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Typing of *Candida* species isolated from blood cultures and analysis of their *in vitro* antifungal susceptibilities

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The aim of the present study was to investigate antifungal susceptibility of Candida strains isolated from blood cultures in our tertiary hospital. Patients hospitalized between December 2008 and April 2011 whose more than one blood cultures revealed growth of *Candida* species and Candida strains isolated in these cultures were included in the study. In order to identify isolated yeast species, appearance and configuration of the colonies, germ tube test results and their morphological appearance in corn flour Tween 80 agar were evaluated. Within the study period, among 65 Candida strains isolated, 36 (55.4%) *Candida parapsilosis*, 17 (26.2%) *Candida albicans*, 6 (9.2%) *Candida glabrata*, 4 (6.2%) *Candida tropicalis*, and 2 (3.1%) *Candida lusitaniae* isolates were identified. According to Fungifast susceptibility panel, antifungal susceptibility rates were as follows: amphotericin B and flucytosine (100%) fluconazole (93.8%), itraconazole (87.6%) and voriconazole (96.9%). Antifungal susceptibility rates of Candida isolates based on E-test method were as follows: amphotericin B (100%), voriconazole (92.3 %) itraconazole (53.8%) and fluconazole (89.2%). In consideration of higher morbidity, mortality and economic burden of the cases with fungemia, measures against emergence of these infections convey crucial importance. Typing of fungi isolated from intensive care units in particular, and their antifungal susceptibility tests should be done regularly to reveal resistance patterns of pathogens, and any increase in resistance (if any) over time, must be determined with scientific methods. We think that similar studies will guide the clinician in planning treatment of Candida infections especially in patients at risk.

Key words: Candidemia, candida, *in vitro*, microbial sensitivity tests.

INTRODUCTION

Hematologic infections due to *Candida* species in the world are becoming increasingly important. In the United States, *Candida* spp. yeasts ranked fourth among the most frequently isolated microorganisms from blood cultures (Martin et al., 2005). In recent years, the incidence of nosocomial Candida infections increased due to increasing number of patients receiving chemotherapy and other immunosuppressive therapies, innovations in transplantation surgery, use of broad-spectrum antibiotics, higher number of patients hospitalized in the intensive care units and invasive procedures performed on patients (Lunel et al.,1999; Cheng et al.,2004). Although fungemias are not frequently encountered clinical entities, these infections present therapeutic challenges with their inherent higher risks of mortality, and requirement for longer hospital stays (Ostrosky-Zeichner et al., 2003).

Due to differences in antifungal use and infection control strategies, distribution and antifungal susceptibility of

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Candida spp. differ between countries and hospitals (Pfaller et al., 2003; Dimopoulos et al., 2008). In species other than *Candida albicans*, especially in *Candida krusei* and *Candida glabrata* resistance against azole group antifungal agents develops which further complicates choice of treatment in candidemias. Antifungal susceptibility tests guide the antifungal therapy during episodes of fungemia.

In this study, our aim was to investigate antifungal susceptibility of Candida strains isolated from blood cultures in our tertiary hospital.

MATERIALS AND METHODS

Our study included patients hospitalized in various services of our tertiary hospital between December 2008 and April 2011 whose more than one blood cultures sent to Medical Microbiology Laboratory revealed growth of *Candida* spp. and also Candida strains isolated in these cultures. Among the same results obtained from the cultures of the same patient, only one blood culture results were included in the analysis.

In order to identify isolated yeast spp., appearance and configuration of the colonies, germ tube test results and their morphological appearance in corn flour Tween 80 agar were evaluated. In addition to these conventional methods, commercial Fungifast (ELİTech France SAS) kit which provided quick identification and antifungal susceptibility results were used in accordance with the manufacturer's instruction.

