# Identification of *Candida* species and susceptibility testing with Sensititre YeastOne microdilution panel to 9 antifungal agents

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## ABSTRACT

**الأهداف**: أجريت هذه الدراسة الاستطلاعية لتحديد مدى انتشار الأنواع ونمط التعرض للعوامل التسعة المضادة للفطريات والخمائر المعزولة من عينات سريرية مختلفة للمرضى الذين عولجوا من البكتيريا المستعمرة أو المعدية و تلقوا العلاج في وحدات العناية المركزة التاجية والجراحية ( ICU ).

**الطريقة**: تم علاج ما مجموعه 421 مريضا في وحدة العناية المركزة في معهد أمراض القلب، جامعة اسطنبول، اسطنبول، تركيا بين يونيو 2013 ومايو 2014، وعُزل 44 نوعا الفطريات المبيضة من الدم والبول والسائل داخل الرغامى الشفطي، والبلغم، والجروح من 16 مريضا من وحدة العناية المركزة. وقد أجريت تحديد الفطريات المبيضة باستخدام CHROMagar. و تم تحديد قابلية مضاد للفطريات عن طريق Sensititre YeastOne colorimetric microdilution panel.

**النتائج**: ( المبيضة البيضاء C ) كانت الأكثر شيوعا ، لوحظ الكائنات الحية الدقيقة 23 ( 54% )؛ كانت الكائنات الدقيقة الأخرى المعزولة المبيضات المدارية 12 ( 27% )، المبيضات الجرداء 5 ( 11% )، المبيضات المرطية 1 ( 2% )، المبيضات ،( 2% ) lusitaniae 1 ( 2% ) Candida sake 1، والتيربية العظم الكبير 1 ( 2% ). وكانت جميع المعازل عرضة amphotericin B و G 5 flucytosine. العظم الكبير بينما المعازل الأخرى المستثناة كانت عرضة لكلاً من micafungin، anidulafungin، وcaspofungin أيضا. وُجد أن المبيضة المرطية C سريعة التأثر لجميع مضادات الفطريات التي شملتها الدراسة. تم العثور على معدلات MIC عالية لمجموعة azole من الأدوية المضادة لفطريات المبيضة البيضاء C و المدارية C، و الجرداء C. كان معدل الاستعمار 3.8% ( 16/421 ) 0.7% فقط ( 3/421 ) من المرضى من أصل ما مجموعه 421 اصيبوا بالمبيضات في الدم.

**الخاتمة**: لقد وجدنا أن معدلات الخميرة المستعمره والعدوى في المرضى في وحدة العناية المركزة منخفضة جداً، ولا تزال المبيضة البيضاء C أكثر الأنواع شيوعاً واكتشفنا قابلية التناقص إلى مكونات azole.

**Objectives:** To determine the species incidence and susceptibility pattern to 9 antifungal agents of yeasts isolated from various clinical specimens of colonized or infected patients treated in the coronary and surgical intensive care units (ICU).

**Methods:** A total of 421 ICU patients were treated at the Cardiology Institute, Istanbul University, Istanbul, Turkey between June 2013 and May 2014, and 44 *Candida* species were isolated from blood, urine, endotracheal aspiration fluid, sputum, and wounds of 16 ICU patients. Identification of *Candida* was performed using CHROMagar. Antifungal susceptibility was determined by a Sensititre YeastOne colorimetric microdilution panel.

**Results:** *Candida albicans* (*C. albicans*) was the most commonly observed microorganism 23 (54%); the other microorganisms isolated were *Candida tropicalis* 12 (27%), *Candida glabrata* 5 (11%), *Candida parapsilosis* 1 (2%), *Candida lusitaniae* 1 (2%), *Candida sake* 1 (2%), and *Geotrichum capitatum* 1 (2%). All isolates were susceptible to amphotericin B and 5-flucytosine. *Geotrichum capitatum* excepted, the other isolates were also susceptible to anidulafungin, micafungin, and caspofungin. *Candida parapsilosis* was found to be susceptible to all the studied antifungals. High MIC rates for azole group of antifungal drugs were found for *C. albicans*, *C. tropicalis*, and *C. glabrata*. The rate of colonisation was 3.8% (16/421). Only 0.7% (3/421) patients out of a total of 421 developed candidemia.

