The High Rate of Multiple-Species Candidemia Detected with Fluorescence *In Situ* Hybridization (FISH) Methods: What are the Clinical and Prognostic Implications?

Fluoresan *İn Situ* Hibridizasyon (FISH) Yöntemi ile Saptanan Yüksek Oranda Çoklu Kandidemi: Klinik ve Prognostik Anlamı Nedir?

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ABSTRACT Objective: The fluorescence in situ hybridization (FISH) with rRNA-targeted probes is a molecular method that allows identification of microorganisms in mixed assemblages from direct clinical samples without cultivitation. The aim of this study was to investigate the clinical features and risk factors in patients with single and multiple-species candidemia detected with FISH method and also to evaluate the episodes of breakthrough candidemia. **Material and Methods:** A total of 325 positive blood culture samples were examined between January and August 2004 in medical microbiology laboratory of Mersin University Hospital. The demographic and clinical data of 45 patients with candidemia detected with conventional culture methods, FISH and PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) were analyzed retrospectively, single and multiple-species candidemia were investigated among patients with candidemia and these patients were compared with respect to risk factors. **Results:** Thirty five (77.8%) of 45 patients with candidemia had a single Candida species and 10 (22.2%) had multiple-species detected by FISH method. Of these 45 cases, 31.1% had associated solid and hematologic malignancies. The mean duration of hospitalization was 27 (2-180) days. All patients had at least one indwelling catheter (urinary catheter: 80%, peripheric venous catheter: 55.6%, central venous catheter: 26.7%). Most of the patients (95.6%) had been treated with broad spectrum antibiotics. The candidemias were mostly caused by non-albicans *Candida* spp., particularly *Candida parapsilosis* (71.1%). The rate of breakthrough candidemia was detected in 11.1% of the patients with candidemia and all of these were in patients with single species candidemia. Surprisingly, in case of single-species candidemia, the mortality rate (77.8%) was higher than that of multiple-species candidemia (22.2%). **Conclusion:** According to our data, the indwelling catheters and treatment with multiple antibiotics were considered as the risk factors for single and multiple-species candidemia. Although the rate of multiple-species candidemia detected with FISH method was found to be high, clinical and prognostic significance of multiple-species candidemia are still debatable.

Key Words: Fungemia; candida; in situ hybridization, fluorescence

ÖZET Amaç: Fluoresan in situ hibridizasyon (FISH) rRNA'yı hedef alan problar kullanarak kültür yöntemlerine başvurmaksızın direkt klinik örneklerden karışık ortamdaki mikroorganizmaların identifikasyonuna olanak sağlayan moleküler bir yöntemdir. Bu çalışmanın amacı FISH yöntemi ile saptanan tekli ve çoklu kandidemisi olan hastaların klinik özelliklerini ve risk faktörlerini araştırmak ve kandidemi ataklarını değerlendirmekti. **Gereç ve Yöntemler:** 2004 yılı Ocak ve Ağustos ayları arasında Mersin Üniversitesi Tıp Fakültesi tıbbi mikrobiyoloji laboratuvarındaki toplam 325 pozitif kan kültürü örneği değerlendirildi. Konvansiyonel kültür yöntemleri, FISH ve PZR-RFLP (polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi) ile saptanan 45 olgunun demografik ve klinik verileri retrospektif olarak incelendi. Kandidemili olgular arasında tekli ve çoklu kandidemiler araştırıldı ve risk faktörleri yönünden karşılaştırıldı. **Bulgular:** Kandidemisi olan 45 olgunun 37'sinde (%77.8) FISH yöntemiyle saptanan tek bir kandida türü ve 10'unda (%22.2) çoklu kandida türleri vardı. Bu 45 olgunun %31.1'inde aynı zamanda solid ve hematolojik malignensi vardı. Ortalama hastanede kalış süresi 27 (2-180) gündü. Tüm hastaların en az bir kateteri vardı (idrar kateteri: %80, periferik venöz kateter: %55.6, santral venöz keteter %26.7). Hastaların çoğu (%95.6) geniş spektrumlu antibiyotiklerle tedavi edilmişti. Kandidemilerin nedeni çoğunlukla albikans dışı kandida türleri, özellikle de *Candida parapsilosis* (%71.1) idi. Tedavi altındaki kandidemili olgularda kandidemi atağı oranı %11.1 idi ve bunların tamamı tekli kandidemi olan hastalardı. Şaşırtıcı şekilde, tek türün neden olduğu kandidemilerde mortalite hızı (%77.8), çoklu kandidemilere (%22.2) göre daha yüksekti. **Sonuç:** Verilerimiz ışığında tek ve çoklu kandidemiler için kateterler ve çoklu antibiyotik tedavisinin risk faktörü olduğu düşünüldü. Bazı olgularda FISH yöntemiyle çoklu kandidemi saptanması ve bu oranın yüksek bulunmasına rağmen, çoklu kandidemilerin klinik ve prognostik önemi halen tartışmalıdır.

