Jundishapur J Microbiol. 2014 December; 7(12): e13141. DOI: 10.5812/jjm.13141

Published online 2014 December 1. **Research Article**

Distribution of Yeast-Like Fungi at a University Hospital in Turkey

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Received: June 23, 2013; **Revised:** September 25, 2013; **Accepted:** October 14, 2013

Background: The increased life span has led to application of more invasive procedures for diagnosis and treatment of particularly immunosuppressed individuals. This situation drew more attention to fungal infections due to existence of yeast-like fungi. *Candida* infections have increased due to transplant in patients, prolonged intensive care unit (ICU) stays, and invasive procedures. Recently, identification of yeast-like fungi as well as antifungal susceptibility test has been gaining more importance.

Objectives: In our study, we aimed to evaluate the distribution of yeast-like fungi strains isolated from blood, urine, wound and respiratory specimens, which were sent from various departments of Izmir University School of Medicine University Hospital.

Materials and Methods: The 262 yeast strains (of 13860 clinical specimens), isolated during 30.05.2012-20.05.2013, which were sent from various departments of Izmir University School of Medicine to Medical Microbiology Laboratory, were included in this study. Blood, wound, respiratory (sputum, tracheal secretion), and urine specimens were cultivated on blood agar and Sabouraud dextrose agar and incubated for 24-48 hours at 37°C. The isolates were cultivated on CHROMagar *Candida* and Cornmeal Tween 80 medium for identification. Besides, the automatized Vitek version 2.0 system was used for identification of the yeast strains as well as the antifungal susceptibility of blood culture strains.

Results: A total of 262 strains, isolated from the Anesthesiology and Reanimation Unit, as well as from the departments of Hematology, Urology, Infectious Diseases, Gynecology and Obstetrics, and Ear Nose and Throat, were included in this study. The most common isolated yeast-like species was *Candida albicans*. *C. parapsilosis* was the most common yeast-like fungus isolated from blood cultures. All the blood culture strains were susceptible to amphotericin B, flucytosine, fluconazole and voriconazole.

Conclusions: *Candida* strains isolated from newborns, elderly patients, and intensive care patients, identified and isolates from blood cultures, should be studied for antifungal susceptibility for management of the treatment. Our University Hospital is a recently opened center and these are the first data of our center. Gradually, as the number of patients increases, this data will be evaluated further.

Keywords: Yeast-Like Fungi; *Candida* Species; Antifungal Susceptibility

1. Background

Fungal agents are prevalent in nature, besides in gastrointestinal system and skin flora. They can gain pathogenicity under appropriate conditions, which may lead to infection. Hematological malignancy, immunosuppressive therapy, bone marrow transplantation, organ transplantation, radiotherapy, surgical procedures, burn, and long stay in intensive care units are the major risk factors for fungal infections. Recently, opportunistic fungal infections have been increasing, particularly in intensive care units. Mortality due to fungal infections is more than other infections; in addition, the therapeutic doses and toxic limits of antifungal drugs are close to each other. Due to this fact, antifungal susceptibility of clinical yeast-like isolates of these patients should be carried out. Identification of *Candida* species is important both for detection of intrinsic resistance and choosing appropriate therapy (1-5).

Studies held in the US and Europe reported that nosocomial bloodstream infections due to candidemia, particularly non-*albicans* cases, have increased. Before 1990s, non-*albicans* isolates causing fungal infections constituted 10-40% of the strains; after this period, this rate increased up to 35-63% (6-8). *Candida* species are prevalent in nature and belong to *Cryptococceae* family. They are anamorphic yeast-like fungi. Approximately, 200 species of *Candida* exist in the world (9). Primarily, they are located in gastrointestinal system and mucocutaneous membranes (10). Colonization in healthy subjects increases, starting from mouth to colon. Vagina is intensely colonized with *Candida*. It is reported that healthy women are 30% colonized with *Candida* in vagina. *Candida albicans* is the most commonly isolated strain. *Candida* species are not common on intact skin; but, *C. albicans* is usually isolated from superficial mycoses. Environmental cultures may reveal non-*albicans* strains (10). Treatment is unnecessary in cases of isolation from sputum, feces, urine and vagina, and if there are no clinical symptoms. In cases with clinical signs, isolation from sterile body fluids and high number of colonies are indications for treatment initiation (11).