Antifungal susceptibility tests were performed using E test method. For this purpose, CLSI M27-A M44-A guidelines published by CLSI were followed. As a culture medium, Mueller-Hinton agar (Oxoid CM0337) (GM-MH) containing 2% glucose and 5 µg/ml methylene blue (RM 956-Himedra) was used. In the study, for E-test application, yeasts was suspended in 0.85% NaCl so as to achieve 0.5 McFarland turbidity standard. Surface of GM-MH Agar were scratched in zigzags with sterile cotton swab to provide homogenous growth of Candida. E-test strips (bioMerieux AB, Sweden) containing fluconazole, itraconazole, amphotericin B and voriconazole were applied on agar surface using sterile forceps. E-test strips containing culture medium were incubated at 35°C. Incubation was maintained up to 24 or 48 h till distinct appearance of inhibition ellipse which indicated growth of Candida. The first intersection point between the inhibition ellipse and the scale on the strip where a significant inhibition was observed was evaluated as minimal inhibitor concentration (MIC).

RESULTS

Within the study period, among 65 Candida strains isolated, 36 (55.4%) *Candida parapsilosis*, 17 (26.2%) *Candida albicans*, 6 (9.2%) *Candida glabrata*, 4 (6.2%) *Candida tropicalis*, and 2 (3.1%) *Candida lusitaniae* isolates were identified. In addition, two standard Candida strains (*C. krusei* ATCC 6258 and *C. albicans* ATCC 90 029) were included in the study. Distribution of the patients among services is shown in Table 1, and of isolates of *Candida* spp. in Table 2.

In the study, according to Fungifast susceptibility panel, antifungal susceptibility rates were as follows: amphotericin B and flucytosine (100%) fluconazole (93.8%), itraconazole (87.6%), and voriconazole (96.9%).

Table 1. Distribution of the patients among hospital departments.

Department	Number of patients (%)
Anaesthesia Intensive Care Unit	37 (56.9)
Internal Medicine	8 (12.3)
Cardiovascular Surgery	7 (10.8)
Neurosurgery	1 (6.2)
General Surgery	4 (6.2)
Haematology	2 (3.1)
Urology	2 (3.1)
Emergency Medicine	1 (1.5)
Infectious diseases	1 (1.5)
Nephrology	1 (1.5)
Neurology Intensive Care Unit	1 (1.5)

Table 2. *Candida* spp. isolated from blood cultures and their distribution.

Candida spp.	Number of patients (%)
C. parapsilosis	36 (55.4)
C. albicans	17 (26.2)
C. glabrata	6 (9.2)
C. tropicalis	4 (6.2)
C. lusitaniae	2 (3.1)

Antifungal susceptibility rates of Candida isolates based on E-test method were as follows: amphotericin B (100%), voriconazole (92.3%) itraconazole (53.8%) and fluconazole (89.2%).

Using E-test in the study, MIC range of amphotericin B for *C. albicans* was 0004-0125 µg/ml, and for non-albicans *Candida spp*. it was 0.003-0.38 µg/ml, respectively. In our study, amphotericin B resistance was not observed in none of the 65 isolates. MIC_{50}, and MIC_{90} values of amphotericin B against *C. albicans* were 0.047 and 0.125 µg/ml, while for non-albicans *Candida* spp. MIC50, MIC 90 values were 0.064 and 0.19 µg/ml, respectively.

MIC range of fluconazole for *C. albicans* and non-albicans *Candida* spp. were 0.38-64 and 0.19-256 µg/ml, respectively. Seven of the sixty five isolates in our study (10.7%) were resistant to fluconazole. Four *C. albicans*, one *C. tropicalis* and two *C. glabrata* strains were resistant strains to fluconazole. In our study, a dose-dependent susceptible strain for fluconazole was not detected. Fluconazole MIC 50 and MIC 90 values for *Candida albicans*, and non-albicans *Candida* spp. were detected as 0.75 vs. 64 µg/ml, and 0.50 vs. 4 µg/ml, respectively.

MIC range of itraconazole was 0023-32 µg/ml for *C. albicans*, and 016-32 µg/ml for non-albicans *Candida* spp. In our study, 17 of 65 isolates (26.1%) were resistant to itraconazole. However, 13 (20%) of these strains were susceptible to itraconazole in a dose-dependent manner. Five strains of *C. albicans* (29.4%) twelve (25%) of non-

albicans *Candida* spp. were resistant. Among all non-albicans *Candida* spp., all *C. glabrata* (100%), three *C. tropicalis* (75%), and three *C. parapsilosis* (8.3%) strains were resistant to itraconazole. One *C. albicans*, two *C. lusitaniae*, and ten *C. parapsilosis* strains were susceptible to itraconazole in a dose-dependent manner. MIC50 and MIC 90 values of itraconazole against *C. albicans* were 0125 and 32 µg/ml, and for non-albicans *Candida* spp. MIC 50 and MIC 90 values of the drug were 0125 and 32 µg/ml, respectively.

