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IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA* ISOLATES FROM VARIOUS CLINICAL SPECIMENS IN DOCTOR BEHÇET UZ HOSPITAL

DOKTOR BEHÇET UZ HASTANESİNDE ÇEŞİTLİ KLİNİK ÖRNEKLERDEN İZOLE EDİLEN CANDİDALARIN İDENTİFİKASYONU VE ANTIFUNGAL DUYARLILIKLARI

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Özet

Bu çalışmanın amacı çeşitli klinik örneklerden izole edilen 101 maya suşunun tür tanımlanması ve antifungal duyarlıklarının belirlenmesidir. İzolatların identifikasyonu API 20 C AUX (Biomérieux, France) kiti ile yapılmış olup, API ATB (Biomérieux, France) fungus kiti ile suşların flusitozin, amfoterisin B, flukonazol, itrakonazol ve vorikonazola karşı antifungal duyarlılıkları belirlenmiştir. Yüz bir klinik suşun 48'i idrar, 46'sı kan, 5'i kateter ve 2'si yaradan izole edildi. Suşların türlere göre dağılımında en sık C. albicans (54.46%), ikinci sıklıkta C. parapsilosis (33.66%), üçüncü sıklıkta C. famata (6.93%), dördüncü sıklıkta C. tropicalis (2.97%) ve beşinci sıklıkta C. pelliculosa (1.98%) izledi. C. albicans suşlarında vorikonazol ve flukonazola karşı direnç oranları sırasıyla %5.45 ve % 3.63 olarak saptandı. Diğer Candida suşlarında ise özellikle itrakonazola karşı farklı oranlarda direnç saptandı. Candida infeksiyonlarının etkin tedavisi için antifungal duyarlılığın ve tür tanımlamalarının yapılması önerilmektedir. (Anatol J Clin Investig 2009:3(3);170-173).

Abstract

The purpose of this study was to identify and determine antifungal susceptibility patterns of 101 yeast strains isolated from various clinical specimens. Identification of the isolates were determined by API 20 C AUX kit (Biomérieux, France) and antifungal susceptibilities of these species to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole were determined by API ATB Fungus kit (Biomérieux, France). Of the 101 strains, 48 were isolated from urine, 46 from blood, 5 from catheter and 2 from wound. C. albicans (54.46%) was the most frequently isolated species, followed by C. parapsilosis (33.66%), C. famata (6.93%), C. tropicalis (2.97%) and C. pelliculosa (1.98%). Rates of resistance to voricanozole and fluconazole were 5.45% and 3.63%, respectively in C. albicans. Different rates of resistance against especially itraconazole were detected in other Candida strains. Species definition and determination of antifungal susceptibility patterns are advised for the proper treatment of Candida infections. (Anatol J Clin Investig 2009:3(3);170-173).

Introduction

Though a healthy immune system keeps *Candida* spp. under control, they can lead to significant infections with high mortality and morbidity in immunocompromised hosts. In the last two decades a steady increase in nosocomial infections caused by *Candida* species has been seen. The majority of cases of fungal infections are caused by *C. albicans*; however, episodes due to non-albicans species of *Candida* appear to be increasing [1-3]. A high variability in the susceptibility of clinical isolates to antifungal agents has been documented among these *Candida* spp. emphasizing the importance of performing species identification and antifungal susceptibility testing [4,5]. Importantly, many non-*albicans* spp have decreased susceptibility to antifungal agents over the past decades.

Little is known about the distribution of *Candida* species and antifungal susceptibility in Turkey. In this study, we aimed to determine the species level distribution and antifungal susceptibilities of *Candida* strains from various clinical samples to five antifungal agents, flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole.

Material & Methods

A total of 101 *Candida* strains were isolated from January 2006 to January 2008 in Doctor Behçet Uz Hospital for Child Diseases Education and Research from İzmir, Turkey. Clinical specimens from patients in different departments were sent to the Microbiology Laboratory of this hospital for characterization and susceptibility testing. Of the 101, 48 were isolated from urine, 46 from blood, 5 from catheter and 2 from wound. Urine, catheter and blood samples were placed in culture media of sheep blood agar (Blood Agar Difco), Potato Dextrose Agar (PDA Difco) and Sabouraud Dextrose Agar (SDA Difco), and left to incubate at 35^{o}C for 3 days. Blood samples

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were collected in sterile conditions from each patient in the service, planted into 2 to 5 ml blood culture bottles (BD BacTec Peds Plus/F, USA) and placed into blood culture equipment (BD BacTec 9240, USA). The bottles incubated at 37^{o}C for 7 days in the equipment. The bottles which were reproduction positive were transferred to PDA and SDA culture media and left to incubate at 35^{o}C for 3 days.

