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In vitro activity of voriconazole, itraconazole, caspofungin, anidulafungin (VER002, LY303366) and amphotericin B against *aspergillus* spp

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Abstract

Voriconazole, anidulafungin (VER002, LY303366) and caspofungin are promising antifungal agents which provide a good protection against a variety of fungi, including yeasts and filamentous fungi. In this study, we tested the in vitro efficacy of voriconazole, itraconazole, caspofungin, anidulafungin (VER002, LY303366) and amphotericin B, against different species of *Aspergillus* spp. isolated from clinical specimens, using a microdilution broth method and following the NCCLS guidelines (document M38-P). We also evaluated the effect that time readings have on MIC results. For caspofungin, we determined the minimun effective concentration (MEC), defined like the lowest concentration of caspofungin causing abnormal hyphal growth. Anidulafungin (VER002, LY303366) was the most active antifungal agent tested with MIC_{90} of ≤0,03 mg/L. The activity of voriconazole, and itraconazole very similar with MIC_{90} of 0,12 mg/L, 0,12 mg/L respectively. For caspofungin the MEC_{90} was of 0,25 mg/L. Amphotericin B was the lest active antifungal agent studied with MIC_{90} of 1 mg/L. There were no differences between MIC values at 48 and 72 h. These data demonstrate promising activity of voriconazole, anidulafungin (VER002, LY303366) and caspofungin against *Apergillus* spp.

1. Introduction

The incidence of invasive aspergillosis, have increased considerably in the past few decades. This is mainly due to the fact that the number of immunocompromised patients has also increased considerably as a result of the following: the use of new and more aggressive therapies to treat solid tumors, myelomas, lymphomas and leukemia; the chronic use of corticosteroids; the increasing number of patients who undergo organ transplant; and, finally, the spread of AIDS (Denning, 1996, 1998). These infections are associated with significant morbidity and mortality despite therapy with amphotericin B, which remains the drug of choice (Patterson et al., 2000; Stevens et al., 2000). The problem of this drug is the high toxicity, so newer antifungal therapies with improved efficacy and reduced toxicity are needed to improve the treatment of invasive aspergillosis.

Voriconazole is a monotriazolic antifungal agent which is effective with a wide spectrum of fungi, including yeasts (Arikan et al., 1999; Barry & Brown, 1996; Chávez et al., 1999; Marco et al., 1998; Pfaller et al., 1999; Uzun et al., 2000) and filamentous fungi (Abraham et al., 1999; Cuenca-Estrella et al., 1998; Johnson et al.,1998; Manavathu et al., 2000; Pfaller et al., 2002; Radford et al., 1997; Verweij et al., 1998). Anidulafungin (VER002, LY303366) and caspofungin are antifungal drugs included in the echinocandine group which act as a non competitive inhibitor of the (1,3)-β-D-glucan synthetase, enzyme that produces glucan polymers, the main component of the cellular wall of many pathogenic fungi. These agents present an excellent efficacy against *Candida* spp. (Chávez et al., 1999; Cuenca-Estrella et al., 2000; Espinel-Ingroff, 1998; Pfaller et al., 1997; Uzun et al., 1997), *Aspergillus* spp. and other pathogenic fungi (Arikan et al., 2001; Espinel-Ingroff ,1998; Oakley et al., 1998; Pfaller et al., 1998; Zhanel et al., 1997). The aim of the present study is to evaluate the in vitro activity of these new antifungal agents (voriconazole, anidulafungin (VER002, LY303366), and caspofungin) and two others

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(amphotericin B and itraconazole) against 68 clinical isolates of *Aspergillus* spp. samples obtained from patients attended in a general hospital area.

