

Susceptibility of Mexican isolates of yeasts and moulds to amphotericin B and triazole antifungals

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Abstract

Background: Resistance to antifungal drugs, especially towards triazoles, is commonly referred to by clinicians, but data on its prevalence in developing countries is limited.

Methodology: To determine the prevalence of triazole-resistance amongst pathogenic yeasts and moulds, we assessed the *in vitro* susceptibility of 250 isolates from hospitalized patients at five Mexican cities towards amphotericin B, fluconazole and voriconazole, by E-test.

Results: All yeasts were susceptible to voriconazole, according to E-test interpretive criteria (MIC \leq 1 $\mu\text{g/mL}$), and all filamentous or dimorphic fungi also had voriconazole MIC \leq 1 $\mu\text{g/mL}$, except for one isolate each of *Mucor* sp. and *Acremonium* sp. *Candida krusei* and one isolate of *C. glabrata* were resistant to fluconazole, a drug that had MIC \geq 192 $\mu\text{g/mL}$ for filamentous fungi. Although no breakpoints for amphotericin B are available, all three *C. krusei*, 2/25 *C. glabrata*, 3/22 *C. parapsilosis* and 1/108 *C. albicans* had MIC \geq 2 $\mu\text{g/mL}$.

Conclusion: *In vitro*, voriconazole is active against yeasts and moulds commonly causing severe mycoses in Mexico.

Keywords: *Candida* spp., moulds, *in vitro* susceptibility, azoles, amphotericin B

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Introduction

The prevalence and susceptibility of the etiological agents of fungal infections is, compared to bacterial infections, controversial and much less reported, especially in developing countries. Technical difficulties in detecting, and susceptibility testing of fungal etiological agents [1], as well as a wider diversity of fungi in warm and humid climates, make for an obscure picture of the therapeutic options against mycoses. The notion of resistance, particularly towards azole drugs, is widespread but almost always anecdotal, and physicians often use amphotericin B out of fear of resistance, despite the adverse effects of this drug.

A previous multicenter study (RedMic) provided information on the prevalence of yeasts and moulds causing nosocomial infections in several Mexican cities. A total of 455 isolates from 393 patients were analyzed: 56% were *Candida albicans*, 37% were non-*albicans Candidae* (of which 47% were *C. tropicalis*, 13% *C. parapsilosis*, 12% *C. glabrata*, 10% *C. krusei*) and 7% were moulds and dimorphic fungi (47% *Aspergillus* spp., 27% *Coccidioides immitis*)

[2]. Here we report the results of a second stage (RedMic2) of this study, with less focus on prevalence, but including susceptibility testing using the E-test.

Material and methods

Yeast and mould isolates

A total of 250 isolates from fungal infections considered to be hospital-acquired by attending physicians, or severe mycoses that required hospitalization, collected between July and November 2007, were tested. These included 108 isolates of *Candida albicans*, 43 of *C. tropicalis*, 25 of *C. glabrata*, 22 of *C. parapsilosis*, 24 of other yeasts (*C. krusei*, *Cryptococcus neoformans*, *Trichosporon asahii*); 15 of *Aspergillus* spp., 4 of *Coccidioides immitis*, 9 of other moulds or dimorphic fungi (*Mucor* spp., *Penicillium* spp., *Acremonium* spp., *Exserohilum rostratum*, *Geotrichum capitatum*). Organisms were first isolated and identified at 14 participating hospitals from five Mexican cities (Mexico City, Guadalajara, Monterrey, Torreon and Puebla), then sent to a central laboratory in agar

slants, where the identification of each isolate was reassessed by colony morphology and biochemical methods. Susceptibility testing was performed immediately after subculture, and a sample of each culture was stored in Mueller-Hinton broth supplemented with 25% glycerol, at -70°C for additional assays.

Antifungal susceptibility testing

Susceptibility towards fluconazole (an older, “narrow-spectrum” triazole drug), voriconazole (a newer, “wide-spectrum” triazole drug), and amphotericin B (as a reference drug used instead of azoles due to perceived resistance) was tested using E-test strips, following the manufacturer’s guidelines, on RPMI 1640 + 2% glucose agar (AB Biodisk), and incubated at 35°C for 24-48 hours. Strains ATCC 90028 (*C. albicans*) and ATCC 6258 (*C. krusei*) were used for quality control purposes, and results were within acceptable ranges. Agreement of E-test results and conventional broth dilution methods have been previously documented [3-4].

Results

Minimal inhibitory concentrations’ ranges, MIC₅₀ and MIC₉₀ values, are shown in Table 1; in order to reach MIC₅₀₋₉₀ values for most isolates, even those for which there were very few isolates, organisms that are clearly different (*e.g.*, *Aspergillus* spp. and *Penicillium* spp.) but share susceptibility profiles were grouped in the table. Wide variations in MIC values were found in *C. albicans* for all three drugs (31-47-fold between higher and lower MIC), and for voriconazole vs. *C. tropicalis* (32-fold), and fluconazole vs. *C. glabrata/C. krusei* (48-fold), *T. asahii* (32-fold) and *C. neoformans* (64-fold). One isolate of *G. capitatum* (not included in Table 1, and mistaken for *Candida* spp. at the hospital) had MICs of 1, 48 and 0.25 µg/mL for amphotericin B, fluconazole and voriconazole, respectively.