Pathogenesis was accepted as 10^4-10^5 cfu/ml reproduction in urine cultures, and pure reproduction in other clinical cultures [6]. Strains with a positive germ tube test were considered *Candida albicans* in microbiological description.

Identification: The cultures were microscopically examined (direct examination and gram staining) and the fermented cultures were isolated. The yeasts were put into ready bead tubes (Cryobank, Mast Diagnostics, Germany) and kept at -20^{o}C in a deep freezer. Isolated yeasts were typed using API 20 C AUX (BioMérieux, France) kit, which consists of 24 different cupules, each containing dehydrated carbohydrate substrates, and the typing was based on the carbohydrate using property of each yeast.

Antifungal susceptibility testing: Antifungal sensitivity was determined using API ATB Fungal 3 (BioMérieux, France) kit. The results were read by a Mini API apparatus (BioMérieux, France). Antifungal agents were used as flucytosine 4-32 mg/l, amphotericine B 0.5-2 mg/l, fluconazole 8-64 mg/l, itraconazole 0.125-1.0 mg/l and voriconazole 1-4 mg/l. *C. albicans* (ATCC 90028) and *C. parapsilosis* (ATCC 22019) microorganisms were used as control strains in this study.

Results

As it is seen in Table 1, typing performed with API ID 20C AUX kit revealed that, 55 strains (54.46%) were *C. albicans*, 34 (33.66%) were *C. parapsilosis*, 7 (6.93%) were *C. famata*, 3 (2.97%) was *C. tropicalis* and 2 (1.98%) was *C. pelliculosa*. *C. albicans* was found the most common *Candida* species isolated from various clinical samples.

Table 2 presents the antifungal sensitivity tests carried out using ATB Fungal 3 kit that 55 (100%) *C. albicans* strains were sensitive to flucytosine, itraconazole and amphotericine B while 2 (3.63%) of *C. albicans* strains were resistant to fluconazole and 3 (5.45%) to voriconazole. Of the *C. parapsilosis* strains, 34 (100%) were sensitive to flucytosine, amphotericine B and voriconazole, whereas two strains (5.88%) were resistant to itraconazole and one strain (2.94%) was less sensitive to fluconazole. Among the *C. famata* strains, seven (100%) were found sensitive to flucytosine, amphotericine B, fluconazole and voriconazole, while three strains (42.85%) were found sensitive to itraconazole, two strains (28.57%) were less sensitive, however two (28.57%) of the strains were found resistant to itraconazole. *C. tropicalis* and *C. pelliculosa* strains were found sensitive to all antifungal agents.

Discussion

As the number of fungal infections increase, the diversity of species causing these infections change. Although *C. albicans* is the most common nosocomial fungal infection due to its endogenous origin, rate of encounter with non-albicans species like *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis* and *C. lusitaniae*, which are known to be more resistant to treatment, is also increasing rapidly. Due to these reasons it is of utmost importance to define species and determine their resistance profiles [5,7].

In this present study, we isolated 101 strains of which were 54.46% *C. albicans,* 45.54% non-albicans *Candida* species. These results are in agreement with those of Gültekin & Aydın [8] who described that 56.3% *Candida* strains were isolated from various clinical samples as *C. albicans*, 43.7% as non-*albicans Candida* species. A remarkable point in our study is that the most commonly isolated species in blood samples was *C. parapsilosis* (Table 1). It is seen here that *C. parapsilosis* is more common than *C. albicans*. In a similar study of Pina-Vaz et al. [9] noted that of the yeasts they isolated from blood were mostly 33.8% *C. parapsilosis* and 30.9% *C. albicans*. The data obtained in this study, as well as results of other studies suggest that *C. parapsilosis* besides *C. albicans* species can pose a serious threat.