2. Material and methods

2.1. Organisms

A total of 68 strains of *Aspergillus spp.* were evaluated. These included 28 *A. fumigatus,* 19 *A. flavus,* 9 *A. niger,* 8 *A. glaucus,* 2 *A. terreus* and 2 *A. flavipes.* They were all isolated from clinical specimens, received at the microbiology service of the University Hospital of Valme in Seville over a two years period. The identification of each strain was performed by using routine mycological techniques. The mold isolates were stored as spore suspensions in sterile distilled water with 25% glycerol at −80°C until the study was done. Before testing, each isolate was subcultured in Potato dextrose agar (PDA) (Difco, Detroit, Mich) to ensure its viability and purity.

2.2. Quality control

The two quality control strains recommended in the NCCLS document M27-A (NCCLS, 1997), *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 and *A.fumigatus* NCPF 7099 and NCPF 7100, were included in each run of the study.

2.2.1. Antifungal agents

In order to carry out the in vitro susceptibility test, the standard powders, with known potency, provided by the different drug manufacturers were used. Voriconazole (Pfizer Central Reseach, Sandwich, UK), itraconazole (Janssen Research Foundation, Beerse, Belgium), anidulafungin (VER002, LY303366) (Ely Lilly & Co., Versicolor, Ind), caspofungin (Merck Research Laboratories, Rahway, N.J.) and amphotericin B (Squibb, Madrid, Spain). All the drugs were dissolved in dimethyl sulfoxide (DMSO), except caspofungin which was disolved in sterile water, in order to obtain a 1.280 mg/L stock solution and were kept in 2 mL aliquots at −80°C.

2.3. Antifungal susceptibility testing

We used the microdilution broth method following the NCCLS (M38-P) guidelines for in vitro susceptibility (NCCLS, 1998), although for echinocandin and pneumocandin there's no standard susceptibility testing method established.

Each *Aspergillus* spp. strain, previously frozen, was subcultured in PDA for 7 days at 35°C.

Seven-day-old colonies were covered with approximately 1 mL of sterile 0.85% saline, and the suspensions were made by gently probing the colonies with the tip of a Pasteur pipette and we added one drop of Tween 20 to facilitate the preparation of the inocula. The resulting mixture of conidia and hyphal fragments was withdrawn and transferred to a sterile tube. After heavy particles were allowed to settle for 3 to 5 min, the upper homogeneous suspension was collected and mixed with a vortex mixer for 15 s. The densities of the conidial suspension were read and adjusted spectrophotometricaly to an optical density that all range from 0.09 to 0.11 (80 to 82% transmittance) for all *Aspergillus* species. These suspensions were diluted 1:50 in RPMI 1640 (Sigma, Spain) medium, yielded a double-strength inoculum containing 0.4×10^4 to 5×10^4 CFU/ml. Inoculum quantification was performed by plating 0.01 mL of a 1:100 dilution of the adjusted inoculum on modified Sabouraud glucose agar to determine the viable number of CFU per milliliter. The inoculum concentration for the two QC strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, was of 0,5-2,5 $\times 10^3$ CFU/ml prepared from a 24h old culture.

At the time of the study, the frozen drugs were exposed to room temperature and were later vigorously shaken in order to dissolve the remaining crystals. These drugs were diluted in a liquid medium RPMI 1640 supplemented with L-glutamine and MOPS (morpholineproponelsulfonic acid) buffer. The final concentration rates for each antifungal agent was 0.03-16 mg/L.

Each well was inoculated on the day of the test with 0.1 mL of the 2× conidial inoculum suspension. The growth control wells contain 0.1 mL of the corresponding diluted inoculum suspension and 0.1 mL of the drug diluent (2%) without antifungal agent.

2.4. MIC determinations

Microdilution trays were incubated at 35°C and examined at 24, 48 and 72 h for MICs determination. For voriconazole and itraconazole, the MIC was the lowest concentration showing prominent growth inhibition (approximately ≥50%); for anidulafungin (VER002, LY303366) (Krishnarao & Galgiani, 1997) and amphotericin B, the MIC was the lowest concentration showing 100% growth inhibition (NCCLS, 1998). For caspofungin, we determined the minimum effective concentration (MEC), defined like the lowest concentration of caspofungin causing abnormal hyphal growth with short abundant branchings observed microscopically. These values were coincident with the lowest concentration of drug producing a substantial reduction of growth and the presence of microcolonies (Arikan et al., 2001).

