In Vitro Activities of Fluconazole and Voriconazole against Clinical Isolates of Candida spp. Determined by Disk Diffusion Testing in Turin, Italy

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The in vitro activities of fluconazole and voriconazole against 1,024 clinical isolates of Candida spp. were determined by the agar disk diffusion test using the Clinical and Laboratory Standards Institute (CLSI) M44-A guidelines. The results of this investigation demonstrated the broad-spectrum in vitro activity of voriconazole, relative to that of fluconazole, against yeasts tested, in particular fluconazole-resistant isolates, such as Candida krusei that showed high susceptibility to voriconazole. The situation in Turin, Italy, is quite similar to that of the rest of Italy, reflecting the worldwide trend.

Candida species represent the most common cause of fungal infections. Candida albicans remains the predominant agent of candidemia and is usually susceptible to azoles, such as fluconazole. Recently, the incidence of invasive candidiasis and candidemia and is usually susceptible to azoles, such as fluconazole, increased, while those due to non-C. albicans strains markedly increased. Routine prophylactic and therapeutic use of fluconazole in hospitalized and immunocompromised patients can be associated with a shift of species and resistance patterns. Reduced fluconazole susceptibility was observed for C. glabrata, and C. krusei appeared to be intrinsically resistant to fluconazole (4, 14, 16, 18). The increased antifungal resistance to antifungal agents most commonly used underscores the need for new antifungal agents. The more recent triazoles, such as voriconazole, ravuconazole, and posaconazole, and the echinocandins, such as caspofungin, are new drugs that broaden the available therapeutic armamentarium for the treatment of invasive fungal infections. In particular, voriconazole shows broad-spectrum activity with antifungal activity against clinically relevant yeasts and molds. Its key property is a potential activity against some fluconazole-resistant isolates, and it can be considered a salvage treatment for patients with refractory candidiasis (11, 18).

With the reference laboratory in Turin, Italy, part of the Institute (CLSI) in association with the BIOMIC system (Giles Scientific Inc., Santa Barbara, CA) (8). The BIOMIC system provides a cost-effective means to electronically read and interpret test plates and to collect and transfer data; moreover, the BIOMIC system provides improved intra- and interlaboratory reading consistency (5, 7, 13, 19).

A total of 1,024 yeast isolates were collected from various specimens (blood, normally sterile body fluids, deep tissue, genital tract, gastrointestinal tract, and respiratory tract) from three participating hospitals in Turin and Biella, Italy, between January 2002 and December 2006. All strains were identified by ID32C panels (bioMérieux, Rome, Italy).

Fluconazole and voriconazole susceptibility testing was performed in accordance with CLSI document M44-A for yeasts (8). For quality control, C. albicans ATCC 90029 was used in accordance with CLSI document M44-A (8). Fluconazole (25 μg) and voriconazole (1 μg) disks were manufactured and supplied by Becton Dickinson, Sparks, MD. Zone diameter endpoints were automatically read at 80% growth inhibition and interpreted using the BIOMIC image analysis plate reader system (Giles Scientific, Inc.) (5, 9, 13). The interpretive criteria for fluconazole and voriconazole disk diffusion tests were performed in accordance with CLSI (2, 8). The MICs were determined by the BIOMIC system that uses species-specific linear regression analysis to correlate all zone diameters from the disk diffusion test with corresponding MICs from the reference dilution method (12). The corresponding MIC breakpoints were those of CLSI (2, 8). An excellent correlation between the MIC and the zone diameter has been observed in previous studies (5, 12). Hence, fluconazole and voriconazole MIC₉₀% and MIC₅₀% were calculated and reported.

The species distribution and the in vitro susceptibilities of 1,024 Candida isolates to fluconazole and voriconazole are summarized in Table 1. In agreement with the international literature (1, 5, 13), we found that in Turin, Italy, C. albicans was the most common yeast species but the C. albicans frequency (55.6%) was slightly lower than that reported by other...
TABLE 1. In vitro susceptibilities of Candida spp. to fluconazole and voriconazole as determined by CLSI disk diffusion method