Anahtar Kelimeler: Fungemi; kandida; in situ hibridizasyon, flöresans

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Bloodstream fungal infections are the major healthcare problems in hospitals all over the world and are associated with significant morbidity and mortality.[1-3] The infections caused by *Candida* species has increased in recent years, being the fourth most common nosocomial pathogen. Moreover, studies from United States and Europe indicated that they are similarly the fourth most common bloodstream pathogen in these countries.[2,4,5] *Candida albicans* and other *Candida* species are responsible from 70% to 80% of invasive bloodstream fungal infections.[2,6,7] Most of the *Candida* species, including *Candida albicans*, are susceptible to systemic antifungal agents, such as fluconazole and amphotericin B.[1,2] The rates of candidemia caused by non-*albicans Candida* spp. are reported to be increased in several studies. They are associated with a resistance to antifungals and risk of increased mortality rates.[7-9] Non-*albicans Candida* spp. accounted for 60% of the candidemia episodes in cases receiving antifungal prophylaxis.[1,10]

Bloodstream infections involving either bacteria or fungi are generally caused by a single species.[1,2,11] Bacteriemic episodes with multiple-species are generally nosocomial in origin and their mortality rate is higher than the episodes with a single species. However, multiple-species fungemia is an infrequent clinical entity and it is rarely reported in the literature.[3,12] The rates of multiple-species candidemia (MSC) was reported as 1.7-11.6% in publications on candidemia.[3,13-15] The multiple-species and single-species candidemia (SSC) are found to be similar in respect to their mortality rates.[15] Recent studies revealed an increase in incidence of candidemia during antifungal prophylaxis or therapy. The rate of breakthrough candidemias ranged between 10.5-25% in several studies.[16-18] The mortality rate reached to 70-80% in leukemia and in other cancer patients.[17,19,20] However, studies on breakthrough candidemia in patients with multiple-species candidemia are limitted in the literature.

Fluorescence *in situ* hybridization (FISH) with rRNA-targeted fluorescently labelled oligonucleotide probes is a powerful molecular technique for the identification of microorganisms in clinical and environmental samples.[21] Several studies have shown that mixtures of isolates in cultures can be detected by FISH method and FISH is more sensitive than conventional methods in the detection of mixtures of isolates in blood cultures.[22-24]

The aims of this study were first to investigate the clinical features and risk factors in patients with single and multiple-species candidemia detected by FISH method. In addition, the episodes of breakthrough candidemia were evaluated.

MATERIAL AND METHODS

MICROBIOLOGY

This study was carried out in Mersin University Medical Faculty Hospital, a 200-bed-hospital in Mersin Turkey. Three hundred twenty five (13%) out of 2492 blood cultures positive for a micro-organism and *Candida* spp. were isolated in 50 (15.4%) of these samples between January 2004 and August 2004 in medical microbiology laboratory. Each one of the positive blood cultures was obtained from different patients and samples were withdrawn directly from a vein, not through a catheter. BACTEC 9050 system (BD Diagnostic Systems, UK) was used for blood cultures. Sabouraud dextrose agar (Merck, Germany) and CHROMagar *Candida* (Becton Dickinson, France) medium were used for the identification of *Candida* spp. from positive blood cultures with conventional culture methods. Then, the *Candida* spp. on agar plates were identified by standard laboratory methods detecting germ tube formation, chlamydospore formation on cornmeal agar, and with API ID 32C (bioMerieux, France) test.

