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Short Communications

Distribution and antifungal susceptibility of *Candida* species causing nosocomial candiduria

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The aim of the study was to investigate the distribution of *Candida* species isolated from urine specimens of hospitalized patients in Akdeniz University Hospital, Antalya, Turkey, as well as their susceptibilities to antifungal agents. A total of 100 patients who had nosocomial candiduria between March 2003 and May 2004 at the facility were included in the study. Organisms were identified by conventional methods and the use of API ID 32C strips. Susceptibilities of the isolates to amphotericin B were determined by Etest, whereas the minimum inhibitory concentration (MIC) values of these same strains to fluconazole, voriconazole and caspofungin were assessed using the broth microdilution method. The most common species recovered was *C. albicans* 44% of all yeasts, followed by *C. tropicalis* (20%), *C. glabrata* (18%), *C. krusei* (6%), *C. famata* (5%), *C. parapsilosis* (4%), *C. kefyr* (2%) and *C. guilliermondii* (1%). A total of nine (9%) of the isolates, including five *C. krusei* and four *C. glabrata* isolates were susceptible dose-dependent (SDD) to fluconazole. In constrast, only two *C. glabrata* and one *C. krusei* isolates were resistant to this antifungal. The voriconazole MICs for all *Candida* isolates were ≤0.5 μg/ml, except for one *C. glabrata* isolate with a MIC value of 2 μg/ml. Among all isolates, 94% were susceptible to amphotericin B with MIC values of <1 μg/ml and all isolates were susceptible to caspofungin with MIC values of ≤0.5 μg/ml. Future studies are needed to define better treatment regimens for those patients who have fluconazole-resistant *Candida* urinary tract infections.

Keywords Nosocomial candiduria, antifungal susceptibilities, *Candida* species

Introduction

The interpretation of the significance of the recovery of *Candida* from urine specimens is often difficult. Candiduria may represent contamination, colonization of the urinary catheter or of the bladder. In people who are infected, all anatomic levels of the urinary tract may be involved. Most cases of candiduria are asymptomatic and treatment is rarely necessary. *Candida albicans* has been the most common species isolated from urine samples, followed by *C. glabrata* and *C. tropicalis* [1]. The development of resistance to antifungal drugs has become increasingly important and there is growing evidence of shifts to more resistant strains [2]. Therefore, isolation and identification of *Candida* species causing urinary tract infections are extremely important for their appropriate treatment [3]. We conducted a descriptive study of the distribution of *Candida* species isolated from urine specimens of hospitalized patients in Akdeniz University Hospital, Antalya, Turkey, as well as their susceptibilities to antifungal agents.

Materials and methods

One hundred *Candida* spp. isolates from 100 patients who had nosocomial candiduria between March 2003 and May

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2004 at Akdeniz University Hospital, Antalya, Turkey, were included in the study. Nosocomial infections were defined by Centers for Disease Control and Prevention (CDC) criteria used by infection control physicians. Candiduria was considered nosocomial if the infection becomes evident 48 or more hours after admission. The patients were eligible for the study if two consecutive cultures of urine samples were positive for *Candida* species [3]. Demographic data and underlying diseases of the patients were collected.

Specimens were collected by using midstream urine, straight catheters or from indwelling catheters. The second specimen was obtained after replacing the first catheters in patients with indwelling catheters.

Identification of the isolates was obtained by standard microbiologic procedures and the use of API ID 32C strips (Biomerieux, France). Organisms were identified to the species level by use of the germ tube test, evaluation of morphologic properties on corn meal tween 80 agar and carbohydrate assimilation tests with API ID 32 C strips (Biomerieux, France) [4,5]. The isolates were stored as suspensions in water until they were used. Each isolate was subcultured at least twice on Sabouraud dextrose agar (SDA) and incubated at 35°C prior to testing to ensure purity.

Susceptibilities of the isolates to amphotericin B were determined by the Etest (Biomerieux, France) method according to the manufacturer's instructions. Broth microdilution method, specified by Cinical Laboratory and Standards Institute (CLSI; formerly NCCLS) document M27-A3 and M27-S3, were used to evaluate the susceptibilities of the isolates to fluconazole, voriconazole and caspofungin [6,7]. Reagent grade powder of fluconazole, voriconazole (Pfizer, Inc, New York, USA) and caspofungin (Merck, Australia) were obtained from their respective manufacturers. Stock solutions were prepared in dimethyl sulfoxide (DMSO; voriconazole) or water (fluconazole and caspofungin). Antifungal susceptibility testing was performed by using RPMI 1640 medium with a final inoculum suspension of 0.5×10^3 to 2.5×10^3 yeasts per ml. Plates were incubated at 35°C and read 24 h after inoculation for caspofungin, and at 48 h for fluconazole and voriconazole [6,7]. Minimum inhibitory concentrations (MICs) were determined visually by two separate investigators. The MIC was defined as 50% reduction in turbidity compared to that of the growth control.