3. Results

Table 1 summarizes the MIC/MEC range, the MIC/MEC 50/90 and the geometric mean MIC/MEC for all the 68 *Aspergillus* spp. isolates to voriconazole, itraconazole,

Table 1
MICs(mg/L) values and geometric mean(GM) obtained at 48–72 hours.

Species	Antifungals	48 hours			72 hours		
		Range	MIC 50/90	GM	Range	MIC 50/90	GM
	Voriconazole	≤0.03–0.12	≤0.03/0.12	0.05	0.03–0.25	0.06/0.25	0.07
	Itraconazole	≤0.03–0.25	0.06/0.12	0.05	≤0.03–0.25	0.06/0.12	0.05
A.fumigatus (28)	Caspofungin**	≤0.03–0.25	0.06/0.25	0.08	—	—	—
	Anidalofungin	≤0.03	≤0.03	—	≤0.03	≤0.03	—
	Amphotericin	0.06–2	0.5/1	0.4	0.25–2	0.5/1	0.6
	Voriconazole	≤0.03–0.12	0.06/0.06	0.05	0.03–0.12	0.06/0.12	0.08
A.flavus (19)	Itraconazole	≤0.03–0.12	≤0.03/0.06	0.02	≤0.03–0.12	0.03/0.12	0.03
	Caspofungin**	≤0.03–0.25	0.06/0.12	0.05	—	—	—
	Anidalofungin	≤0.03	≤0.03	—	≤0.03	≤0.03	—
	Amphotericin	0.06–2	0.25/1	0.2	0.12–2	0.25/1	0.3
	Voriconazole	≤0.03–0.12	0.06/0.12	0.07	0.03–0.25	0.25/0.25	0.08
A.niger (9)	Itraconazole	≤0.03–0.25	0.06/0.25	0.06	≤0.03–0.25	0.06/0.25	0.05
	Caspofungin**	≤0.03–0.25	0.06/0.25	0.06	—	—	—
	Anidalofungin	≤0.03	≤0.03	—	≤0.03	≤0.03	—
	Amphotericin	0.12–0.5	0.12/0.5	0.2	0.06–1	0.25/1	0.2
	Voriconazole	0.06–0.12	0.06/0.06	0.08	0.03–0.12	0.06/0.12	0.08
A.glaucus (8)	Itraconazole	≤0.03–0.25	0.06/0.25	0.05	≤0.03–0.25	0.06/0.25	0.05
	Caspofungin**	≤0.03–0.12	0.12/0.12	0.08	—	—	—
	Anidalofungin	≤0.03	≤0.03	—	≤0.03	≤0.03	—
	Amphotericin	0.06–1	0.5/1	0.3	0.06–1	0.5/1	0.4
	Voriconazole	≤0.03–0.12	—	—	0.03–0.12	—	—
Aspergillus spp. (4)*	Itraconazole	≤0.03–0.12	—	—	≤0.03–0.12	—	—
	Caspofungin**	0.06–0.12	—	—	—	—	—
	Anidalofungin	≤0.03	—	—	≤0.03	—	—
	Amphotericin	0.12–1	—	—	0.25–1	—	—
	Voriconazole	≤0.03–0.25	0.06/0.12	0.05	0.03–0.25	0.06/0.12	0.07
All organisms (68)	Itraconazole	≤0.03–0.25	≤0.03/0.12	0.04	≤0.03–0.25	0.06/0.12	0.04
	Caspofungin**	≤0.03–0.25	≤0.03/0.25	0.07	—	—	—
	Anidalofungin	≤0.03	≤0.03	—	≤0.03	≤0.03	—
	Amphotericin	0.03–2	0.25/1	0.3	0.06–2	0.5/1	0.4

** Aspergillus spp.*: 2 *A.terreus*, 2 *A.flavipes*.
***Caspofungin dates are expressed in MEC (Minimal effective concentration)*

caspofungin, anidulafungin (VER002, LY303366) and amphotericin B, comparing the results obtained at 48 and 72 h of incubation. Because of the insufficient growth at 24 h of incubation to determine the end-points, these results were overlooked. MIC values tended to remain the same or increase only onefold at 48 h compared with the MIC at 72 h for most isolates and antifugal agents, so for the rest of the study, we are going to consider the results obtained at 48 h of incubation.