FISH and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods were the molecular methods that were performed for identification of particular *Candida* spp. FISH method was applied as described in previous studies.[25,26] For each hybridization reaction, 10 µl was dropped on microscope slide per positive blood culture and dried at 45°C for 20 min. The slides were dehydrated in 50%, 80% and 100% ethanol for 3 min each. Fluorescein isothiocyanate

(FITC) and CY3-labelled oligonucleotide probes for *Candida* spp. were used to identify following particular species: Caal for *C. albicans,* Ckrus for *C. krusei*, Cpara for *C. parapsilosis*, Cagl for *C. glabrata*. Beside these, universal probe for all yeast (PF2) and negative control (nonEub388) were applied simultaneously. One microliter of probe (50 ng) as well as 9 µl of hybridization buffer containing 20% formamide were added into each well and incubated in a moist chamber at 46°C for 1.5 h. Following this, the slides were washed in washing buffer at 46°C for 15-20 min and then 10 µl of 0.001% DAPI staining was added. After the slides were air-dried, Citifluor (Citifluor Ltd., London) was added as a mounting medium over the slides. Finally, the slides were examined under microscope (NIKON E 1000) equipped with a standard filter set and imaging analysis system (Applying Image).

For PCR technique, DNA isolation was performed in the MagNA pure LC system (Roche Diagnostics; Germany) by using MagNA Pure LC DNA isolation kit III (Roche, Germany). The PCR-RFLP method was implemented as described by Mirhendi et al.[27] ITS1 and ITS4 (Fungus-specific universal primer pairs) primers (Molbio, Germany) were used for PCR-RFLP. All reactions for PCR were carried out in a total volume 50 µl. For each reaction the following were used Template DNA (1 µl), forward (ITS1) and reverse (ITS4) primer (0.2 µM), deoxynucleocide triphosphate (dNTP) (0.1 mM), 10X PCR buffer (5 µl) and of *Taq* DNA polymerase (2.5 U). The first denaturation step was carried out at 94°C for 5 min and followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 1 min and final extension at 72°C for 7 min. PCR products were visualized by 2% agarose gel electrophoresis in TBE buffer stained ethidium bromide (0.5 µg ml^{-1}). *Msp*I enzyme (Fermentas, EU) was used to obtain the species-specific length patterns. A 10 µl aliquot of PCR product was combined with 10U *Msp*I and the final reaction volume, 25 µl, was incubated at 37°C for 2 h. Restriction fragments were separated by 3% agarose gel electrophoresis in TBE buffer at 80 V for 45 min and visualized by staining with ethidium bromide.

DEFINITIONS

Candidemia was defined as the isolation of any *Candida* spp. from one or more blood cultures taken from a patient with symptoms and signs of an infection.[8] The presence of two or more *Candida* spp. from a single or different sets of blood cultures obtained within a 72-h period was termed as multiple-species candidemia.[15] Antifungal treatment was administeted to all patients, except the neutropenic patients with candidemia following isolation of microorganisms from the clinical specimens on cultures. Breakthrough candidemia indicated candidemia in a patient who had received at least three days of systemic antifungal therapy.[16]

PATIENTS

The demographic and clinical data of patients with single, multiple-species (from our unpublished study using FISH technique) and breakthrough candidemia were analyzed retrospectively in the Department of Clinical Microbiology and Infectious Diseases, Mersin University Hospital. The medical records of 45 patients were reviewed for the demographic and clinical features, management and outcomes. Single and multiple-species candidemia cases were compared based on the following risk factors; history of solid and hemotologic malignancy, the mean duration of hospitalization/catheterization, antibiotic treatments, nutritional support with total parenteral nutrition and corticosteroid use. Five patients were excluded from the study because of insufficient medical records.

