of specimens were from the upper and lower respiratory tract (16 and 24, respectively), but wound, blood and genital isolates were also obtained. Inpatients were treated at 10 separate clinics at different times.

Identification of the isolates was carried out as described earlier.1 The reference BMD method was carried out according to the guidelines of the NCCLS document M27-A2. The pure fluconazole powder (Pfizer) was dissolved in sterile distilled water, and the final concentration range was 0.25–128 mg/L. Yeast suspensions were prepared in RPMI 1640 medium and were adjusted to a final concentration of $10^3$ cells/mL (standard inoculum size), or in the modified BMD method $10^4$ cells/mL (large inoculum size). Drug-free purity controls and growth controls were included in each plate. The plates were incubated at 35°C and read visually after 24 and 48 h. Fluconazole MIC was defined as the lowest concentration that produced a prominent decrease in turbidity compared with that of the drug-free growth control.5 Quality control strains of Candida parapsilosis (ATCC 22019) and Candida krusei (ATCC 6258) were included in each test.

Etest (AB Biodisk, Solna, Sweden) and Fungitest (Bio-Rad SDP, formerly Sanofi Diagnostics Pasteur, France) methods were carried out according to the manufacturers’ instructions. Etest MICs were recorded at 24 and 48 h. Fungitest was read visually at 48 h. Quality control strains were included in all tests.

The isolates were tested using the four methods: NCCLS M27-A2, BMD (24 h and 48 h), Etest (24 h), and Fungitest (48 h).

**Table 1. Categorical agreement and discrepancies of different methods used for fluconazole susceptibility testing of C. inconspicua isolates \((n = 48)\) compared with the standard broth microdilution method (NCCLS M27-A2)**

<table>
<thead>
<tr>
<th>Test method</th>
<th>Inoculum/ incubation time</th>
<th>Overall agreementa</th>
<th>S</th>
<th>S-DD</th>
<th>R</th>
<th>Percentage of isolates by categoryb</th>
<th>Percentage of discrepant results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S-D versus S</td>
<td>S-D versus R</td>
</tr>
<tr>
<td>NCCLS M27-A2</td>
<td>$10^3$ cells/mL 48 h</td>
<td>32/32</td>
<td>0</td>
<td>89.5</td>
<td>10.5</td>
<td>6.3</td>
<td>0</td>
</tr>
<tr>
<td>BMDb</td>
<td>$10^3$ cells/mL 24 h</td>
<td>100%</td>
<td>32/32</td>
<td>6.3</td>
<td>89.5</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>BMDd</td>
<td>$10^3$ cells/mL 24 h</td>
<td>100%</td>
<td>32/32</td>
<td>2.1</td>
<td>87.4</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Etest</td>
<td>24 h</td>
<td>97.9%</td>
<td>32/64</td>
<td>0</td>
<td>58.3</td>
<td>41.7</td>
<td>0</td>
</tr>
<tr>
<td>Fungitest</td>
<td>48 h</td>
<td>58.3%</td>
<td>64/128</td>
<td>6.3</td>
<td>56.2</td>
<td>37.5</td>
<td>0</td>
</tr>
</tbody>
</table>

aPercentage agreement represents the percentage of MIC values within ±1 dilution compared with the reference method (NCCLS M27-A2).
bPercentage of isolates classified in the different categories.
cPercentage of isolates classified within the same category as with the standard broth microdilution method.
dBroth microdilution method.

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after 48 h. Isolates were classified as S, S-DD or R according to the NCCLLS guidelines.5

Fluconazole MICs obtained with the modified BMD tests and Etest read at 24 and 48 h were compared with the reference BMD MICs read at 48 h. Discrepancies between MICs of no more than ±1 dilution were calculated to be percentage of agreement. Categorical agreement was defined when the applied method gave the same category result compared with the reference BMD category read at 48 h.

Results are summarized in Table 1. In vitro susceptibility test results demonstrated relatively high fluconazole MICs for C. inconspicua clinical isolates by all applied methods. Compared with the standard method, overall agreement of MICs obtained with the studied methods was excellent with the exception of the Etest read at 48 h. After 24 h of incubation, categorical agreement of the normal and large inoculum BMD method was very good, but out of five R strains three and two, respectively, were misdiagnosed as S-DD. Using a large inoculum BMD method read at 48 h and Etest at 24 and 48 h, poor categorical agreements were observed, though all R isolates were diagnosed correctly. The Fungitext gave good categorical agreement compared with the standard method, but four of five R isolates were misdiagnosed as S-DD.

This study demonstrates, for the first time, the applicability of alternative fluconazole susceptibility testing methods in the case of C. inconspicua. Etest read at 24 h is a good choice for screening the fluconazole resistance of C. inconspicua, though Etest results tended to be higher (generally within one dilution) than the standard BMD results.

Correct identification is a major step to adequate drug choice, mainly in the case of rare Candida species as we demonstrated in our previous study of C. inconspicua.1 However, if a clinician urgently needs fluconazole susceptibility results, before the correct identification becomes available, Etest read at 24 h safely detects the decreased fluconazole susceptibility of C. inconspicua. In a proven life-threatening C. inconspicua infection, the empirical fluconazole therapy should, of course, be switched to amphotericin B.6 Though the efficacy of caspofungin and voriconazole has not been tested in a systematic study, they seem to be reasonable alternatives.

**References**


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**Activity of a peptide deformylase inhibitor LBM415 (NVP PDF-713) tested against recent clinical isolates from Japan**

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Sir,

Peptide deformylase (PDF) has been recognized as a new target for antibacterial agents and several PDF inhibitors have been developed.1 LBM415 (NVP PDF-713) is a new PDF inhibitor with documented activities against Gram-positive organisms.2–5 The aim of this study was to evaluate the potency of LBM415 against key Gram-positive pathogens, as well as Haemophilus influenzae, from Japan where antimicrobial resistance levels are very high among clinically significant Gram-positive organisms and community-acquired respiratory pathogens.6,7

A total of 695 clinical isolates originally collected in Japan included Staphylococcus aureus (n = 222), Haemophilus influenzae (n = 119), Streptococcus pneumoniae (n = 122), coagulase-negative staphylococci (CoNS; n = 119), Enterococcus spp. (n = 65) and Streptococcus spp. (n = 48). No vancomycin-resistant enterococci were detected during this period. LBM415 (Novartis Pharmaceuticals, Basel, Switzerland) was diluted in broth microdilution trays or agar using NCCLS methods and media supplements as required.3 NCCLS quality control strains with established MIC ranges were included throughout the study.

The MIC distribution, MIC50 and MIC90 values are shown in Table 1. Oxacillin-resistant S. aureus had slightly lower LBM415 MIC values than oxacillin-susceptible strains. MIC50 and MIC90 values for LBM415 against oxacillin-resistant S. aureus were 2 log10 dilutions lower than those observed by Credito et al.,2 although they tested a smaller number of strains. CoNS