MIC range, MIC50 and MIC 90 of voriconazole against *C. albicans*, and non-albicans *Candida* spp. were 0012-32, 0 094 and 32 vs. 0012-32, 0 032 and 12:25 µg/ml, respectively. A total of five (7.6%) strains were found to be resistant among 65 strains investigated for their susceptibilities to voriconazole. Four of these resistant strains belonged to the *C. albicans*, and one to the *C. tropicalis* spp. None of the strains of *C. parapsilosis*, *C. glabrata* and *C. lusitaniae* spp. were resistant to voriconazole.
According to Fungifast susceptibility panel, all (100%) strains of *C. albicans*, and non-albicans *Candida* spp. were susceptible to amphotericin B, while the corresponding susceptibility rates for fluconazole (88.2 vs. 95.8%), itraconazole (82.3 vs. 89.6%) and voriconazole (94.1 vs. 97.9%) were also estimated as indicated in parentheses.

We used E-test in our study, and detected rates of susceptibility of *C. albicans* and non-albicans Candida spp. to amphotericin B (100 vs. 100%), fluconazole (76.5 vs. 93.7%), itraconazole (64.7 vs. 50%) and voriconazole (76.5 vs. 97.9%) as indicated in parentheses.

DISCUSSION

Since fungemias are often severe, rapidly progressive, and treatment-resistant diseases which are difficult to diagnose, they cause serious morbidity and mortality. Literature studies have been emphasizing potential differences among regions, and patient groups served by hospitals (Martin et al, 2005; Cheng et al., 2004; Ostrosky-Zeichner et al., 2003).

In many studies performed, various causative agents of candidemia varying from country to country, annually in the same country, and also among the hospitals were reported. According to some population based studies in the United States and Europe four strains of Candida, namely *C. albicans, C. glabrata, C. parasilosis* and *C. tropicalis* are responsible for about 95% of the cases of candidemia (Tan et al., 2008). In a study performed by the International Fungal Surveillance Group which included 32 nations, and related data recorded between years 1992 and 2001, observed that *C. parapsilosis* candidemia took the first place among cases of candidemia (Pfaller et al., 2004). In our study in 36 (55.4%) of 65 strains of Candida, *C. parapsilosis* was identified and ranked first with more than twice the number of *C. albicans* isolates. *C. albicans* was identified in 17 (26.2%) isolates and took the second place. Subsequently 6 (9.2%) *C. glabrata*, 4 (6.2%) *C. tropicalis*, 2 (3.1%) *C. lusitaniae* strains were identified in order of decreasing frequency.

The International Fungal Surveillance Group isolated 6082 strains of *Candida* spp. during a study lasting for 12 years, and detected susceptibility of *Candida* spp. to fluconazole as 90%, while rates of dose-dependent susceptibility, and resistance to this antifungal medication were 7 and 3%, respectively. In the same study, *C. glabrata* was determined as the least fluconazole susceptible strain (Pfaller et al., 2004). Matta et al. (2007) used microdilution antifungal susceptibility test in their investigation in Brazil conducted between 1995 and 2003 with 1000 Candida isolates obtained from blood cultures, and reported susceptibility rates for amphotericin B, fluconazole, itraconazole, and voriconazole as 100, 97, 93, and 99.7%, respectively. In our country, resistance to amphotericin B seems to have changed among geographic regions. Yucesoy et al. (2000) investigated *in vitro* susceptibilities of blood culture isolates of *Candida* spp. to antifungal agents, and all strains of *Candida* spp. were susceptible to amphotericin B. However, according to another study performed by Dograman et al.(2000) resistance against amphotericin B was reported for ten *C. tropicalis* spp, two *C. albicans* spp, and one *C. parapsilosis* spp. Strains. In studies performed in our country, regions with higher resistance against azole antifungals were detected. In an investigation, significant *in vitro* resistance against azole antifungals was not detected in isolates identified (Arikan et al., 2001). Koc et al. (1999) retrospectively evaluated yeast growth in blood cultures for one year, and found MIC 50 vs. MIC 90 values of fluconazole for *C. glabrata* and *C. krusei*, which are mostly encountered *Candida* spp. after *C. albicans* as 64 vs.64 and 64 vs. 128 µg/ml, respectively.