STATISTICAL ANALYSIS

Statistical analysis were performed using SPSS software (SPSS 11.5 for Windows). Descriptive statistics and Likelihood Ratio Chi-Square tests were performed for defining the basic features of the data and the comparing the two groups; single and multiple-species candidemia. Likelihood Ratio Chi-Square method can be more effective than Chi-square statistics in case of observed and expected frequencies in the cells are less than five. In addition, two proportions test was performed to compare the proportions in two groups by using Minitab 6.1 statistical software.

RESULTS

Among 45 patients with candidemia; 35 (77.8%) and 10 (22.2%) patients had SSC and MSC, respectively. The most commonly isolated *Candida* spp. from the blood cultures was *Candida parapsilosis.* Of these samples, 48.9%, 53.3% and 48.9% were detected with FISH, PCR-RFLP and conventional methods, respectively. In mixed cultures, *C. albicans* and *C. parapsilosis* (11.1%) were the most common *Candida* spp. determined by FISH (Table 1). The incidence of MSC detected with FISH, PCR-RFLP and conventional methods were 22.2%, 6.7% and 4.4%, respectively.

The general characteristics of patients with single and multiple-species candidemia are presented in Table 2. All patients with candidemia had at least one catheter; urinary, peripheral venous and central venous catheters were present in 80%, 55.6%, and 26.7% of the patients, respectively. Beside these, 95.6% had been receiving antibiotics. Single and multiple-species candidemia cases were compared based on the following risk factors; history of solid and hemotologic malignancy, the mean duration of hospitalization/catheterization, antibiotic treatments, nutritional support with total parenteral nutrition and corticosteroid use. The statistical analysis revealed no differences between the two groups ($p > 0.05$). Of the patients with candidemia, 55.6 % were treated with antifungal drugs (Liposomal amphotericin B [40%], and fluconazole [32%]). There was no statistically significant difference between two groups in respect to treatment with antifungal drugs ($p > 0.05$). In two of three patients who were on caspafungin therapy MSC was determined (Table 3) and all patients died on treatment of caspafungin. The incidence of single species episodes was higher in internal, medical, surgical and intensive care units than that of multiple-species episodes ($p < 0.05$). However, the multiple-species candidemias were higher particularly in internal medicine ($p < 0.05$) and intensive care units ($p < 0.05$) than medical and surgery units. The overall mortality rate in candidemia was 40%, the rates being higher in SSC patients (77.8%) than MSC patients (22.2%). Non-albicans *Candida* spp., especially *C. parapsilosis* ($p < 0.05$) dominated the candidemia episodes in both groups.

TABLE 1: The types of *Candida* spp. with FISH, PCR-RFLP and conventional culture methods.

Candida spp.	FISH n (%)	PCR-RFLP n (%)	Culture methods n (%)
Candida parapsilosis	22 (48.9)	24 (53.3)	22 (48.9)
Candida albicans	10 (22.2)	13 (28.9)	13 (28.9)
Candida tropicalis	ND	4 (8.9)	8 (17.8)
C. albicans + *C. parapsilosis*	5 (11.1)	-	1 (2.2)
C. tropicalis + *C. parapsilosis*	-	3 (6.7)	-
C. parapsilosis + *R. mucilaginosa*	-	-	1 (2.2)
Candida spp + *C. parapsilosis*	5 (11.1)	-	-
Candida spp.	3 (6.7)	-	-
Negative	-	1 (2.2)	-

ND: Not determined because *C. tropicalis* specific probe was not used.
FIS: Fluorescence in situ hybridization.
PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.

Characteristics of patients with breakthrough candidemia are presented in Table 3. Breakthrough candidemia was detected in 11.1% of the candidemic patients. Moreover, it was noted that breakthrough candidemia was diagnosed only in patients with SSC. All of the patients had at least one catheter and they were treated with broad-spectrum antibiotics (three patients with multiple antibiotics; two with a single antibiotic). Most of the breakthrough episodes occurred in patients receiving amphotericin B.