Anidulafungin (VER002, LY303366), was the most powerful antifungal agent showing MIC values of ≤ 0,03 mg/L in all the strains studied.

Voriconazole was as active as itraconazole for *A.fumigatus* and *A.flavus*. For the other species, voriconazole showed $MICs_{90}$ lower than itraconazole (0,12 mg/L vs 0,25 mg/L for *A.niger* and 0,06 mg/L vs 0,25 mg/L for *A.glaucus*).

Although the GM for voriconazole, itraconazole and caspofungin were very similar, caspofungin results can't be comparable with those obtained with the other two antifungal agents since different parameters were employed to read the results (MEC vs MIC).

Amphotericin B was the antifungal that showed the lowest activity, with MIC_{90} of 1 mg/L for *A.fumigatus, A.flavus* and *A.glaucus* and of 0,5 mg/L for *A.niger*.

4. Discussion

Voriconazole, anidulafungin (VER002, LY303366) and caspofungin have been proven to be powerful antifungal agents which provide protection against a wide spectrum of fungi (Abraham et al., 1999; Arikan et al., 1999, 2001; Chávez et al., 1999). Voriconazole has been reported to have both fungistatic and fungicidal activity against *Candida* and *Cryptococcus* spp. and most fungi. Its efficacy against *Aspergillus* spp. has been recently demonstrated both in vitro and in vivo in laboratory tests with animals (Chandrasekar et al., 2000; Kirkpatrick et al., 2000; Murphy et al., 1997) and in humans (Denning, 2002). However, although anidulafungin (VER002, LY303366) and caspofungin show a good in vitro efficacy rate against *Candida* spp., *Pneumocystis carinii*, and filamentous fungi, including *Aspergillus* spp., these agents did not provide any protection against *Cryptococcus neoformans*.

Our in vitro studies with anidulafungin (VER002, LY303366) have shown lower MIC_{90} values ($\leq$0.03 mg/L), against *Aspergillus* spp. than voriconazole (0,12 mg/L), itraconazole (0,12 mg/L) and amphotericin B (1 mg/L). These results also been found by other authors (Espinel Ingroff, 1998; Oakley et al., 1998; Pfaller et al., 1998; Zhanel et al., 1997). We did not observe differences between species, only Oakley et al. (Oakley et al., 1998) showed MIC_{90} of 1 mg/L for *A.flavus.* At the moment, there are no many studies of the new antifungal agent, caspofungin, against Aspergillus isolates, our results (MEC 0.25 mg/L) are very similar to those observed by Pfaller et al. (Pfaller et al., 1998) (MIC_{90} of 0,25 mg/L vs 0,12 mg/L for *A.fumigatus* and MIC_{90} of 0,12 mg/L vs 0,12 mg/L for *A.flavus*) and Del Poeta et al. (Del Poeta et al., 1997), (GM of 0.08 mg/L vs $\leq$0.09 mg/L for *A.fumigatus*) but lower than those observed by Espinel Ingroff and Arikan et al. (Arikan et al., 2001; Espinel Ingroff, 1998), with GM of 0,08 mg/L vs 2,15 mg/L and 0,3 mg/L for *A. fumigatus* and GM of 0,05 mg/L vs 0,5 mg/L and 0,3 mg/L for *A.flavus.* For this specie, Del Poeta et al. (Del Poeta et al., 1997), showed GM higher than ours too (GM 0,20 mg/L vs 0,05 mg/L).

