SHORT COMMUNICATION/COURTE COMMUNICATION

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

Sensibililité à la caspofungine de souches de Candida sp. isolées en Ceará, Nord-est Brésilien

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Received 1 August 2011; received in revised form 15 September 2011; accepted 23 September 2011
Available online 9 November 2011

KEYWORDS
Caspofungin; Candida spp.; Microdilution; Susceptibility tests

Summary Caspofungin is an echinocandin prescribed for the treatment of invasive fungal infections caused by Candida spp. and Aspergillus spp. The aim of this study is to assess the degree of susceptibility of Candida spp., isolated from blood cultures in the state of Ceará (Brazil) to caspofungin by the broth microdilution method. Thirty-three strains of Candida spp. were selected for the test (seven C. albicans, nine C. tropicalis and 17 C. parapsilosis); these strains are the most commonly isolates of fungal infections in Ceará. The results of susceptibility tests by broth microdilution can be read at 24 or 48 hours after testing, without compromising test interpretations. C. parapsilosis exhibited the highest MICs when compared with the MICs of caspofungin against C. albicans and C. tropicalis.

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MOTS CLÉS Caspofungine ; Candida sp. ;

Résumé La caspofungine est une échinocandine prescrite pour le traitement des infections fongiques invasives causées par Candida sp. et Aspergillus sp. Le but de cette étude est d’évaluer le degré de sensibilité à la caspofungine de Candida sp., isolé à partir d’hémocultures dans l’état du Ceará (Brésil) par la méthode de microdilution. Trente-trois souches de Candida sp. ont été
Microdilution ;
Tests de sensibilité

sélectionnées pour le test (sept C. albicans, neuf C. tropicalis et 17 C. parapsilosis) ; ces souches sont le plus souvent isolées dans des infections fongiques en Ceará. Les résultats des tests de sensibilité par microdilution peuvent être lus à 24 ou 48 heures après les tests, sans compromettre les interprétations du test. C. parapsilosis montre les CIM les plus élevées comparative-ment à celles de C. albicans et C. tropicalis pour la caspofungine.

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Introduction

Candida albicans is the species most commonly isolated from patients with invasive fungal infections (IFIs); however, C. tropicalis and C. parapsilosis are increasingly prevalent and are the principal species isolated in Brazil [8,14,22,16].

Echinocandins are the newest class of antifungal drugs approved by the Food and Drug Administration (FDA) for the treatment of systemic fungal infections. This was the first class of antifungal agents to exhibit action on the cell wall, by inhibiting the enzyme (1,3) beta-glucan synthase. Echinocandin exhibit a limited spectrum of action compared to azoles. However, they are excellent agents for the treatment of candidiasis. The primary constituents of this group are: caspofungin, micafungin and anidulafungin [11,3,5,9,18,12].

There is no in vitro susceptibility testing with echinocandins against Candida spp. isolated from blood cultures in Ceará. The aim of this study is to evaluate the susceptibility of strains of C. albicans, C. tropicalis and C. parapsilosis against caspofungin by the broth microdilution method, and to compare the effectiveness of caspofungin against these strains.

Materials and methods

Isolation and identification of fungal strains

We used 33 strains of Candida spp. (seven C. albicans, nine C. tropicalis and 17 C. parapsilosis), the strains were isolated from blood cultures, between 2009 and 2010, and are part of the Collection of Yeasts of the Laboratory of Bioprospection and Experiments in Yeast (LABEL), Department of Clinical and Toxicological Analysis, College of Pharmacy, Federal University of Ceará. 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 [7,2].

Susceptibility testing

Caspofungin (Sigma, St Louis, MO) was prepared in water, and diluted in RPMI 1640 medium (Sigma, St. Louis, MO) buffered to pH 7.0 with 0.165 M MOPS (morpholinopropanesulfonic acid) buffer (Sigma). The microdilution testing was performed in accordance with the guidelines in CLSI document M27-A3 (CLSI 2008). MICs were determined visually, after 24 h and 48 h of incubation, as the lowest concentration in microgram per millilitre of drug that caused a significant diminution of 50% and 90% inhibition of growth below control levels. Quality control was performed by strains C. krusei ATCC 6258 and C. parapsilosis ATCC 22019.