**Conclusion:** We found that the yeast colonization and infection rates of patients in our ICUs are very low. *Candida albicans* is still the most common species. We detected a decreasing susceptibility to azole compounds.

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Over the last decade, fungal infections due to *Candida* species and other fungi have increased dramatically and candidemia is a major risk factor for morbidity and mortality in intensive care units (ICUs).[1,2] Fungi are saprophytic microorganisms that have evolved mechanisms to survive in the mammalian hosts. *Candida* species are capable of a wide spectrum of infections in human hosts, ranging from benign colonisation of the skin and mucosal surfaces to invasion of the bloodstream and integral organs. Most of the fungal infections have been accidental and systemic fungal infections are a rarity that may result in high mortality. In systemic fungal infections, the outcome of the disease depends more on the host factors rather than the fungal virulence. The most common risk factors for invasive candidiasis include major surgery, especially involving the abdomen, immunosuppression, and supportive care measures used in the critically ill patients, such as long term broad spectrum antimicrobial therapy, central venous catheters, total parenteral nutrition, and renal replacement therapies. These factors explain, in part, the continuing high prevalence of *Candida* infections in transplant, cancer, and ICU patient population.[3-7] Identification of yeasts isolated from clinical samples has become increasingly important. *Candida albicans* (*C. albicans*) remains the most common cause of human candidiasis. Non-albicans invasive infections have also increased. These species are also shown to have reduced susceptibility to antifungal agents. The diagnosis was simply based on whether it was *C. albicans* or non-*albicans Candida* species that indicated the appropriate treatment for these fungal infections.[1-6] Rapid identification of yeast isolates to the species level is essential to optimise the antifungal treatment. CHROMagar *Candida* (CC) is a selective and differential medium. This medium contains chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentations. These enzymes are species specific, allowing yeasts to be identified to the species level by their colour and colony characteristics.[5,6,8] The aim of this study was to evaluate the fungal colonisation and infection rates of ICU patients at the Cardiology Institute, Istanbul University during a one-year period, to investigate the identification of *Candida* species using CHROMagar, conventional methods, and use the ID 32C system and the Sensititre YeastOne colorimetric microdilution panel to determine the in vitro susceptibility to anidulafungin, micafungin, caspofungin, 5-flucytosine, amphotericin B, posaconazole, voriconazole, itraconazole, and fluconazole.

**Disclosure**. Authors have no conflict of interest, and the work was not supported or funded by any drug company.

**Methods.** A total of 1296 surveillance cultures were taken from 421 non-neutropenic ICU patients of Istanbul University Cardiology Institute between June 2013 and May 2014 in the prospective study. A total of 44 yeasts were isolated from blood, urine, endotracheal aspiration fluid, sputum, and wounds of 16 ICU patients. Eleven patients had undergone cardiac operations and 5 patients suffered heart attacks. Specimens from the patients in the coronary ICU were taken 72 hours after hospitalisation and then twice weekly until discharge. Samples from the surgical patients were taken before an operation and 6 days after surgery or on discharge. Demographic and clinical data, including date of hospitalisation and discharge, gender, age, length of hospitalisation, underlying disease, use of antibiotics, total duration of antibiotic therapy before surgery, duration of central venous catheters and other medical devices, causes of admission to ICU, and length of stay in the ICU were recorded; patients who stayed in the ICUs for less than 5 days were excluded from the study. Ethics approval informed consent was obtained from each patient after approval of the study protocol by the Local Ethics Committee. Colonization and infection were determined according to established criteria.[9-11] Colonization was defined when yeasts were isolated from the endotracheal aspiration fluid, throat swab, sputum, urine, and central venous catheter specimens in the absence of signs or symptoms of infection. Infection was indicated as and when yeasts were isolated from normally sterile sites; patients receiving antifungal treatment for specific signs of clinical infection were associated with repeated positive yeast cultures. The characteristics of the patients are shown in **Table 1**. All yeasts were identified by conventional methods, CC (CHROMagar *Candida*, France) and the ID 32C system (BioMerieux, France) system according to the manufacturer's procedure. All specimens first obtained where Gram stained, inoculated on to the Sabouraud dextrose agar (SDA)(Oxoid, UK), and incubated at 37°C for 24 hours. Germ tube test was performed and further was classified as albicans and non-albicans. The germ tube positive was further incubated at 45°C to look for the growth. These strains from SDA were inoculated on corn meal agar (CMA)(HiMedia, India) for morphological examination of the production