DISCUSSION

There are only a few published studies on multiple-species candidemia. Recent studies on MSC indicate that this entity is an uncommon event.[3,12,28] Pulimood et al. reported that the incidence was 5.2% during a nine year period.[15] Guerra-Romero et al. indicated multiple-species was present in 3.4% of patients with fungemia.[28] Yamamura et al. observed that MSC occurred in 1.7% of cases.[3] However, a recent study indicated a higher incidence in patients with cancer (the incidence being 33% and 52% in case of acute leukemia and solid tumors, respectively).[29] In the present study, the incidence was found higher by using molecular methods (22.2% with FISH; 6.7% with PCR-RFLP) compared to conventional methods (4.4%). The

TABLE 2: The general characteristics of patients with candidemia and comparison of patients with single and multiple-species candidemia detected with FISH.

Variable	Candidemia (n= 45)	Single species candidemia (n= 35)	Multiple-species candidemia (n= 10)	P value[a,b]
Candidemia	45 (100)	35 (77.8)	10 (22.2)	
Age (mean, years)	42.80 ± 23.55	44.57 ± 23.72	36.50 ± 22.98	
Age groups				0.248[a]
<20	9 (20)	6 (66.7)	3 (33.3)	
21-40	8 (17.8)	7 (87.5)	1 (12.5)	
41-60	17 (37.8)	12 (70.6)	5 (29.4)	
<61	11 (24.4)	10 (90.9)	1 (9.1)	
Sex				0.366[a]
Female	17 (37.8)	12 (70.6)	5(29.4)	
Male	28 (62.2)	23 (82.1)	5 (11.1)	
Underlying Diseases				0.148[a]
Haemotologic malignancy	4 (8.9)	4 (100)	0	
Solid malignancy	10 (22.2)	9 (90)	1 (10)	
Others	31 (68.9)	22 (71)	9 (29)	
Hospitalization /day (mean)	35.96 ± 30.60	39.20 ± 32.47	25.30 ± 20.76	
<10	3 (6.7)	1(33.3)	2 (66.7)	0.338[a]
11-30	22 (48.9)	17 (77.3)	5 (22.7)	
31-50	13 (28.9)	11 (84.6)	2 (15.4)	
>51	7 (15.6)	6 (85.7)	1 (14.3)	
Catheter use	45 (100)	35 (77.8)	10 (22.2)	
Single	10 (22.2)	9 (90)	1 (10)	0.292[a]
Multiple	35 (77.8)	26 (74.3)	9 (25.7)	
Urinary catheter				0.370[a]
Yes	36 (80)	27 (75)	9 (25)	
No	9 (20)	8 (88.9)	1 (11.1)	
Periferic venous catheter				
Yes	25 (55.6)	20 (80)	5 (20)	0.688[a]
No	20 (44.4)	15 (75)	5 (25)	
Central venous catheter				
Yes	12 (26.7)	9 (75)	3 (25)	0.787[a]
No	33 (73.3)	26 (78.8)	7 (21.2)	
Jugular catheter				
Yes	12 (26.7)	7 (58.3)	5 (41.7)	0.058[a]
No	33 (73.3)	28 (84.8)	5 (15.2)	
Antibiotic use				
Yes	43 (95.6)	34 (79.1)	9 (20.9)	0.377[a]
No	2 (4.4)	1 (50)	1 (50)	
Single	12 (27.9)	9 (75)	3 (25)	0.624[a]
Multiple	31 (72.1)	25 (80.6)	6 (19.4)	
Antibiotic				
Glycopeptide	1 (2.3)	1(100)	0	0.181[a]
Anti-pseudomonal	19 (44.2)	15 (78.9)	4 (21.1)	
Glycopeptide+beta lactam	8 (18.6)	8 (100)	0	
Others	15 (34.9)	10 (66.7)	5 (33.3)	

