Short Communication

Revista da Sociedade Brasileira de Medicina Tropical 46(1):106-107, Jan-Feb, 2013
http://dx.doi.org/10.1590/0037-868210502013

Correlation between microdilution, Etest, and disk diffusion methods for antifungal susceptibility testing of fluconazole against *Candida* sp. blood isolates

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ABSTRACT

Introduction: Antifungal susceptibility testing assists in finding the appropriate treatment for fungal infections, which are increasingly common. However, such testing is not very widespread. There are several existing methods, and the correlation between such methods was evaluated in this study. **Methods**: The susceptibility to fluconazole of 35 strains of *Candida* sp. isolated from blood cultures was evaluated by the following methods: microdilution, Etest, and disk diffusion. **Results**: The correlation between the methods was around 90%. **Conclusions**: The disk diffusion test exhibited a good correlation and can be used in laboratory routines to detect strains of *Candida* sp. that are resistant to fluconazole.

Keywords: Disk diffusion. Antifungal susceptibility. Fluconazole.

Antifungal susceptibility testing is a notable breakthrough in the treatment of fungal infections and is the primary tool in determining the appropriate antifungal therapy[1]. Several methods are available to evaluate the antifungal susceptibility of *Candida* sp., and the main goal of these methods is to detect resistance *in vitro*. The most common methods are: broth microdilution (BMD), disk diffusion (DD), and Etest[2-4]. BMD is a test that can be somewhat troublesome to conduct in a laboratory routine, and Etest is still an expensive method. Therefore, DD is the most appropriate test to be applied in the detection of resistant strains due to its simplicity and ease of execution[5].

The aims of this study were to assess the susceptibility to fluconazole of *Candida* sp. strains, isolated from blood cultures of patients in the state of Ceará, using three different methods and to measure the correlation between these methods.

We evaluated 35 isolates of *Candida* sp. (16 *Candida tropicalis* and 19 *Candida parapsilosis*) isolated from blood cultures of ICU (Intensive Care Unit) patients at *Hospital Geral de Fortaleza*, Ceará, Brazil. The yeasts were isolated on a potato agar and incubated at 35°C for 24/48h. The presumptive identification was performed on HiCrome *Candida*® agar medium (Mumbai, India). The identification was confirmed by a microculture on rice agar with Tween 80 and with the API 20C AUX® kit (BioMérieux, Marcy-I'Étoile, France)[5].

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Received in 30/03/2011
Accepted in 06/12/2011

Antifungal susceptibility tests were conducted by the methods of broth microdilution in RPMI, Etest, and disk diffusion in accordance with the Clinical Laboratory Standards Institute (CLSI) documents. The break points used in this study were those suggested in protocols M27-A3 and M44-A2[2-4]. The break points for susceptibility interpretation are as follows: for fluconazole, Minimum Inhibitory Concentration (MIC) of ≤ 8µg/ml for susceptible (S); MICs of 16 to 32µg/ml for susceptible dose dependent (SDD); and MICs of ≥ 64µg/ml for resistant (R) for BMD and Etest methods. The interpretive criteria for the fluconazole disk diffusion tests were: (S), zone diameters ≥ 19mm; SDD, 15 to 18mm; (R), zone diameters ≤ 14mm[2,3].

A comparison of methodologies was performed by using analysis of the error as recommended by the CLSI. The definitions of error used in this study were as follows: the Very Major Error (VME) that occurs when one strain is detected (R) by the method of BMD (considered standard) and this same strain appears as sensitive when tested by the DD method, the Major Error (ME) that is observed when a strain is shown as (S) by BMD and (R) by DD, and the Minor error (M) that occurs when the result shows BMD as susceptible dose dependent (SDD) and DD is (S) or (R). Essential Agreement (EA) is calculated by using the formula: EA = 100 - VME + ME + M. In general, the methods showed a good correlation to the VME, which should be < 1.5%, and the agreement between the methodologies should be > 90%[2].

Table 1 shows the results obtained in this study: the DD method showed a good agreement with the other methods tested. In studies with *Candida* sp. isolated in Brazil, a correlation approaching 70% between the methods was found. In our study, the agreement was higher, exceeding 90%[6,7].

Based on these results and on studies that evaluated a high number of strains of *Candida* sp., we can conclude that the disk diffusion test correlated well with the other methods of assessing susceptibility. It is inexpensive, easy to perform, and can be implemented in laboratory routines that provide results in 24h[5,8].

***TABLE 1 - Correlation between methods for antifungal susceptibility testing of fluconazole against* Candida sp.**

Strains (n) and method	Range[a]	S/SDD/R[b]	Essential agreement (%)
Candida parapsilosis (19)			
BMD	0.12-64	16/1/2	-
Etest	1.0-256	15/1/3	90.0
DD	0-37	15/1/3	90.0
Candida tropicalis (16)			
BMD	0.12-32	15/1/0	-
Etest	1.0-2.0	16/0/0	93.0
DD	23-38	16/0/0	93.0

n: number of strains; **S/SDD/R:** susceptible, susceptible dose dependent, and resistant, respectively**; BMD:** broth microdilution; **DD:** disk diffusion**; [a]:** ranges of BMD, Etest, and DD are given as µg/ml, µg/ml, and halo mm, respectively; [b]**:** number of isolates.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

This study was funded by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

RESULTS

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