Voriconazole showed a similar activity to all *Aspergillus* spp., with $CMI_{50/90}$ values of 0,06 mg/L and 0,12 mg/L. These values were lower than those obtained in other previous studies in which a large number of *Aspergillus* spp. isolates were included (Abraham et al., 1999; Arikan et al., 1999; Cuenca-Estrella et al., 1998; Johnson et al., 1998; Manavathu et al., 2000, 2001; Murphy et al., 1997; Pfaller et al., 2002; Verweij et al., 1998), but if we have a look at the MIC values at 72 h of incubation, we see that there was no more than one or twofold of differences. If we compare the activity of both azoles studied, we found that there was no more than onefold up or down of differences. The same values have seen by other authors (Abraham et al., 1999; Arikan et al., 1999; Cuenca-Estrella et al., 1998; Johnson et al., 1998; Manavathu et al., 2000, 2001; Murphy et al., 1997; Pfaller et al., 2002; Verweij et al., 1998). In all these studies, *A.niger* showed the highest MIC_{90} of all the strains studied. Although in our study we did not find any case of resistance to itraconazole, it has been demonstrated that the strains which are resistant to itraconazole tend to be susceptible to voriconazole (Abraham et al., 1999; Manavathu et al., 2000).

When we analyzed the results taking the reading times into account, we found that there were no differences between MIC values at 48 and 72 h like in other studies (Arikan et al., 1999; Verweij et al., 1998). Therefore, we can conclude that MIC values could be read after 48 h of incubation.

Today, the most common drugs of choice to treat invasive aspergillosis is amphotericin B. The lipid formulation are a good alternative treatment as they can prevent the nephrotoxocity caused by amphotericin deoxycholate. The other problem of amphotericin B is the rising of resistance, so clearly there is a need for alternative antifungal agents to erradicate these serious infections. In this study we observed that voriconazole, anidalofungin (VER002, LY303366) and caspofungin present low MICs and MECs respectively, but this don't mean a guarantee to consider them a good alternative treatment of the invasive aspergillosis infections, pharmacokinetic, pharmacodinamic and host factors are full key players too. Apart from their proven in vitro and in vivo efficacy, they also present low toxicity and good tolerability. However, although the level of efficacy of these antifungal agents is fairly proven, we believe that more research, especially in vivo, needs to be carried out in this field.

References

Abraham, O. C., Manavathu, E. K., Cutright, J. L., & Chandrasekar, P. H. (1999). In vitro susceptibilities of *Aspergillus* species to voriconazole, itraconazole, and amphotericin B. *Diagn Microbiol Infect Dis*, *33*, 7–11.

Arikan, S., Lozano-Chiu, M., Paetznick, V., Nangia, S., & Rex, J. H. (1999). Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against isolates of *Aspergillus* and *Fusarium* species. *J Clin Microbiol*, *37*, 3946–3951.

Arikan, S., Lozano-Chiu, M., Paetznick, V., & Rex, J. H. (2001). In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. *Antimicrob Agents Chemother*, *45*, 327–330.

Barry, A. L., & Brown, S. D. (1996). In vitro studies of two triazole antifungal agents (voriconazole [UK-109,496] and fluconazole) against *Candida* species. *Antimicrob Agents Chemother*, *40*, 1948–1949.

Chandrasekar, P. H., Cutright, J., & Manavathu, E. (2000). Efficacy of voriconazole against invasive pulmonary aspergillosis in a guinea-pig model. *J Antimicrob Chemother*, *45*, 673–676.

Chávez, M., Bernal, S., Valverde, A., Gutierrez, M. J., Quindos, G., & Martín Mazuelos, E. (1999). In-vitro activity of voriconazole (UK-109,496), LY303366 and other antifungal agents oral *Candida spp*. Isolates from HIV-infected patients. *J Antimicrob Chemother*, *44*, 697–700.