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Unnecessary use of broad spectrum antibiotics, catheterization, and mucosal colonization in patients with risk factors are important predisposing factors (12). Initiation of azole group antifungal drugs for prophylaxis and treatment led to increased isolation of resistant non-*albicans Candida* strains (13).

2. Objectives

Recently, identification of fungi and antifungal susceptibility test have been gaining importance. In our study, we aimed to evaluate the distribution of *Candida* strains isolated from blood, urine, wound and respiratory specimens, which were sent from various departments and assess the antifungal susceptibility results of *Candida* species isolation from blood cultures at Izmir University School of Medicine University Hospital.

3. Materials and Methods

The 262 yeast strains (of 13860 clinical specimens), isolated during 30.05.2012-20.05.2013, which were sent from various departments of Izmir University School of Medicine to Medical Microbiology Laboratory, were included in this study. Blood, wound, respiratory (sputum, tracheal secretion), and urine specimens were cultivated on blood agar and Sabouraud dextrose agar (SDA) (Salubris, Turkey) and incubated for 24-48 hours at 37°C. The isolates were cultivated on CHROMagar *Candida* (Salubris, Turkey) and Cornmeal Tween 80 mediums (Salubris, Turkey) for identification. Germ tube test was also performed to differentiate between *C. albicans* and non-*albicans Candida strains*. The colonies from CHROMagar and SDA were plated on cornmeal agar with Tween 80 for morphological examination of production of chlamydospores, blastospores, true hyphae, and branched pseudohyphae. The results were again compared with the colonies grown on CHROMagar. This medium was ready to use. The appearance of colonies, including color, size, and textures on CHROMagar *Candida* was analyzed.

Automatized Vitek version 2.0 system (Biomerieux, France) was used for identification, using biochemical parameters. Finally, the results were again compared with the culture results and automatized Vitek version 2.0 system results and *Candida* species were identified. Antifungal susceptibility of the blood culture strains was studied on Vitek version 2.0 system (Biomerieux, France). The Vitek 2 cards, containing serial twofold dilutions of amphotericin B (concentration range: 0.25-16 μg/mL), flucytosine (concentration range: 1-64 μg/mL), and voriconazole (concentration range: 0.125-8 μg/mL) were provided by the manufacturer. The minimum inhibitory concentration (MIC) results, obtained by Vitek 2 system following 9.1 to 27.1 hours of incubation, were evaluated (14, 15).

4. Results

A total of 262 strains, isolated from the Anesthesiology and Reanimation Unit, as well as the departments of Hematology, Urology, Infectious Diseases, Gynecology and Obstetrics, and Ear Nose and Throat, were included in this study. Distribution of the yeast isolates according to the specimen type is shown in Table 1. Distribution of the hospital departments is as follows: 174 Anesthesiology and Reanimation Unit, 28 Infectious Diseases, 20 Hematology, 16 Urology, 8 Ear Nose and Throat, and 16 Gynecology and Obstetrics. Distribution of *Candida* species according to different departments of the hospital is shown in Table 2. *C. albicans*, *C. parapsilosis* and *C. glabrata* strains, isolated from the blood cultures, were all susceptible to amphotericin B, flucytosine, fluconazole, and voriconazole.

5. Discussion

Candida species led to superficial and deep infections in the past decade. Superficial infections are usually detected in the community and deep systemic infections are nosocomial (16). *C. albicans* is the most common strain isolated from the gastrointestinal system. This strain causes 90-100% of mucosal infections and 50-70% of candidemia cases. *C. tropicalis* and *C. glabrata* have the second place after *C. albicans*. *C. parapsilosis* is in the skin flora, accompanying *C. albicans* (16, 17).

Table 1. Distribution of *Candida* Isolates According to the Specimen Type [a]

Clinical Specimen/Isolated Species	Urine (n = 110)	Wound (N= 48)	Sputum (n = 46)	Tracheal Secretion (n = 46)	Blood (n = 12)	Total (n = 262)
C.albicans	62 (56.3)	30 (62.5)	32 (69.5)	30 (65.2)	2 (6.7)	156 (59.5)
C.krusei	28 (25.4)	-	8 (17.5)	-	-	36 (13.7)
C.glabrata	10 (9.09)	14 (29.2)	4 (8.7)	8 (17.4)	2 (16.7)	38 (14.4)
C.parapsilosis	-	4 (8.3)	-	2 (4.3)	8 (66.6)	14 (5.2)
C.famata	2 (1.8)	-	-	-	-	2 (0.8)
C.tropicalis	4 (3.7)	-	2 (4.3%)	6 (13.1)	-	12 (4.8)
C.kefyr	4 (3.7)	-	-	-	-	4 (1.6)

[a] Data are presented as No. (%).