Discussion

Microbial resistance emerges and spreads as a natural consequence of the use of antimicrobial drugs. However, there are clear differences between the usage of antibacterial and antifungal drugs: the former are widely abused for both agricultural use and self-prescription in developing countries, while systemic polyenes and triazoles are not commonly used without medical prescription. Furthermore, the lack of extrachromosomal mobile genetic elements, a bacterial feature that seems responsible for most

clinically relevant resistance in bacteria, limits the spread of resistance among fungi to vertical transmission [6]. However, the growing incidence of fungal infections due to AIDS and other immunocompromising conditions, demands increased use of antifungal therapies that can foster resistance [7]. Nevertheless, the incidence of acquired resistance to polyenes is low and often linked to long-term therapy; fungistatic azoles, especially fluconazole, face a somewhat higher incidence of acquired resistance, while some yeasts (*e.g.*, *C. glabrata* and *C. krusei*) and filamentous fungi are intrinsically resistant [1,8]. For instance, a recent report of a worldwide surveillance that included more than 200,000 yeast isolates found that only 2.4% of 10,288 Latin American *C. albicans* isolates were resistant to fluconazole (1.5% global, 5.1% North America) [9]. Another study performed at a large Mexican city did not find resistance towards fluconazole among 337 isolates of *C. albicans*, *C. parapsilosis* and *C. tropicalis* [10].

According to E-test interpretive criteria, all of the *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates included here were susceptible to fluconazole (MIC ≤ 8 µg/mL) and to voriconazole (MIC ≤ 1 µg/mL). Voriconazole was fully-active against all *C. glabrata* and *C. krusei* isolates (as well as *T. asahii* and *C. neoformans*, extending *Candida* spp. interpretive criteria to these other yeasts), while only 5/25 *C. glabrata* isolates were susceptible to fluconazole, and 1/25 and all 3 *C. krusei* isolates were resistant to this drug. Although no breakpoints for amphotericin B are provided for E-test, taking resistance criteria for dilution methods, all three *C. krusei*, 2/25 *C. glabrata*, 3/22 *C. parapsilosis* and 1/108 *C. albicans* were resistant to this polyene (MIC ≥ 2 µg/mL; E-test results of 1.5 µg/mL were included).

No interpretive criteria are available for filamentous fungi; it is clear, however, that voriconazole is much more potent, *in vitro*, than fluconazole against moulds. Three isolates of *A. niger* were the less voriconazole-susceptible of these organisms (MIC 0.5-1 µg/mL), while other species of *Aspergillus*, *Penicillium* spp. and *E. rostratum* had much lower MICs (0.016-0.19 µg/mL). These results are comparable with those reported for *Aspergillus* spp. using the European Committee on Antimicrobial Susceptibility Testing (EUCAST); E-test is considered to yield results with good correlation to dilution methods [11]. When growing as mycelia, *C.*

Table 1. Minimal inhibitory concentrations of antifungal agents ($\mu\text{g/mL}$)

species (n)	amphotericin B			fluconazole			voriconazole		
	range	MIC ₅₀	MIC ₉₀	range	MIC ₅₀	MIC ₉₀	range	MIC ₅₀	MIC ₉₀
<i>C. albicans</i> (108)	0.032-1.5	0.38	0.75	0.25-8	1	3	0.012-0.38	0.047	0.094
<i>C. tropicalis</i> (43)	0.25-0.75	0.5	0.5	0.75-6	1.5	4	0.023-0.75	0.064	0.19
<i>C. parapsilosis</i> (22)	0.38-2	0.5	1.5	0.75-6	1	6	0.012-0.125	0.047	0.125
<i>C. glabrata</i> (25)	0.38-4	0.75	1.5	4-192	12	64	0.125-1	0.38	0.75
<i>C. krusei</i> (3)									
<i>T. asahii</i> (13)	0.19-0.75	0.25	0.75	4-128	4	8	0.094-1	0.125	0.25
<i>C. neoformans</i> (7)	0.125-0.5	-	-	4->256	-	-	0.064-0.094	-	-
<i>C. immitis</i> (4)	0.5-1.5	-	-	2-12	-	-	0.002-0.003	-	-
<i>Aspergillus</i> spp. (15)									
<i>E. rostratum</i> (4)	0.75-32	1.5	24	192->256	>256	>256	0.016-1	0.125	0.75
<i>Penicillium</i> spp. (2)									
<i>Mucor</i> spp. (2)									
<i>Acremonium</i> sp. (1)	3	-	-	>256	-	-	6-12	-	-

immitis showed the highest susceptibility to voriconazole; however, this observation is of dubious clinical relevance, as the infective form of *C. immitis* is most often a spherule. On the other hand, *Mucor* spp. and *Acremonium* spp. (species causing 3% of nosocomial mycoses, as found in the previous prevalence study), were not affected by triazole drugs. While fluconazole is active against the *Candida albicans/tropicalis/parapsilosis* group, and resistance towards amphotericin B is slowly emerging amongst this group, voriconazole is active against all yeasts tested, and apparently also against most filamentous fungi.

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