Susceptibility of clinical isolates of *Candida lusitaniae* to five systemic antifungal agents

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Received 22 September 2003; returned 22 October 2003; revised 21 November 2003; accepted 10 December 2003

**Objectives:** The aim of the present study was to expand the MIC database for *Candida lusitaniae* in order to further determine its antifungal susceptibility pattern.

**Methods:** The activities of amphotericin B, fluconazole, itraconazole, voriconazole and flucytosine were determined *in vitro* against 80 clinical isolates of *C. lusitaniae*. A set of 59 clinical isolates of *Candida albicans* and of 51 isolates of *Candida glabrata* was included to compare the susceptibilities to amphotericin B. The MICs were determined by Etest with RPMI 1640 agar, and with both this medium and antibiotic medium 3 (AM3) agar for testing of amphotericin B.

**Results:** All isolates were highly susceptible to fluconazole. The susceptibility to itraconazole was good; only 4% of isolates had dose-dependent susceptibility (MICs 0.25–0.5 mg/L). Voriconazole was very active *in vitro* (100% of isolates were inhibited at ≤0.094 mg/L). Flucytosine MICs ranged widely (0.004–32 mg/L). The set included 19% of flucytosine-resistant isolates. For amphotericin B, 100% of isolates were inhibited at ≤0.75 mg/L (MIC50 0.047 mg/L; MIC90 0.19 mg/L) and at ≤4 mg/L (MIC50 0.25 mg/L; MIC90 0.75 mg/L) on RPMI and on AM3, respectively. A single isolate was categorized as resistant to amphotericin B (MIC 0.75 and 4 mg/L on RPMI and on AM3, respectively). Amphotericin B thus appeared very active *in vitro* against *C. lusitaniae*. Whatever the test medium, the level of susceptibility of *C. lusitaniae* to amphotericin B did not differ much from those of *C. albicans* and *C. glabrata*.

**Conclusion:** *C. lusitaniae* appears to be susceptible to amphotericin B, azole antifungal agents, and, to a lesser extent, flucytosine.

Keywords: *C. lusitaniae*, antifungals, susceptibility patterns, Etest

**Introduction**

Among the rare non-albicans *Candida* species, *Candida lusitaniae* has emerged during the last 20 years as an important nosocomial pathogen.1 From the first well documented case of infection in 1979, amphotericin B resistance has frequently been reported, in its different expressions: clinical resistance and microbiological (initial or acquired) resistance.1 This has largely contributed to the distinguishing of *C. lusitaniae* from other *Candida* species. In 1997, we provided the first evaluation of its *in vitro* antifungal susceptibility pattern.2 Since then, few studies have focused on this topic.3–6 To further determine the antifungal susceptibility pattern of *C. lusitaniae*, we report here the *in vitro* activities of four conventional systemic antifungal agents (amphotericin B, flucytosine, fluconazole and itraconazole) and of the new triazole voriconazole against a panel of clinical isolates, mostly colonizing ones. MICs were determined by the Etest, which is now well established as a reliable method for *in vitro* testing of antifungal agents, especially amphotericin B.7

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Materials and methods

The 80 isolates of *C. lusitaniae* were obtained from 80 patients hospitalized in five medical centres in France. The patients had not received antifungal prophylaxis or treatment. Isolates were recovered from upper respiratory tract (37 isolates), urine (16), stool (11), mouth (five), catheter (four), vagina (three), blood (two) and skin (two). A panel of 59 clinical isolates of *Candida albicans* and 51 isolates of *Candida glabrata* was also included. All isolates were identified to the species level by standard methods and the Auxacolor system (Bio-Rad, Marnes la Coquette, France). The MICs of amphotericin B, fluconazole, itraconazole, flucytosine and voriconazole were determined by Etest (AB Biodisk, Solna, Sweden) on RPMI 1640 agar (American Bioorganics, Buffalo, NY, USA) according to Etest technical guide no. 4. For amphotericin B, Etest was also performed on antibiotic medium 3 (AM3) (Difco Laboratories, Detroit, MI, USA) supplemented with 2% glucose and 1.5% agar (Difco). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality control strains, and *C. lusitaniae* ATCC 200951 was used as an amphotericin B-resistant reference strain. The inoculum suspensions were adjusted spectrophotometrically at 530 nm to match the turbidity of a 0.5 McFarland standard. Agar plates were inoculated with a cotton swab and allowed to dry for at least 15 min before the Etest strips were applied. Etest agar plates were incubated at 35°C and read at 48 h. The MIC interpretive criteria used for fluconazole, itraconazole and flucytosine were those published by the NCCLS.8 Interpretive breakpoints have not yet been established for voriconazole. For amphotericin B, we used a resistance breakpoint of ≥0.38 mg/L on RPMI and >1 mg/L on AM3.5,7