Interpretive breakpoints established by the CLSI were used for fluconazole [Susceptible (S), ≤ 8 μg/ml; susceptible dose-dependent (SDD), 16–32 μg/ml; Resistant (R), ≥ 32 μg/ml)], voriconazole (S, ≤ 1 μg/ml; SDD, 2 μg/ml; R, ≥ 4 μg/ml) and caspofungin (S, ≤ 2μg/ml) [6,7]. For amphotericin B which has no established interpretive breakpoints, a breakpoint of ≤ 1 μg/ml was used [8].

Quality control isolates, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in all runs. The study was approved by Akdeniz University Medical Faculty Ethical Committee with B.30.2.AKD.0.01.00.00/ Etik/484 protocol number.

Results

Of the 100 patients with nosocomial candiduria 60 were female. The mean age of the study population was 43.5 ± 26.6 (range 0–94 years). Underlying diseases included trauma 24%, neurologic disorders 21%, diabetes mellitus 18%, malignancy 13%, pulmonary and heart diseases 11%, urinary system abnormalities 10%, prematurity 8%, organ transplantation 4%, burn 2% and others 7%. The majority of the patients (88%) had indwelling bladder catheters. The most common species isolated was *C. albicans* 44%, followed by *C. tropicalis* (20%), *C. glabrata* (18%), *C. krusei* (6%), *C. famata* (5%), *C. parapsilosis* (4%), *C. kefyr* (2%) and *C. guilliermondii* (1%).

MIC ranges, MIC_{50}/MIC_{90} values of antifungal agents for urinary *Candida* isolates are shown in Table 1. A total of nine (9%) of the isolates including five *C. krusei* and four *C. glabrata* isolates were susceptible dose dependent (SDD) to fluconazole, whereas only two *C. glabrata* and one *C. krusei* isolates were resistant.

For all *Candida* isolates, the voriconazole MIC's were ≤ 0.5 μg/ml, except for one *C. glabrata* isolate with a MIC value of 2 μg/ml for voriconazole. This isolate was also resistant to fluconazole.

Ninety-four percent of the isolates were susceptible to amphotericin B with MIC's lower than 1 μg/ml. Amphotericin B MIC values of higher than 1 μg/ml were only found with *C. krusei* (four isolates) and *C. glabrata* (two isolates). All isolates were susceptible to caspofungin with MIC values of 0.5 μg/ml or lower.

Discussion

Recent studies highlight the changing epidemiology of candiduria in hospitalized patients [2,9,10]. In our investigation, *C. albicans* was the prominent species isolated from urine cultures. However, non-*C. albicans Candida* species accounted for more than 50% of all isolates in our center which suggests a shift to these species. Kobayashi *et al.* reported a high incidence of candiduria caused by species other than *C. albicans*. *C. tropicalis* (22.2%) was the most common isolate in their study [11]. De Oliviera *et al.* recovered *C. tropicalis* from 53% of their 166 candiduria patients [9]. In a case-control study assessing the significance of candiduria in renal transplant recipients, *C. glabrata* which was recovered from speciments of

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Table 1 *In vitro* susceptibilities of urinary *Candida* isolates to fluconazole, voriconazole amphotericin B and caspofungin.