Cuenca-Estrella, M., Rodriguez-Tudela, J. L., Mellado, E., Martinez-Suarez, J. V., & Monzon, A. (1998). Comparison of the in-vitro activity of voriconazole (UK-109,496), itraconazole and amphotericin B against clinical isolates of *Aspergillus fumigatus*. *J Antimicrob Chemother*, *42*, 531–533.

Cuenca-Estrella, M., Mellado, E., Diaz-Guerra, T. M., Monzon, A., & Rodríguez-Tudela, J. L. (2000). Susceptibility of fluconazole-resistant clinical isolates of *Candida spp.* to echinocandin LY303366, itraconazole and amphotericin B. *J Antimicrob Chemother*, *46*, 475–477.

Del Poeta, M., Schell, W. A., & Perfect, J. R. (1997). In vitro antifungal activity of pneimocandin L-743,872 against a variety of clinically important molds. *Antimicrob Agents Chemother*, *41*, 1835–1836.

Denning, D. W. (1996). Therapeutic outcome invasive aspergillosis. *Clin Infect Dis*, *23*, 608–615.

Denning, D. W., del Favero, A., Gluckman, E., Rhunke, M., Yonren, S., Troke, P., & Sarantis, N. (2002). The efficacy and tolerability of UK 109,496 (voriconazole) in the treatment of invasive aspergillosis. *Clin Infect Dis*, *34*, 563–571.

Denning, D. W. (1998). Invasive aspergillosis. *Clin Infect Dis*, *26*, 781–805.

Espinel-Ingroff, A. (1998). Comparison of in vitro activities of the new triazole SCH56592 and the echinocandins MK-0991(743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. *J Clin Microbiol*, *36*, 2950–2956.

Johnson, E. M., Szekely, A., & Warnock, D. W. (1998). In-vitro activity of voriconazole, itraconazole and amphotericin B against filamentous fungi. *J Antimicrob Chemother*, *42*, 741–745.

Kirkpatrick, W. R., McAtee, R. K., Fothergill, A. W., Rinaldi, M. G., & Patterson, T. F. (2000). Efficacy of voriconazole in a guinea pig model of disseminated invasive aspergillosis. *Antimicrob Agents Chemother*, *44*, 2865–2868.

Krishnarao, T. V., & Galgiani, J. N. (1997). Comparison of the in vitro activities of echinocandin LY303366, the pneumocandin MK-0991, and fluconazole against *Candida* species and *Cryptococcus neoformans*. *Antimicrob Agents Chemother*, *41*, 1957–1960.

Manavathu, E. K., Cutright, J. L., Loebenberg, D., & Chandrasekar, P. H. (2000). A comparative study of the in vitro susceptibilities of clinical and laboratory-selected resistant isolates of *Aspergillus spp.* To amphotericin B, itraconazole, voriconazole and posaconazole(SCH 56592). *J Antimicrob Chemother*, *42*, 229–234.

Manavathu, E. K., Abraham, O. C., & Chandrasekar, P. H. (2001). Isolation and in vitro susceptibility to amphotericine B, itraconazole and posaconazole of voriconazole-resistant laboartory isolates of *Aspergillus fumigatus*. *Clin Microbiol Infect*, *7*, 130–137.

Marco, F., Pfaller, M. A., Messer, S., & Jones, R. N. (1998). In vitro activities of voriconazole (UK-109,496) and four other antifungal agents against 394 clinical isolates of *Candida spp*. *Antimicrob Agents Chemother*, *42*, 161–163.

Murphy, M., Bernard, E. M., Ishimaru, T., & Armstrong, D. (1997). Activity of Voriconazole (UK-109,496) against clinical isolates of *Aspergillus* species an its effectiveness in an experimental model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother*, *41*, 696–698.

National Committee for Clinical Laboratory Standards. (1997). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast-Approved Standard. M27A. NCCLS, Villanova, PA.

National Comittee for Clinical Laboratory Standards (1998). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi; Proposed Standard. NCCLS document M38-P.