Results and discussion

Treatment of systemic mycoses is one of the foremost problems in the field of medical mycology; these infections affect patients with predisposing factors. Such pathologies are difficult to detect and the delay in diagnosing them is the reason for high mortality. The antifungal agents available today often show secondary and side effects because their therapeutic margin is narrow, which limits usage of such agents [20]. Testing for susceptibility to antifungal agents has become very important due to the emergence of fungi exhibiting some degree of resistance to drugs commonly used in clinical medicine [23,21]. In northeastern Brazil, there are very few studies that assess the incidence of fungal infections and the profile of drug susceptibility. What we observed in clinical practice, on a cultural basis, was the prescription of antifungal drugs without prior susceptibility testing. There is an overall lack of trained professionals and reference laboratories with qualified and knowledgeable personnel, and most importantly, a lack of integration between laboratories and hospitals. Echinocandins are attractive therapeutic options for the treatment of IFIs. Although these agents have a narrow spectrum, they cover the two most common IFIs, candidiasis and aspergillosis [3]. Echinocandins exhibit few interactions with other drugs, low toxicity, and are broadly active against azole-resistant Candida spp. [5]. Table 1 shows the geometric mean (GM) of MICs 50% and 90% at 24 and 48 hours of caspofungin against C. albicans, C. parapsilosis and C. tropicalis. From a microbiological viewpoint, we see that the GM of the 50% and 90% MICs at 24 and 48 hours of C. parapsilosis is higher when compared to the GM of the 50% and 90% MICs at 24 and 48 hours of C. albicans and C. tropicalis; this difference was statistically significant with P < 0.05, 24 and 48 hours of caspofungin vis-a-vis C. albicans, C. parapsilosis and C. tropicalis. Epidemiological studies conducted in different regions of Latin America, including Brazil, have indicated that C. albicans is the most commonly isolated species, followed by C. tropicalis and C. parapsilosis [22]. C. parapsilosis is one of the foremost causes of invasive candidiasis [14]. In our study, it was the principal strain isolated (17 strains) (Table 1). Treatment of IFIs requires an understanding of the epidemiology of specific infections. Echinocandins represent one of the mainstays in the treatment against candidiasis, and should be monitored by identifying the most frequent species, as well as the suscepti-bility of such species to drugs used previously [5]. In our study, the GM of the MICs of C. parapsilosis compared to C. albicans and C. tropicalis was higher, showing that C. parapsilosis
exhibits higher MICs for caspofungin; similar observations have been described in the literature [9,18,1,4,19].

The Pearson coefficient (R) was used to assess the correlation of the laboratory results at 24 and 48 hours. One can see a strong correlation between the results read after these periods of time, suggesting that there is no difference in the release of results at 24 or 48 hours after testing, thereby speeding up not only diagnosis, but also establishment of the appropriate therapy. This coefficient is a measure of the degree of linear relationship between two quantitative variables, used in several studies to correlate laboratory procedures and time of operationalization [6,13,17].

Prescribing caspofungin without prior susceptibility testing may compromise the treatment of infections caused by C. parapsilosis, which in our study and in other studies have been shown to be less susceptible to caspofungin [9,18,1,4,19]. The union between susceptibility testing and prior identification of the causative agent is essential for establishing the correct treatment. The GM of the high MICs found in the strains of C. parapsilosis can be explained by the presence of the mutation on the fks gene already reported in other studies, C. parapsilosis, Candida metapsilosis, and Candida orthopsilosis, which harbor a naturally occurring amino acid substitution in the equivalent position of Fks1p. [10,15]. Future studies are required to evaluate this mutation and the MICs found.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

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