Table 2. Distribution of *Candida* Species According to Hospital Departments [a]

Hospital Ward/Isolated Species	Anesthesiology and Reanimation Unit	Infectious Diseases	Hematology	Urology	Ear Nose	Gynecology and Obstetrics
C.albicans **(n = 156)**	124 (71.2)	8 (28.5)	6 (30)	4 (25)	2 (25)	12 (75)
C.krusei **(n = 36)**	20 (16.1)	6 (21.4)	6 (30)	4 (25)	-	-
C.glabrata **(n = 38)**	16 (9.2)	8 (28.5)	-	4 (25)	6 (75)	4 (25)
C.parapsilosis **(n = *14*)**	4 (2.29%)	4 (14.2)	6 (30)	-		-
C.famata **(n = *2*)**	2 (1.14)	-	-	-	-	-
C.tropicalis **(n = *12*)**	6 (3.4)	2 (7.1)	2 (10)	2 (12.5)	-	-
C.kefyr **(n = *4*)**	2 (1.1)	-	-	2 (12.5)	-	-

[a] Data are presented as No. (%).

The most common *Candida* strain is *C. albicans* in the US and Northern and Middle Europe. Non-*albicans* strains are common in Asia, South Europe, and South America (18). Of blood stream infections, 95-97% is caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis,* and *C. krusei*. The rest (3-5%) are caused by *C. parapsilosis C. lusitaniae*, *C. guilliermondii*, and *C. rugosa* (19). Ece et al. evaluated the distribution of *Candida* isolates at a tertiary care center in Turkey. Yeast-like fungi were isolated from 337 clinical specimens. They were urine, blood, wound, and respiratory specimens. The most isolated yeast strains were *C. albicans* (38.6%), *C. tropicalis* (13.9%), *C. parapsilosis* (28.4%), *C. glabrata* (7.4%), and *C. krusei* (3.8%). The authors concluded that there was an increment in *Candida* infections. In this study, the most common strain was *C. albicans* and the rate of *C. glabrata* and *C. krusei* isolates were lower than expected. *C. parapsilosis* was the most isolated strain in blood cultures which may be due to invasive procedures and the use of indwelling catheters (20). Similar to this data, we also isolated *C. parapsilosis* as the most common yeast-like fungus from blood cultures.

Zer et al. evaluated the distribution of *Candida* species isolated from urine (54%), blood (11%), and tracheal secretion (28%) samples sent from the intensive care unit. The strains consisted of 56.09% *C. albicans*, 11.21% *C. tropicalis*, 10.24% *C. parapsilosis*, 5.83% *C. glabrata*, 4.39% *C. kefyr*, 3.41% *C. lusitaniae*, 2.92% *C. famata*, 2.92% *C. krusei,* and 2.92% *C. guilliermondii* (21). The resistance rates were 19.51% to amphotericin B, 27.31% to fluconazole, and 20% to flucytosine, using E-test method. We found that all of our blood culture isolates were susceptible to amphotericin B, flucytosine, voriconazole, and fluconazole. This might be due to the fact that these are the results of a recently opened center.

Comert et al. investigated the species distribution of the yeast-like fungi isolated from an intensive care unit. The clinical specimens consisted of urine (58%), blood culture (7%), and respiratory tract specimens (15%). The 320 strains consisted of 65.6% *C. albicans*, 11.3% *C. parapsilosis*, 8.8% *C. glabrata*, and 7.8% *C. tropicalis* (22). Antifungal susceptibility test was performed by broth microdilution method. Of all the isolates, 92.9% revealed susceptibility to fluconazole. Susceptibility to fluconazole was the highest for *C. albicans,* followed by *C. parapsilosis* and *C. glabrata*. We performed antifungal susceptibility test by automatized Vitek version 2.0 system and detected 100% susceptibility to fluconazole among the blood culture strains.

