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ORIGINAL ARTICLE/ARTICLE ORIGINAL

In vitro antifungal susceptibility of clinical species belonging to *Aspergillus* genus and *Rhizopus oryzae*

Sensibilité in vitro des antifongiques d'isolats cliniques du genre Aspergillus *et de* Rhizopus oryzae

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KEYWORDS
Antifungal susceptibility;
Clinical specimens;
Aspergillus spp.;
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Summary
Objective. — Among filamentous fungal pathogens, *Aspergillus* spp. and zygomycetes account for highest rates of morbidity and mortality among immunocompromised patients. Recently developed antifungal drugs offer the potential to improve management and therapeutic outcomes of fungal infections. The aim of this study was to analyse the in vitro activities of voriconazole, itraconazole, amphotericin B and caspofungin against clinical isolates of *Aspergillus* spp. and *Rhizopus oryzae*.
Material and methods. — The in vitro antifungal susceptibility of 54 isolates belonging to different clinical isolates of *Aspergillus* spp. and *R. oryzae* was tested for four antifungal agents using a microdilution reference method (CLSI, M38-A2). All isolates were identified by typical colony and microscopic characteristics, and also characterized by molecular methods.
Results. — Caspofungin (MEC range: 0.008—0.25 and MEC50: 0.0023 μg/mL) was the most active drug in vitro against *Aspergillus* spp., followed by voriconazole (MIC range: 0.031—8 and MIC50: 0.5 μg/mL), itraconazole (MIC range: 0.031—16 and MIC50: 0.25 μg/mL), and amphotericin B (MIC range: 0.125—4 and MIC50: 0.5 μg/mL), in order of decreasing activity. The caspofungin, voriconazole, and itraconazole demonstrated poor in vitro activity against *R. oryzae* isolates evaluated, followed by amphotericin B.

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Conclusion. — This study demonstrates that caspofungin had good antifungal activity and azole agents had better activity than amphotericin B against *Aspergillus* species. Although, azole drugs are considered ineffective against *R. oryzae*. This result is just from a small scale in vitro susceptibility study and we did not take other factors into consideration.

MOTS CLÉS
Sensibilité aux antifongiques ;
Des spécimens cliniques ;
Aspergillus spp. ;
Rhizopus oryzae

Résumé
Objectif. — Parmi les agents pathogènes fongiques filamenteux, *Aspergillus* spp. et zygomycètes représentent les plus hauts taux de morbidité et de mortalité chez les patients immunodéprimés. Les antifongiques récemment développés offrent la possibilité d'améliorer la gestion et les résultats thérapeutiques des infections fongiques. Le but de cette étude était d'analyser les activités in vitro du voriconazole, de l'itraconazole, l'amphotéricine B et de la caspofungine contre des isolats cliniques d'*Aspergillus* spp. et *Rhizopus oryzae*.
Matériel et méthodes. — La sensibilité antifongique in vitro de 54 isolats appartenant à différents isolats cliniques d'*Aspergillus* spp. et *R. oryzae* a été testée par quatre agents antifongiques en utilisant une méthode de référence de microdilution (CLSI, M38-A2). Tous les isolats ont été identifiés par l'aspect des colonies typique et les caractéristiques microscopiques, et également caractérisés par des méthodes moléculaires.
Résultats. — La caspofungine (MEC gamme : de 0,008 à 0,25 et MEC50 : 0,0023 μg/mL) était la molécule la plus active in vitro contre *Aspergillus* spp, suivie par le voriconazole (gamme MIC : 0,031 à 8 et CIM50 : 0,5 μg/mL). L'itraconazole (gamme MIC : 0,031 à 16 et CIM50 : 0,25 μg/mL), et l'amphotéricine B (plage MIC : 0,125 à 4 et CIM50 : 0,5 μg/mL), par ordre décroissant de l'activité. La caspofungine, le voriconazole et l'itraconazole ont démontré une faible activité in vitro contre des isolats de *R. oryzae*, suivie par l'amphotéricine B.
Conclusion. — Cette étude démontre que la caspofungine a eu une bonne activité antifongique et que les agents azolés ont une meilleure activité que l'amphotéricine B contre des espèces d'Aspergillus. Toutefois, les médicaments azolés sont considérés comme inefficaces contre *R. oryzae*. Ce résultat est issu d'une étude limitée de la sensibilité in vitro et nous n'avons pas pris d'autres facteurs en considération.

Introduction

The incidence of fungal infections in immunocompromised populations has increased greatly over the past decades [5,11]. Among filamentous fungal pathogens, *Aspergillus* spp. and zygomycetes account for highest rates of morbidity and mortality among immunocompromised patients especially cancer, organ transplant, burn and surgical patients [10]. Recently developed antifungal drugs offer the potential to improve management and therapeutic outcomes of fungal infections [16]. Despite the availability of potent antifungal agents, systemic fungal infections continue to cause significant morbidity and mortality [1]. Itraconazole, voriconazole and posaconazole have been approved, particularly for the treatment of infections caused by *Aspergillus* and other filamentous fungi [17,22]. Due to the life threatening nature of these infections and reports of drug resistance, susceptibility testing of fungal pathogens has become very important [8]. The *in vitro* anti-fungal susceptibility testing of different pathogenic fungi is helpful in defining the activity spectrum of an antifungal and it can be a valuable guide for the clinician for treating infecting agents [24]. However, data about the *in vitro* antifungals against zygomycetes or other moulds are scarce in Iran. Nonetheless, there is little information regarding its susceptibility patterns against currently available antifungal agents. The aim of this study was to analyse the *in vitro* activities of amphotericin B, itraconazole, voriconazole, and caspofungin against clinical isolates of *Aspergillus* spp. and *Rhizopus oryzae*.

Materials and methods

Fungal strains and culture conditions

During September 2010 until October 2014, 54 clinical isolates belonging to the *Aspergillus* genus and *R. oryzae* were collected from patients hospitalized at the teaching hospital in North of Tehran (the isolates obtained from samples are routinely sent to the hospital laboratory for diagnosis and present study was carried out on samples in coordination with the hospital, and according to the hospital it was not required to plan the hospital ethics committee). The isolates were sub-cultured onto potato dextrose agar and incubated at 48 h at 37 °C before testing. All isolates were identified by typical colony and microscopic characteristics, also characterized and determined by molecular methods. Two reference strains, *Candida parapsilosis* (ATCC 22019) and *Aspergillus flavus* (ATCC 204304) were included in all tests as references for quality controls and were tested by both methods in each series of assays.