Comparison of Sensititre YeastOne® with the NCCLS M38-A microdilution method to determine the activity of amphotericin B, voriconazole, and itraconazole against clinical isolates of

Aspergillus fumigatus

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Abstract

The in vitro activities of amphotericin B, itraconazole, and voriconazole against 279 clinical isolates of Aspergillus fumigatus were studied by comparing Sensititre YeastOne® with the reference method NCCLS (CLSI) M38-A. The methods were considered to agree when the results of the MICs by Sensititre were within 2 dilutions of the MICs obtained by NCCLS M38-A. Agreement of readings at 24, 48, and 72 h was as follows: amphotericin B (0%, 31.5%, 82.1%), itraconazole (86.7%, 98.3%, 98.2%), and voriconazole (92.8%, 98.3%, 98.9%). Sensititre YeastOne® is a suitable alternative for the determination of the MICs of itraconazole and voriconazole against A. fumigatus when results are read at 48 h of incubation.

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1. Introduction

Aspergillus fumigatus is the main etiologic agent of invasive aspergillosis (Bouza et al., 2005), an increasingly prevalent disease with high mortality (Denning, 1998; Groll et al., 1996).

In spite of the low rates of resistance of A. fumigatus to azoles and amphotericin B (Guinea et al., 2005), the most commonly used drugs for antifungal therapy, evidence of in vitro activity is important to detect increases in resistance and to select agents for antifungal therapy in special cases.

The NCCLS (CLSI) M38-A procedure is the gold standard for antifungal susceptibility testing in filamentous fungi (NCCLS, 2002). There are alternatives to the NCCLS method, such as Sensititre YeastOne®, a commercial colorimetric method. Sensititre YeastOne® panels are dried and have a 2-year shelf life at room temperature.

Our study compares the in vitro activity of amphotericin B, voriconazole, and itraconazole against A. fumigatus measured by the Sensititre YeastOne® and the NCCLS M38-A procedure.

2. Organisms

We analyzed 279 clinical strains of A. fumigatus from our patients hospitalized in the following units: high-risk units, including intensive care units and hematology wards (36.9%); internal medicine (49.2%); surgery (10.8%); and low-risk units, including psychiatry and obstetrics (3.1%). Our institution cares for all types of patients at risk of acquiring invasive aspergillosis, including solid organ and bone marrow transplant recipients and patients with hematological malignancies or HIV infection.

All strains were cultured on Sabouraud dextrose agar and identified by conventional methods. They were stored as spore suspensions in a solution of sterile distilled water with
glycerol (10%) at −70 °C. Before being tested, each isolate was subcultured on potato dextrose agar to ensure viability and purity.

3. Microdilution method of susceptibility testing

The antifungal drugs used in the study were obtained as reagent-grade powders: amphotericin B (Sigma Chemical, St. Louis, MO), itraconazole (Janssen Pharmaceutical Research and Development, Madrid, Spain), and voriconazole (Pfizer Pharmaceutical Group, New York, NY). The broth microdilution method was performed according to NCCLS guidelines (NCCLS, 2002). Stock solutions of amphotericin B, itraconazole, and voriconazole were prepared in dimethyl sulfoxide (Sigma, Madrid) and diluted 100 times to their final concentration, further diluted in Roswell Park Memorial Institute (RPMI) 1640 medium buffered to pH 7.0 with morpholinepropanesulfonic acid buffer, and dispensed into 96-well microdilution trays. The trays, which contained a 0.1-mL aliquot of the appropriate drug solution (2×final concentration) in each well, were sealed and stored at −70 °C until used. The final concentration of the drugs in the wells ranged from 0.03 to 16 µg/mL. We prepared adjusted suspensions of spores in saline solution using a spectrophotometer with an optical density of 0.15 to 0.17. We then performed a 1:50 dilution in RPMI 1640 to a final concentration of 0.4 × 10⁴ to 5 × 10⁴ colony-forming unit/mL and placed the suspension in the microdilution wells. The inoculated microdilution trays were incubated at 35 °C and read after 48 h. The MIC end point for the azoles and amphotericin B was defined as the lowest concentration that produced complete inhibition of growth.

