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SHORT COMMUNICATION/COURTE COMMUNICATION

Comparison of two antifungal susceptibility testing of *Candida* sp. isolates using agar diffusion method: Neo-sensitabs[®] tablets and Bio-rad[®] disks



Comparaison de deux méthodes d'antifongigramme sur milieu gélosé en utilisant les comprimés Neo-sensitabs[®] et les disques Bio-rad[®] pour tester la sensibilité des isolats de Candida sp.

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KEYWORDS

Antifungal susceptibility methods;
Agar diffusion method;
Concordance;
Candida;
Antifungal agents

Summary

Introduction. – The aim of our study was to evaluate the concordance between the two antifungal susceptibility testing of *Candida* sp. isolates using agar diffusion method: Neo-Sensitabs[®] tablets and Bio-Rad[®] disks.

Materials and methods. – This is a prospective study conducted in the Laboratory of Parasitology and Mycology of the Mohammed V military teaching hospital from February to August 2012. Upon receiving blood cultures and peripheral sites samples, the identification of *Candida* isolates performed using routine phenotypic standard tests and the realization of the antifungal susceptibility was carried out on Neo-sensitabs[®] tablets and Bio-Rad[®] disks.

Results. – A total of 38 *Candida* strains were isolated: 15 *C. albicans* (39%), 13 *C. glabrata* (34%), 5 *C. tropicalis* (13%), 4 *C. krusei* (11%) and 1 *C. dubliniensis* (3%). There were no significant difference ($P > 0.05$) in susceptibility rate between both methods for all antifungal agents tested except for 5-fluorocytosine. The concordance percentage between two methods was 100% for amphotericin B, 97.4% for fluconazole, 94.7% for voriconazole and 73% for 5-fluorocytosine.

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MOTS CLÉS

Méthodes de sensibilité aux antifongiques ;
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Concordance ;
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Antifongiques

Conclusion. — Both methods are easy to perform, rapid and cost effective. Our results showed the best agreement between the two methods for testing the susceptibility of *Candida* isolates to amphotericin B, fluconazole and voriconazole while for the 5-fluorocytosine, the concordance rate was low.

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Résumé

Introduction. — Le but de notre étude était d'évaluer la concordance entre les deux techniques d'antifongigramme en milieu gélosé avec les comprimés Neo-Sensitabs[®] et les disques Bio-rad[®] pour tester la sensibilité des isolats de *Candida* sp.

Matériels et méthodes. — Une étude prospective menée au laboratoire de parasitologie-mycologie de l'hôpital militaire d'instruction Mohammed V de février en août 2012. Dès la réception des hémocultures et des prélèvements des sites périphériques, l'identification des souches de *Candida* a été effectuée et la réalisation de l'antifongigramme a été faite en utilisant les comprimés Neo-sensitabs[®] et les disques Bio-rad[®].

Résultats. — Au total, 38 souches de *Candida* ont été isolées : 15 *Candida albicans* (39 %), 13 *C. glabrata* (34 %), 5 *C. tropicalis* (13 %), 4 *C. krusei* (11 %) et 1 *C. dubliniensis* (3 %). Il n'y avait pas de différence significative ($p > 0,05$) des niveaux de sensibilité entre les deux méthodes pour tous les antifongiques testés, sauf pour la 5-fluorocytosine. Le pourcentage de concordance entre les deux méthodes était de 100 % pour l'amphotéricine B, 97,4 % pour fluconazole, 94,7 % pour voriconazole et 73 % pour 5-fluorocytosine.

Conclusion. — Les deux méthodes testées sont faciles à réaliser, rapides et économiques. Nos résultats ont montré une très bonne concordance entre les deux méthodes pour tester la sensibilité des isolats de *Candida* à l'amphotéricine B, au fluconazole et au voriconazole tandis que pour la 5-fluorocytosine, le taux de concordance était faible.

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Introduction

Candida sp. represents the fourth or fifth agent found in nosocomial septicemia [6,13,14] with an attributable mortality rate ranging from 26 to 54% [15], this requires the use of in vitro susceptibility testing well standardized as part of guiding the clinician in the choice of the antifungal and its dosage. Intense efforts have been made to reach a consensus on antifungal susceptibility testing of yeast, and establishing repeatable and standardized methods for determining their sensitivity and correlation in vivo [2–4]. The reference method CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) for testing the antifungal susceptibility of yeasts (document M27-A2) has shown its limits: the difficulty of identifying strains of *Candida* with reduced susceptibility to amphotericin B, the phenomenon of residual growth observed for the azole and insufficient growth of certain *Candida* species [9,11]. Other methods inspired by the reference one have been developed for testing antifungal susceptibility [7,8]. The reference method M44-A has been proposed recently by CLSI to assess the sensitivity of yeasts using antifungal disk diffusion method [2]. Several studies on the comparison of antifungal susceptibility have been published and some authors compared the Neo-Sensitabs[®] method (Rosco Diagnostic, Denmark) to other methods [4,7,16]. To our knowledge, this study is the first that focuses specifically on the comparison of the Neo-sensitabs[®] method to that of Bio-Rad[®] (Bio-Rad Laboratories, Marnes-la-Coquette, France). The aim of this study was to evaluate the concordance between two antifungal susceptibility testing of

Candida isolates using agar diffusion method: Neo-Sensitabs[®] tablets and Bio-Rad[®] disks.

Materials and methods

This is a prospective observational study conducted in the Laboratory of Parasitology and Mycology of the Mohammed V military teaching hospital from February to August 2012. The isolates of *Candida* sp. obtained from blood cultures and peripheral sites samples of patients hospitalized in the intensive care units were included in this study. The culture was carried out on Sabouraud–chloramphenicol medium with and without actidione. Incubation of these media was done at 37 °C for 24 to 48 hours. The identification of *Candida* species was performed using standard phenotypic methods: morphological appearance of colonies, Candiselect 4[®] (Bio-Rad, France), filamentation test on serum and Auxacolor TM 2 (Bio-Rad, France). The study of the antifungal susceptibility of *Candida* isolates was carried out by the agar diffusion method using the Neo-sensitabs[®] tablets and Bio-Rad[®] disks. The four antifungal agents tested were: 5-fluorocytosine, fluconazole, voriconazole and amphotericin B. For the Neo-sensitabs[®] tablets method, the culture medium used was Mueller–Hinton agar supplemented with 2% glucose and 0.5 µg/mL of methylene blue [4,7,8]. For antifungal susceptibility disk Bio-rad[®]: 3 media were used: semi-synthetic medium for studying the activity of 5-fluorocytosine, casitone agar for polyenes and imidazoles [5] and Mueller–Hinton medium for testing triazoles [2]. The diameters of zones of inhibition were measured to the nearest

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