Ergon and Yucesoy evaluated the distribution of *Candida* species isolated from intensive care units in a four-year period. The strains consisted of 53.3% *C. albicans*, 14.5% *C. tropicalis*, 12.2% *C. glabrata*, 6.5% *C. parapsilosis*, 4.5% *Trichosporun* spp., 3.9% *C. kefyr,* and 1.6% *C. krusei*. The authors emphasized that *C. albicans* was the most common isolated strain and there was an increment for *C. glabrata* and *C. tropicalis* strains (23).

Motta et al. investigated the distribution and antifungal susceptibility of the yeast-like fungi isolated from blood cultures at a tertiary education hospital in Brazil in 2006. The isolated yeast-like fungi were *C. albicans* (52.2%), *C. parapsilosis* (22.1%), *C. tropicalis* (14.8%), and *C. glabrata* (6.6%), respectively. The in vitro activity of amphotericin B, caspofungin, itraconazole, fluconazole, voriconazole, and posaconazole were determined using E-test method. Overall susceptibility for voriconazole, fluconazole, and caspofungin was > 97%. According to the authors, the candidemia incidence is high and the distribution of *Candida* species and their antifungal susceptibilities should be known (24). We reported *C. albicans* (59.5%) as the most common isolated strain. Besides, we detected non-*albicans* isolates with a different ratio compared to other studies. We isolated more *C. glabrata* (14.4%) and *C. krusei* (13.7%) compared to *C. parapsilosis* (5.2%) and *C. tropicalis* (4.8%).

Our data was relevant to that of Ergon and Yucesoy (23), but it differed in rate of *C. tropicalis* being lesser and *C. krusei*. This may be due to taking the clinical samples from areas other than the intensive care unit. *C. albicans* was the most common strain isolated from blood cultures; in addition, non-*albicans* strains were detected in various ratios. We also detected 100% susceptibility to fluconazole, voriconazole, flucytosine, and amphotericin B among blood culture strains. In a study in South Korea, the isolates included: *C. albicans* 38%, *C. parapsilosis* 26%, and *C. tropicalis* 20% in blood cultures (25). Another study in Argentina reported 38.4% *C. albicans*, 26% *C. parapsilosis*, and 15.4% *C. tropicalis* among blood cultures (26). We

reported 66.6% *C. parapsilosis*, 6.7% *C. albicans* and 16.7% *C. glabrata* isolated from blood cultures.

Our data differed from other studies, as *C. parapsilosis* was the most common isolated strain from blood cultures. This might be due to the fact that strains have been more frequently isolated from intensive care units, where a huge number of invasive procedures is performed. *C. parapsilosis* is spread by intravascular devices, prosthetic materials, and hyperalimentation fluids. This strain is most commonly isolated from intensive care units and most of our strains were from this unit. As the progress has been gained in medicine, life span has increased intensive care treatments are applied more immunosuppressive therapy is increased. These factors led to more studies about *Candida* and its risk factors. Yenigun Kocak et al. concluded that 95% of candidemia cases were nosocomial, 34% of which were from intensive care units in Turkey (27). In our study, 66.7% of cases with candidemia were from the intensive care unit. These ratios may indicate that intensive care unit patients should be monitored for risk of candidemia.

Candida is a common pathogen, leading to prophylactic antifungal usage, particularly in intensive care units. This situation may lead to the formation of intermediate to resistant strains to antifungal drugs. Garnacho-Montero et al. reported that previous fluconazole treatment was an independent risk factor for fluconazole resistance (28). Kovacicova et al. reported that three pediatric patients operated for brain tumor had candidemia caused by *C. lusitaniae*, *C. guilliermondii* and *C. parapsilosis* and showed amphotericin B resistance (29). Amphotericin B was previously administered for two of these patients. In our study, all the blood culture isolates were susceptible to amphotericin B, fluconazole, voriconazole, and flucytosine.

As a conclusion, *Candida* strains isolated from newborns, elderly patients and intensive care unit patients, identified and isolated from blood cultures, should be studied for antifungal susceptibility for management of treatment. The most common isolated yeast-like species were *C. albicans* and *C. parapsilosis,* isolated from blood cultures. Besides, non-*albicans* strains were also detected. Our University Hospital is a recently opened center and these are the first data of our center. Gradually as the number of patients increases, this data will be evaluated further.

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