cont'd

TABLE 2: *cont'd.*

Antifungal drug use				
Yes	25 (55.6)	20 (80)	5 (20)	0.688[a]
No	20 (44.4)	15 (75)	5 (25)	
Antifungal drug				
Amphotericin B	3 (12)	3 (100)	0	0.116[a]
Fluconazole	8 (32)	7 (87.5)	1 (12.5)	
Liposomal amphotericin B	10 (40)	9 (90)	1 (10)	
Caspofungin	3 (12)	1 (33.3)	2 (66.7)	
Fluconazole+amphotericin B	1 (4)	0	1 (100)	
Total parenteral nutrition				
Yes	22 (48.9)	19 (86.4)	3 (13.6)	0.175[a]
No	23 (51.1)	16 (69.6)	7 (30.4)	
Corticosteroid				
Yes	12 (26.7)	10 (83.3)	2 (16.7)	0.589[a]
No	33 (73.3)	25 (75.8)	8 (24.2)	
Unit				0.024[a]
Internal medicine units	25 (55.6)	18 (72)	7 (28)	0.001[b]
Medical surgery units	12 (26.7)	12 (100)	0	0.001[b]
Intensive care unit	8 (17.8)	5 (62.5)	3 (37.5)	0.302[b]
Status				
Exitus	18 (40)	14 (77.8)	4 (22.2)	1.00[a]
Discharge	27 (60)	21 (77.8)	6 (22.2)	
Candida spp.				0.001[a]
C. parapsilosis	22 (48.9)	21 (100)	0	
C. albicans	10 (22.2)	10 (100)	0	
C. albicans +*C. parapsilosis*	5	(11.1)	0	5 (100)
Candida spp.[c] + *C. parapsilosis*	5	(11.1)	0	5 (100)
Candida spp.[c]	3 (6.7)	3 (100)	0	
Concomitant non-fungal				
Infection	0			
Breakthrough candidemia				0.101[a]
Yes	5 (11.1)	5 (100)	0	
No	40 (88.9)	30 (75)	10 (25)	

[a]: Chi-squared test. [b]:Two proportions test. [c]. The samples detected as *Candida* spp., with FISH were identified as C. tropicalis with PCR-RFLP and conventional culture methods.

ability to detect mixed infections may be dependent on the preferred method, and conventional methods may be insufficient to determine the mixed incidents. CHROMagar *Candida* medium may be promising in detecting *Candida* spp. in mixed cultures. In a recent study, Jensen et al. reported that no case of mixed fungemia could be detected in 217 episodes prior to CHROM-agar *Candida* medium, whereas 15 episodes of mixed fungemia (2.8%) over 530 episodes were demonstrated by using CHROMagar *Candida* medium.[13] On the other hand, in the following study, conventional methods like CHROMagar *Candida* medium yielded lower rates of MSC. However, molecular methods, particularly FISH method, allowed detection of multiple-species infections. Many studies indicated that FISH method can identify microorganisms with very high sensitivity (100%) and specificity (100%) and mixed infections can also be detected by this method.[22-24] Beside this, other stu-

TABLE 3: The main characteristics of patients with breakthrough candidemia (n= 5).

Age/sex	Underlying disease	Associated factors	*Candida* spp.	HP/day	Antibiotic use	Antifungal use	Status
58/F	Chronic myeolid leukemia	PVC, Corticosteroid	*C. glabrata*	18	Imipenem	AmB	Discharge
63/M	Bladder Tumor	Urinary catheter, PVC, TPN	*C. albicans*	28	Piperacillin Tazobactam Gentamicin	Fluconazole	Discharge
32/M	Acute lymphoblastic leukemia	PVC, TPN Corticosteroid	*C. parapsilosis*	35	Meropenem Amikacin Teicoplanin	AmB	Exitus
46/M	Necrotizing pancreatitis	Urinary catheter Jugular catheter	*C. parapsilosis*	27	Imipenem Vancomycin	AmB	Discharge
7/M	Non Hodgin lymphoma	PVC, TPN Corticosteroid	*C. parapsilosis*	57	Cefepime	AmB	Discharge

HP: hospitalization period; AmB: amphotericin B; PVC: peripferic venous catheter; TPN: total parenteral nutrition; F: female; M: male.

dies have indicated that PCR-EIA and semi-nested PCR were more sensitive than routine phenotypic methods for the detection and identification of microorganisms in mixed cultures.[30,31] Molecular methods may be more useful in the diagnosis of mixed infection than the conventional culture methods. However, the rate of multiple-species detected by FISH method was found to be higher than PCR-RFLP. PCR-RFLP might be affected by negative factors such as storage and transport of samples, inhibitors in blood and the presence of a small amount of the DNA in the sample, these and may be responsible for the diffence. Therefore FISH may be a more suitable method for the detection of multiple-species candidemia.

