Short Communication

Susceptibility to amphotericin B of Candida spp. strains isolated in Ceará, Northeastern Brazil

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ABSTRACT

Introduction: Amphotericin B (AMB) is an antifungal agent used extensively in clinical medicine, yet resistance remains low. This study aims to evaluate the susceptibility of Candida spp. against AMB. Methods: For broth microdilution susceptibility testing, 77 strains of Candida spp. were selected (32 C. albicans, 33 C. tropicalis, and 12 C. parapsilosis). The strains were considered susceptible when they exhibited MIC≤1.0µg/ml. Results: None of the strains showed an MIC greater than 0.25µg/ml. Conclusions: Further works are necessary, with a higher number of strains, to assess the validity of the results used in this study.

Keywords: Amphotericin B. Candida spp. Protocol M27-A3.

Amphotericin B (AMB) is a polycylic macrolide antifungal agent, isolated from Streptomyces nodosus, collected in 1955 from the Orinoco river delta in Venezuela. AMB was quickly introduced in clinical medicine, receiving approval from the Food and Drug Administration (FDA) in 1958, even without the elucidation of its chemical structure. AMB has been and still remains the gold standard for the treatment of IFIs. It presents a broad spectrum of action and low incidence of fungal resistance after a half century of clinical use. Its primary drawback is nephrotoxicity[1,2].

Clinical and Laboratory Standards Institute (CLSI) developed a document in 2006, classified as M27-A2, which describes the ranges and breakpoints of the antifungal agents used to treat invasive fungal infections (IFIs) caused by Candida spp. and Cryptococcus spp.; there is nothing for the other fungal species. For AMB, the document defined a range from 0.03 to 16µg/ml; breakpoints were not defined. In 2008, a new document was published, M27-A3, which did not contain the breakpoints for AMB. The supplement to that document, M27-S3, also includes nothing on the breakpoints, which makes it difficult to assess AMB-resistant strains[3,4]. This study aims to assess the susceptibility to AMB of Candida spp. strains isolated in State of Ceará.

A total of 77 strains of Candida spp. were selected (32 C. albicans, 33 C. tropicalis, and 12 C. parapsilosis); these are the strains most commonly isolated from fungal infections in State of Ceará. The strains were isolated from blood, urine, respiratory tract, and catheter tip between 2009 and 2010 and are part of the collection of yeasts of the Laboratory of Bioprospecting of Megamicrodiversity of Ceará, College of Pharmacy, Federal University of Ceará (LAMBIC/FF/UFC). The strains were inoculated on potato agar (Himedia Mumbai, India) and incubated at 37°C/24 h. They were then plated on CHROMagar Candida (Himedia Mumbai, India) to assess purity. Identification was done by micromorphology on rice agar Tween 80, germ tube production, fermentation, and assimilation of carbohydrates, as well as molecular tests[5,6].

The broth microdilution (BMD) susceptibility testing was performed according to document M27-A3 using the RPMI broth (Cultilab São Paulo, Brazil) (pH 7.0) buffered with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma, USA) to assess susceptibility to amphotericin B. The AMB (Inlab, São Paulo) was dissolved in DMSO and diluted in RPMI, and the range tested was 0.03-16µg/ml. The plates were incubated for 24h and 48h, and the results were read visually as recommended by the CLSI. For AMB, the minimum inhibitory concentration (MIC) was considered as the concentration that inhibits 100% of fungal growth. The CLSI has not determined breakpoints for AMB; the strains were considered susceptible when they had MIC≤1.0µg/ml. The strains C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used as controls[4,7,9].

Table 1 shows the distribution of Candida spp. strains according to the MICs for the periods tested. None of the strains showed MIC greater than 0.25µg/ml in any of the times tested. The numbers in bold type describe the total number of strains with the same MIC. As can be seen, all strains were susceptible to AMB, and no resistance was detected. The table allows for the proposal of new ranges to be tested for AMB. As no strains with MIC≥1 were detected, we propose that the new range for AMB vary from 0.03 to 2µg/ml. The breakpoint adopted in our study has been described by other authors who consider all strains with MIC≤1.0 to be susceptible strains.
Antifungal susceptibility testing has been a notable advance in the treatment of fungal infections and is the primary tool in the application of appropriate antifungal therapy. Treatment of IFIs requires knowledge of the epidemiology of the fungal species involved and should be done in accordance with the identification of species associated with the result of the antifungal susceptibility testing. This testing is laborious, requiring trained personnel, and is only available for a few fungi10-12.

The standardization of susceptibility testing for fungi is still a hotly debated topic. In the past decade, several consensuses have been obtained. However, the ranges of the drugs and the breakpoints are still under discussion4,13. In a study of 5,821 strains of Candida spp., the range tested was from 0.06 to 8µg/mL, and the results were read after 24h. No breakpoints were determined, and no resistance to AMB was detected; none of the strains exhibited MIC≥2µg/ml14. In another study of 1,000 strains of Candida spp. isolated in Brazil - in which arbitrary breakpoint values were adopted - no strains showed MIC≥2 µg/ml for AMB15. In our study, all strains exhibited 24-h MIC of no more than 0.12µg/ml (Table 1). Our results agree with those found for a higher number of strains.

For AMB, the breakpoint has not been defined, and the test range recommended by the CLSI is 0.03 to 16µg/ml. Studies show that the MICs of susceptible strains are no more than 1µg/ml, and it is unnecessary to use such a broad test range7-9. In conclusion, our study has provided additional information on an extremely relevant topic. Further work is necessary, with a higher number of strains, to assess the validity of the ranges and breakpoints used in this study.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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### REFERENCES