Table 2 presented that antifungal susceptibility testing in our study revealed the significant differences in susceptibilities of *Candida* isolates to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole. The results of our study are in accordance with the results of other studies in this respect. As regards the antifungal sensitivity of *Candida* species study by Adiloğlu et al. [10] 31 of *C. albicans* strains were sensitive (100%) to amphotericine B and flucytosine, and resistant to nystatin, miconazole, econazole and ketoconazole at varying rates (%1.0-50.0). Çıtak et al. [11] investigated the antifungal sensitivity of *Candida* isolated from cancer patients and found that 12.5% *C. albicans* were resistant to fluconazole, 5.3% to flucytosine, 14.3% *C. parapsilosis* was resistant to fluconazole, 33.3%

C. tropicalis was resistant to ketoconazole, while *C. pelliculosa* were resistant to amphotericine B, ketoconazole and fluconazole. In a study [12] carried out in Turkey, antifungal sensitivity test revealed that of the 45 isolates, 39 (86%) were sensitive to all antifungal agents and 6 (13.3%) were sensitive to one or more antifungal agents. Interestingly all resistant strains consist of non-albicans *Candida*. It was established in another study with an antifungal sensitivity test using E test of *Candida*, which is an invasive infection agent, that the most sensitive antifungal among *C. albicans* and non-albicans *Candida* was amphotericine B, with the highest resistance to fluconazole in all *Candida* species [13]. The results in these studies are not only in semblance of our findings, but also indicate that *Candida* develop resistance to antifungal agents at various levels.

In conclusion, this is the first report of in-vitro antifungal susceptibility of *Candida* species isolated from patients with invasive infections in this hospital. Intensive and long-term use of antifungals leads to a decline in the sensitivity and resistance development of *Candida* strains. It is known that non-albicans species increase in candida infections and that these species have a high resistance to antifungal drugs [1,2,4,13]. Therefore, we believe that description of active pathogens in candida infections at the species level and research on antifungal sensitivity will be very useful in managing treatment and preventing resistance development.

Table 1. *Candida* species (n=101) isolated from various clinical samples.

Candida spp.	Urine	Blood	Catheter	Wound	Total (%)
C.albicans	32	18	3	2	55 (54.46)
C.parapsilosis	9	25	-	-	34 (33.66)
C.famata	3	2	2	-	7 (6.93)
C.tropicalis	3	-	-	-	3 (2.97)
C.pelliculosa	1	1	-	-	2 (1.98)
Total (%)	48 (47.52%)	46 (45.54%)	5 (4.95%)	2 (1.98%)	101 (100)

Table 2. Antifungal susceptibility determined using ATB Fungus 3 kit.

	Flucytosine				Amphotericin B				Fluconazole				Itraconazole				Voricanozole			
	S	I (mg/ml)	R		S	I (mg/ml)	R		S	I (mg/ml)	R		S	I (mg/ml)	R		S	I (mg/ml)	R	
	≤4	8-16	≥32	%	ND	ND	ND	%	≤8	16-132	≥64	%	≤0.125	0.5-0.5	1	%	≤1	2	≥4	%
***Candida* spp.**																				
C.albicans	55	-	-	0	55	-	-	0	52	1	2	3.63	55	-	-	0	52	-	3	5.45
C.parapsilosis	34	-	-	0	34	-	-	0	33	1	-	0	32	-	2	5.88	34	-	-	0
C.famata	7	-	-	0	7	-	-	0	7	-	-	0	3	2	2	28.5	7	-	-	0
C.tropicalis	3	-	-	0	3	-	-	0	3	-	-	0	3	-	-	0	3	-	-	0
C.pelliculosa	2	-	-	0	2	-	-	0	2	-	-	0	-	2	-	0	2	-	-	0
**C.albicans* (ATCC 90028)	1	-	-	0	1	-	-	0	1	-	-	0	1	-	-	0	1	-	-	0
**C.parapsilosis* (ATCC 22019)	1	-	-	0	1	-	-	0	1	-	-	0	1	-	-	0	1	-	-	0

S: Susceptible; I, Intermediate susceptibility; R, Resistant; ND: Not determined
*Control strains

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