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates (%) tested</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>SDD</td>
</tr>
<tr>
<td>C. albicans</td>
<td>597 (55.6)</td>
<td>587 (98)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>201 (18.7)</td>
<td>164 (82)</td>
<td>19 (9)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>59 (5.5)</td>
<td>54 (92)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>51 (4.7)</td>
<td>49 (96)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>28 (2.6)</td>
<td>3 (11)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>24 (2.2)</td>
<td>23 (96)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>C. norvegensis</td>
<td>14 (1.3)</td>
<td>3 (21)</td>
<td>11 (79)</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>7 (0.6)</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>C. famata</td>
<td>7 (0.6)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>C. valida</td>
<td>7 (0.6)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>C. inconspicua</td>
<td>5 (0.5)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>C. pulcherimia</td>
<td>3 (0.3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>3 (0.3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C. sake</td>
<td>3 (0.3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other Candida spp.</td>
<td>14 (1.4)</td>
<td>13 (92.9)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1,024</td>
<td>918 (90)</td>
<td>41 (4)</td>
</tr>
</tbody>
</table>

a S, susceptible; SDD, susceptible dose dependent; R, resistant.
b Includes isolates of more unusual Candida species, such as C. holmii, C. humicola, C. intermedia, C. lambica, C. lusitaniae, C. membranifaciens, C. rugosa, and C. valida.
c Despite these values of sensitivity, isolates are assumed to be intrinsically resistant to fluconazole and their inhibition zone diameters should not be interpreted using breakpoints established by CLSI (2, 8).

determined for fluconazole.

Despite these values of sensitivity, isolates are assumed to be intrinsically resistant to fluconazole and their inhibition zone diameters should not be interpreted using breakpoints established by CLSI (2, 8). According to Sugita et al. (17), fluconazole resistance was also observed in C. norvegensis, C. valida, and C. inconspicua; these three opportunistic fungal pathogens are rarely isolated from patients but are considered to be emerging pathogens in humans. The uncommon Candida species were all susceptible to the drug (Table 1).

Compared to fluconazole, voriconazole showed an excellent in vitro potency and broad-spectrum activity against all tested species, including many yeasts that were found to be resistant to fluconazole, such as C. krusei, which showed high susceptibility to voriconazole (92%). Although voriconazole was highly active against C. glabrata, resistance to voriconazole was detected (Table 1), as confirmed by the worldwide data reported by Pfaffer et al. (12, 13). This decreased susceptibility of C. glabrata to multiple azole drugs may be ascribed to the broad current usage of fluconazole and/or itraconazole for the treatment of infections due to Candida spp. and other opportunistic yeasts and yeastslike fungi (10). We found that voriconazole was highly active against most rare Candida species, such as C. dubliniensis, C. famata, C. guilliermondii, C. holmii, C. humicola, C. intermedia, C. lusitaniae, C. membranifaciens, C. pulcherimia, and C. utilis. We found that a high percentage of C. valida isolates were resistant to fluconazole but susceptible to voriconazole (Table 1); this species has been emerging in recent years (5, 13).

MIC ranges, MIC995, and MIC995 for fluconazole and voriconazole are listed in Table 2. Voriconazole inhibited 90% of the isolates at a concentration six to eight times lower than that determined for fluconazole. C. krusei and C. glabrata fluconazole MICs were markedly higher than those for voriconazole (256 versus 2 μg/ml and 64 versus 4 μg/ml, respectively).

There was very little variation in the rates of resistance of Candida isolates to either fluconazole and voriconazole over the study period (2002 to 2006) (data not shown). Only C. kefyr...
Other FLU 0.125–256 2 128
C. norvegensis
C. kefyr

established by CLSI (2, 8). resistant to fluconazole, and MICs should not be interpreted using breakpoints
C. krusei
C. tropicalis
C. glabrata

exhibited a trend of decreased fluconazole susceptibility over the study period, from 100% (2002 to 2005) to 86% in 2006 (data not shown).

In conclusion, our data revealed that the resistance to fluconazole among pathogenic yeasts is still a restricted phenomenon in Turin, Italy. We confirm that voriconazole is a promising antifungal triazole that appears to be more active against yeasts intrinsically resistant to fluconazole and by many less-common species. The susceptibility patterns and trends observed in Turin are similar to those observed elsewhere in Italy, reflecting the worldwide patterns (7, 9, 12).

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REFERENCES
2. Clinical and Laboratory Standards Institute. 2007. Zone diameter interpretative standards, corresponding minimal inhibitory concentration (MIC) interpretive breakpoints, and quality control limits for antifungal disk diffusion susceptibility testing of yeasts; 17th informational supplement. CLSI M44-S2. Clinical and Laboratory Standards Institute, Wayne, PA.