The high rate of MSC in the present study can be related to catheter-related nosocomial infections, because all patients had catheters and *C. parapsilosis* was the most common *Candida* spp. in mixed cultures. Levin et al. reported an outbreak of *C. parapsilosis* fungemia related to long-term central venous catheters and hand hygiene of health care workers were questioned because of slime production of the strains.[32] Similarly, Otag et al. showed that the most frequently observed species were *C. parapsilosis* (51.8%) in blood cultures.[33] These reports are compatible with our findings indicating an increase in the incidence of non-*albicans Candida* spp. in the intensive care units of our hospital. In the future, as the molecular methods are used for the identification of *Candida* spp. in blood cultures, the incidence of mixed episodes may increase in incidence.

The mortality rate of MSC was 22.2% in our study. Verghese et al., Pulimood et al. and Guerra-Romero et al. found the mortality rates in mixed septicemia as 78%, 43% and 59%, respectively.[15,28,34] However, the mortality rate in the patients with MSC was not higher than those with SSC. Similar to the present study, Pulimood et al. and Guerra-Romero, et al. have observed the same tendency with similar mortality rates.[15,28]

The rate of MSC in patients treated with caspofungin was higher than those with SSC in this study. However, although all the patients treated with caspofungin had died, resistance pattern of isolates from patients with MSC treated with caspofungin were not determined in this study. Moreover, the number of patients with MSC treated with caspofungin was small for a statistical analysis. Further prospective studies would be helpful in understanding of this matter.

Breakthrough candidemia occurred in 11.1% of the patients and all of these had SSC in this study. Mortality rate was 20% in these patients. Similar to the present study, Uzun et al. found that the incidence was 10.2% in cancer patients.[20] In

one study on cancer patients, Chung et al. observed breakthrough candidemia in 18.5%.[17] These authors stated that the mortality rates were 85.7% and 42.9% in breakthrough and non-breakthrough candidemia groups, respectively. Boktour et al. reported that the rate of breakthrough candidemia was higher in MSC compared to single-agent ones.[29] The episodes of breakthrough candidemia were reported to be caused by non-*albicans Candida* spp.[17,35] In the present study, the most prevalent *Candida* spp. was *C. parapsilosis* (60%) in patients with breakthrough candidemia. Similarly, Pasqualotto et al. observed similar figures indicating that non-*albicans* C*andid*a spp. (mainly *C. parapsilosis* 30%) were responsible from 75% of these infections.[16] In this study, the following risk factors were correlated with breakthrough candidemia; serious underlying diseases, catheter use, use of antibiotics and antifungal drugs, and prolonged hospitalization.

In conclusion, the incidence of MSC was unexpectedly high in our hospital. Yet, MSC had a less severe outcome than SSC. The distinctive diagnosis of the MSC is clinically difficult and it may be missed by conventional methods. Therefore, molecular methods, especially FISH may be preferred for identification of *Candida* spp. Although, the affect of MSC is not clear on the prognosis of the patient and the development of breakthrough candidemia, FISH method may be important for the identification of resistant fungus and tailoring successful and effective treatment. There are only a few studies in literature on MSC, and the clinical importance of this entity is still to be recognized. In the future, as the number of studies increases and molecular methods are routinely used in the diagnosis, the clinical importance of MSC may be understood better. In our hospital, indwelling catheters and treatment with multiple antibiotics are considered as the main risk factors for candidemias. In order to reduce the morbidity and mortality of candidemia, limited use of broad spectrum antibiotics, removal of indwelling catheters and initiation of antifungal therapy are crucial for management in hospitals.

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