4. Sensititre YeastOne® method for susceptibility testing

Sensititre YeastOne panels (Trek Diagnostic Systems, East Grinstead, UK) containing serial 2-fold dilutions of the drug (0.008–16 µg/mL) were used. Inoculum suspensions were prepared in the same way for the M38-A method, and the adjusted suspensions were diluted 1:100 in YeastOne RPMI medium. The dried Sensititre YeastOne panels were rehydrated, and 100 µL of the working suspension was added to each well. The panels were covered with sealing strips, incubated at 35 °C, and read after 24, 48, and 72 h.

5. Quality control

Quality control was ensured by testing the following strains: Aspergillus flavus ATCC 204304 and A. fumigatus ATCC 204305. (MICs were always within the range of the NCCLS M38-A procedure). All results were within the recommended limits of the NCCLS procedure.

6. Data analysis

The MIC distributions for the 2 azoles and amphotericin B against each clinical isolate of A. fumigatus were converted to log MICs. We compared the results of Sensititre YeastOne® after 24, 48, and 72 h with the NCCLS results. Both methods were considered to agree when the results of the MICs by Sensititre were within 2 dilutions of the MICs obtained by NCCLS M38-A (Castro et al., 2004).

Table 1 shows the activity of amphotericin B, itraconazole, and voriconazole against the 279 clinical isolates. Voriconazole was the most active antifungal drug tested, followed by...
itraconazole and amphotericin B. The table also shows the different MICs obtained using Sensititre after 24, 48, and 72 h of incubation. The MIC90’s achieved using the reference method were higher than those obtained by Sensititre.

The percentage of strains with Sensititre MICs, which agreed with those of the NCCLS at 24, 48, and 72 h of incubation, is shown in Table 2.

Overall, 6 strains were classified as amphotericin B “resistant” (MIC >2 µg/mL) by the reference method (Lass-Flori et al., 1998), but Sensititre classified only 1 of these 6 strains as “resistant” after 72 h of incubation; the remaining 273 strains were classified as amphotericin B “susceptible” by both methods. According to the previously established breakpoints, none of the strains were classified as azole resistant by both methods (Denning et al., 1997a, 1997b; Espinel-Ingroff et al., 2001).

Our study shows that the Sensititre YeastOne® procedure compares well with the NCCLS procedure for the determination of MICs against A. fumigatus of voriconazole and itraconazole when the determinations were read at 48 h. The correlation for amphotericin B is much weaker.

The main disadvantage of the M38-A procedure is that the preparation of trays is time consuming. Therefore, methodologically easier systems, such as Sensititre YeastOne, are necessary. Other studies comparing Sensititre YeastOne and M38-A showed that the 2 procedures agreed, but they were performed with a lower number of A. fumigatus clinical isolates (<30 isolates) (Castro et al., 2004; Martin-Mazuelos et al., 2003; Meletiadis et al., 2002). We compared the Sensititre YeastOne® method with the NCCLS M38-A procedure using 279 clinical isolates. The results presented here are from the largest series of A. fumigatus isolates tested using both methods.

Our study shows that Sensititre YeastOne® is not a good method for in vitro detection of resistance to amphotericin B in A. fumigatus. A potential shortcoming of our study is the lack of azole-resistant strains of A. fumigatus. We did not find resistance to azoles either in our series or in a recent study on antifungal susceptibility testing of 596 strains of A. fumigatus (Guinea et al., 2005). The very low incidence of the resistant strains makes it difficult to assess the capability of Sensititre YeastOne® to detect azole resistance.

In conclusion, Sensititre YeastOne® is a good alternative method for testing the antifungal susceptibility of A. fumigatus to itraconazole and voriconazole. Amphotericin B showed a poor correlation, and Sensititre failed to detect amphotericin-resistant strains.

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