In our study, we evaluated antifungal susceptibilities using E-test method, and commercial Fungifast susceptibility panel. Based on standardization techniques, and experiences gathered, correlation between E-test, and referenced methods as for *Candida* spp and azole antifungals is at an acceptable level. Besides, E-test is a relatively valuable method in the determination of MIC value for amphotericin B, and it is one of the reliable methods for the identification of resistant isolates. In a multicenter study, Pfaller et al. (2000) detected a 86-100% concordance between results of E-test, and macrodilution test methods with respect to amphotericine B, fluconazole, flucytosine, and itraconazole. In our study, we performed E-test method using GM-MH agar instead of RPMI 1640 culture medium. In a study, the authors reported that reference macrodilution method, and E-test method used with RPMI 1640 medium are far from being practical methods that can be used routinely by every laboratory (Lee et al., 2009). In this study, for 182 isolates, GM-MH of fluconazole was used, and in comparisons with E-test and reference macrodilution methods, MIC values of these tests were concordant at a level of 82.9%. In our study, antifungal susceptibility was evaluated

by E-test method and 100, 92.3 53.8, and 89.2% of Candida isolates were found to be susceptible to amphotericin B, voriconazole, itraconazole, and fluconazole, respectively. This 100% susceptibility to amphotericin was in agreement with other studies performed in this country and abroad. Although higher rates of resistance against fluconazole were detected in our study when compared with rates in developed countries, these rates are closer to those reported in research centers localized in different regions of our country.

Susceptibility rate of all *Candida* spp. to itraconazole was 53.8%. Concordance of this susceptibility rate detected for itraconazole in literature results is controversial. In our study, itraconazole-resistance detected in all strains of *C. glabrata* spp. when compared with other drugs, resistance of all *C. glabrata* strains to itraconazole and higher rates of itraconazole-resistance among strains of non-albicans *Candida* spp. to itraconazole is a striking phenomenon. Besides, detection of 10 (27.7%) dose-dependent susceptible and three (8.3%) resistant strains of *C. parapsilosis* spp., higher incidence of nasocomial infection in our hospital caused by this agent, and also the fact that it was the most commonly isolated strain in this study increase the importance of this etiologic factor. Susceptibility rates for voriconazole appear to be in concordance with the literature findings. In our study, all strains were susceptible to both itraconazole and fluconazole at the same time. All five strains resistant to voriconazole were also resistant to fluconazole, and itraconazole.

Fungifast susceptibility panel, and E-test results were compared, and a p value of 0.001 was determined for susceptibility estimations of non-*C. albicans* to itraconazole, and $p < 0.05$ was accepted as the level of significance. Therefore, when compared with the E-test method, Fungifast susceptibility rating method was found to be insufficient for the determination of susceptibility to itraconazole. A statistical difference was not found between two tests with respect to amphotericin B, fluconazole and voriconazole.

When susceptibilities of all isolates identified in our study were evaluated, susceptibility of 89.2% of Candida isolates to fluconazole demonstrates drug's suitability for initial antifungal therapy for patients with candidemia in our hospital in consideration of its relative lack of toxicity, ease of use, affordable cost and availability. All strains of *Candida* spp. responsible for candidemia were found to be susceptible to amphotericin B. In this regard, it is a suitable drug for patients with higher risk of mortality.

In conclusion, in consideration of higher morbidity, mortality, and economic burden of the cases with fungemia, measures against emergence of these infections convey crucial importance. Typing of fungi isolated from intensive care units in particular, and their antifungal susceptibility tests should be done regularly to reveal resistance patterns of pathogens, and any increase in resistance (if any) over time, must be determined with scientific methods. We think that similar studies will guide the clinician in planning treatment of Candida infections especially in patients at risk.

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