Species (*n*)	Antifungal agents	Range (48 h) (μg/ml)	MIC_{50}/MIC_{90} (24 h) (μg/ml)	MIC_{50}/MIC_{90} (48 h) (μg/ml)
C. albicans (44)	Fluconazole	0.125–8	0.25/0.25	0.25/0.25
	Voriconazole	0.03–0.25	0.03/0.03	0.03/0.03
	Amphotericin B	0.008–1	0.047/0.25	0.047/0.25
	Caspofungin	–	0.06/0.06	–
C. tropicalis (20)	Fluconazole	0.125–0.25	0.125/0.25	0.125/0.25
	Voriconazole	0.03–0.06	0.03/0.06	0.03/0.06
	Amphotericin B	0.008–1	0.094/0.5	0.094/0.5
	Caspofungin	–	0.03/0.06	–
C. glabrata (18)	Fluconazole	0.125–64	2/64	2/64
	Voriconazole	0.03–2	0.06/1	0.06/1
	Amphotericin B	0.008–2	0.125/1.5	0.125/1.5
	Caspofungin	–	0.03/0.06	–
C. krusei (6)	Fluconazole	16–32	16/32	16/32
	Voriconazole	0.03–0.25	0.03/0.25	0.03/0.25
	Amphotericin B	0.008–2	2/2	2/2
	Caspofungin	–	0.06/0.125	–
C. famata (5)	Fluconazole	0.125–1	0.25/1	0.25/1
	Voriconazole	0.03–0.06	0.03/0.06	0.03/0.06
	Amphotericin B	0.047–0.38	0.19/0.38	0.19/0.38
	Caspofungin	–	0.03/0.06	–
C.parapsilosis (4)	Fluconazole	0.25–1	0.25/1	0.25/1
	Voriconazole	0.03/0.03	0.03/0.03	0.03/0.03
	Amphotericin B	0.008–0.064	0.023/0.064	0.023/0.064
	Caspofungin	–	0.125/0.5	–
Other* (3)	Fluconazole	0.125–4	0.125/4	0.125/4
	Voriconazole	0.03–0.25	0.03/0.25	0.03/0.25
	Amphotericin B	0.094–1	0.125/1	0.125/1
	Caspofungin	–	0.125/0.125	–

Other*: *C. Kefyr* (2), *C. guilliermondii* (1).

98 (51%) of 191 patiens was the most common isolate [12]. Thus, several published series, along with ours, provide evidence of more resistant strains associated with candiduria in hospitalized patients [2,9,10,12].

Because of its safety and the pharmacokinetic and pharmacodynamic properties, fluconazole is preferred for the treatment of *Candida* urinary tract infections. Other treatment options include flucytosine and low-dose amphotericin B. The role of echinocandins and newer azoles that do not achieve measurable concentrations in the urine is not clear [13].

In our study, high levels of resistance to fluconazole (MIC ≥ 64 μg/ml) was found in 3% of all species but restricted to isolates of *C. glabrata* and *C. krusei*. Sience *C. krusei* is intrinsically resistant to fluconazole, it is expected that 100% of strains would be reistant to this antifungal independent of the *in vitro* susceptibility studies. The fluconazole susceptibilities of *C. glabrata* strains vary widely [14]. *C. glabrata* may be intrinsically resistant to fluconazole and urinary tract infections caused by this species are the most difficult to treat. It is suggested that fluconazole therapy may be succesful for strains with MIC's in the range of 8–32 μg/ml and as a result, susceptibility testing should be performed in this situation [3].

In our study, all *Candida* spp. were susceptible to voriconazole except one *C. glabrata* strain which was intermediate susceptible (MIC = 2 μg/ml). This isolate was also resistant to fluconazole. Cross resistance between fluconazole and voriconazole is well described among isolates of *C. glabrata*. Although *C. krusei* is intrinsically resistant to fluconazole, in contrast to *C. glabrata*, *in vitro* cross resistance to voriconazole is distinctly uncommon because voriconazole binds much more effectively to the cytochrome P450 isoenzyme target in *C. krusei* than does fluconazole [14]. In spite of excellent *in vitro* activity of voriconazole against *Candida* spp., no studies of the use of voriconazole for urinary candidiasis have been published to date [3].

Amphotericin B deoxycolate is another treatment option for *Candida* urinary tract infections. Amphotericin B deoxycolate bladder irrigation is recommended only for patients whose infection is caused by refractory fluconazole-resistant organisms [3,15]. A single intravenous dose of 0.5–1 mg/kg body weight of amphotericin B deoxycolate has been shown to be effective for candiduria [16].

In addition to fluconazole, *C. glabrata* is innately less susceptible to amphotericin B than most other species of *Candida* [17]. In our study, two isolates had MICs > 1 μg/ml for amphotericin B. Reduced susceptibility to amphotericin B may be associated with some strains of *C. krusei* as noted for four isolates in this investigation.

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Flucytosine, which is considered to be as another treatment option in cases of fluconazole treatment failure is not available in our country.

In conclusion, fluconazole is still the drug of choice for the treatment of urinary candidiasis in our hospital. It is clear that future studies are needed to define better treatment regimens for those patients who have fluconazole-resistant *Candida* urinary tract infections. The changing trends in the epidemiology of candiduria in hospitalized patients, especially with risk factors, should be closely monitored. Species identification and antifungal susceptibility testing may have therapeutic implications.

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