Results and discussion

MICs for the quality control strains were always within the described limits (data not shown).9 High MICs (1.5–2 mg/L and 3–12 mg/L for RPMI and AM3, respectively) were obtained for the amphotericin B-resistant reference strain, *C. lusitaniae* ATCC 200951. Table 1 summarizes the *in vitro* susceptibilities of 80 *C. lusitaniae* clinical isolates to fluconazole, itraconazole, voriconazole and flucytosine (5FC) as determined by the Etest method. As shown, all the isolates were highly susceptible to fluconazole. Voriconazole was also very active in vitro. The susceptibility to itraconazole was good; only 4% of isolates had dose-dependent susceptibility (MICs 0.25–0.5 mg/L). These results confirm and extend those previously reported regarding the excellent activity of the triazoles against *C. lusitaniae*.2,3,5 With regard to flucytosine, the MIC range was broad (0.004–>32 mg/L). The distribution of MICs was clearly bimodal, with 81% of isolates inhibited at ≤4 mg/L and 19% of isolates not inhibited at 32 mg/L. Hence, the set included a significant percentage of flucytosine-resistant isolates. These results confirm and extend those of our previous study.2 Among the rare studies that have addressed this issue recently, only a few reported, as we have, a high prevalence of isolates resistant to flucytosine (up to 50%).3–6 The substantial differences in susceptibility to flucytosine may be linked to the difference in the geographical origin of the isolates. This quite high level of resistance to flucytosine is important, since the drug is often used in combination with amphotericin B or with azoles as treatment for infection with this species.1 Susceptibility to amphotericin B was investigated by using the two agar media that are relevant with the Etest, i.e. RPMI 1640 and AM3, both supplemented with 2% glucose.5,7 As shown, on both media, MIC ranges were broad and AM3 agar generated an upward shift of the MICs (Table 2). A single isolate showed elevated MICs (0.75 and 4 mg/L on RPMI and AM3, respectively) and was clearly distinct.
from the other clinical isolates. It was thus easily categorized as resistant to amphotericin B with the interpretive breakpoints proposed above, i.e. ≥0.38 mg/L on RPMI 1640 and >1 mg/L on AM3.3,7 Note that the susceptibility status of this isolate has been confirmed by an analysis of the cell sterol composition.10 As previously reported7 with Etest on AM3, some C. lusitaniae isolates showed slightly elevated MICs (0.75–1 mg/L), and could have decreased susceptibility to amphotericin B. However, amphotericin B appeared very active in vitro against C. lusitaniae. These results confirm those of Pfaffer et al.3 who noted that primary resistance to amphotericin B was uncommon among incident isolates of C. lusitaniae. The amphotericin B susceptibility profile of the panel was further investigated by testing, for comparison, a set of C. albicans and C. glabrata colonizing isolates (Table 2). Like C. lusitaniae, C. glabrata has a diploid genome in which genetic alterations leading to resistance are more likely to be expressed than in diploid yeasts such as C. albicans. On RPMI 1640 agar, the in vitro susceptibility of C. lusitaniae to amphotericin B was similar to that of C. albicans, and C. lusitaniae appeared to be more susceptible to amphotericin B than C. glabrata (Table 2). Note that, whatever the species, MICs yielded on RPMI 1640 agar were overall lower than those usually reported.3,6 This could reflect brand-to-brand variability of the agar medium, and prevented the establishment of a reliable interpretive breakpoint for Candida spp. and amphotericin B. On AM3, overall, the distributions of amphotericin B MICs were highly similar for the three species. However, C. lusitaniae appears to be slightly less susceptible to amphotericin B than the two other species. Its susceptibility level is closer to that of C. glabrata, for which amphotericin B resistance was clearly detected in two isolates, on AM3 as well as on RPMI. Although the influence of both the test medium and the species makes it difficult to compare the levels of susceptibility to amphotericin B, it is obvious that C. lusitaniae is not as singular a species as was stated initially.1

In conclusion, the present study expands the database of MICs for C. lusitaniae, making its antifungal susceptibility pattern more explicit. C. lusitaniae appears susceptible to amphotericin B, azole antifungal agents and, to a lesser extent, flucytosine. However, this issue should be further investigated by testing large panels of geographically diverse clinical isolates and, of course, with new antifungal agents.

Acknowledgements

We are grateful to AB Biodisk for its support of this work.

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