Oakley, K. L., Moore, C. B., & Denning, D. W. (1998). In vitro activity of the echinocandin antifungal agent LY303,366 in comparison with itraconazole and amphotericin B against *Aspergillus spp*. *Antimicrob Agents Chemother*, *42*, 2726–2730.

Patterson, T. F., Kirkpatrick, W. R., White, M., Hiemenz, J. W., Wingard, J. R., Dupont, B., Rinaldi, M. G., Stevens, D. A., & Graybill, J. R. (2000). Invasive aspergillosis: Disease spectrum, treatment practices, and outcomes. *Medicine*, *79*, 250–260.

Pfaller, M. A., Messer, S. A., & Coffman, S. (1997). In vitro susceptibilities of clinical yeast isolates to a new echinocandin derivative, LY303366, and other antifungal agents. *Antimicrob Agents Chemother*, *41*, 763–766.

Pfaller, M. A., Marco, F., Mecer, S. A., & Jones, R. N. (1998). In vitro activity of two equinocandin derivatives, LY303366 and MK-0991 (L-743,792), against clinical isolates of Aspergillus, Fusarium, Rhizopus, and other filamentous fungi. *Diagn Microbiol Infect Dis*, *30*, 251–255.

Pfaller, M. A., Jones, R. N., Doern, G. V., et al. (1999). International surveillance of bloodstream infections due to *Candida* species in the European SENTRY Program: Species distribution and antifungal susceptibility including the investigational triazole and echinocandin agents. *Diagn Microbiol Infect Dis*, *35*, 19–25.

Pfaller, M. A., Messer, S. A., Hollis, R. J., & Jones, R. N., & The Sentry Participants Group. (2002). Antifungal activities of posaconazole, ravuconazole and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: Report from SENTRY antimicrobial surveillance program, 2000. *Antimicrob Agents Chemother*, *46*, 1032–1037.

Radford, S. A., Johnson, E. M., & Warnock, D. W. (1997). In vitro studies of activity of voriconazole (UK-109,496), a new triazole antifungal agent, against emerging and less-common mold pathogens. *Antimicrob Agents Chemother*, *41*, 841–843.

Stevens, D. A., Kan, V. L., Judson, M. A., Morrison, V. A., Dummer, S., Denning, D. W., Bennett, J. E., Walsh, T. J., Patterson, T. F., & Pankey, G. A. (2000). Practice guidelines for diseases caused by *Aspergillus*. *Clin Infect Dis*, *30*, 696–709.

Uzun, O., Kocagoz, S., Cetinkaya, Y., Arikan, S., & Unal, S. (1997). In vitro activity of a new echinocandin, LY303366, compared with tose of amphotericin B an fluconazole against clinical yeast isolates. *Antimicrob Agents Chemother*, *41*, 1156–1157.

Uzun, O., Arikan, S., Kocagoz, S., Sancak, B., & Unal, S. (2000). Susceptibility testing of voriconazole, fluconazole, itraconazole and amphotericin B against yeast isolates in a Turkish University Hospital and effect of time of reading. *Diagn Microbiol Infect Dis*, *38*, 101–107.

Verweij, P. E., Mensink, M., Rijs, A. J., Donnelly, J. P., Meis, J. F., & Denning, D. W. (1998). In-vitro activities of amphotericin B, itraconazole and voriconazole against 150 clinical and environmental *Aspergillus fumigatus* isolates. *J Antimicrob Chemother*, *42*, 389–392.

Zhanel, G. G., Karlowsky, J. A., Harding, G. A. J., Balko, T. V., Zelenitsky, S. A., Friesen, M., Kabani, A., Turik, M., & Hoban, D. J. (1997). In vitro activity of a new semisynthetic echinocandin, LY-303366, against systemic isolates of *Candida* species, *Cryptococcus neoformans, Blastomyces dermatitidis*, and *Aspergillus* species. *Antimicrob Agents Chemother*, *41*, 863–865.