of chlamydospores, blastospores, true hyphae, and branched pseudohyphae. They were also inoculated on to CHROMagar from SDA; identification was made by color and morphology of the colonies according to the manufacturer's instructions. These isolates from SDA were subjected to biochemical analysis by an ID 32C system. The colours and colony morphology were observed at 24 and 48 hours on CHROMagar and compared with the conventional identification and the ID 32C system results. Appearances of *Candida spp.* on CHROMagar were as follows: *C. albicans* - bluegreen, *Candida tropicalis (C. tropicalis)* - dark blue; *Candida parapsilosis (C. parapsilosis)* - cream colored; *Candida glabrata (C. glabrata)* - pale purplish pink; *Candida lusitaniae (C. lusitaniae)* - cream pink colored; *Candida sake (C. sake)* - cream colored, and *Geotrichum capitatum* (*G. capitatum*) - pale pink-lavender colored. Susceptibility to anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole, and amphotericin B was evaluated using colorimetric microdilution panel (SENSITITRE YeastOne Trek Diagnostic Systems, Cleveland, OH, USA). The Sensititre YeastOne panels trays containing serial 2-fold dilutions of anidulafungin (0.015 to 8 µg/ml), micafungin and caspofungin (0.008 to 8 µg/ml), 5-flucytosine (0.06 to 64 µg/ml), posaconazole and voriconazole (0.008 to 8 µg/ml), itraconazole (0.015 to 16 µg/ml), fluconazole (0.12 to 256 µg/ml), and amphotericin B (0.12 to 8 µg/ml) were provided by TREK Diagnostic Systems. The YeastOne panels were shipped in sealed packages and stored at room temperature until testing was performed. Stock inoculum suspensions of the *Candida spp.* were obtained from 24 h culture on SDA at 35°C. The turbidity of each yeast suspension was adjusted by the spectrophotometric method.[12] On the day of the test, a working yeast suspension was prepared to a final turbidity of 0.5 McFarland standards. The dried YeastOne panels were rehydrated with the working yeast suspension using an appropriate multichannel pipetting device by dispensing 100 µl into each well. Panels were recovered with adhesive seals and incubated at 35°C for 24 to 48 hours in a non-CO2 incubator. Minimum inhibitor concentrations (MICs) endpoints were read after 24 hours of incubation. Yeast growth was evident as a color change from blue (negative, indicating growth) to red (positive, indicating growth) was observed. We determined the MICs for both growth inhibition of 50% and 90% of the strains, for all strains, at the recommended endpoints and time intervals. *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as control strains.

**Table 1 -** Predisposing factors of Candida colonized/ infected patients in the intensive care units.

| Characteristics | Number of patients |
|---|---|
| ***Gender*** | |
| Male | 7 |
| Female | 9 |
| ***Underlying disease*** | |
| Diabetes | 6 |
| ***Hospitalization*** | |
| Coronary intensive care unit | 5 |
| Surgical intensive care unit | 11 |
| Parenteral feeding | 7 |
| Use of broad-spectrum antibiotics* | 14 |
| Bacterial infection | 11 |
| Intravenous drug use | 0 |
| HIV infection | 0 |
| Central venous catheterization | 16 |
| Urinary catheter | 14 |
| Invasive mechanical ventilation | 13 |
| Renal replacement therapy | 12 |
| Fluconazole prophylaxis | 10 |
| Colonization with yeasts | 16 |
| Candidemia | 3 |

*Third-genaration cephalosporins (ceftriaxone, ceftazidime, cefodizime, cefoperazone-sulbactam, cefotaxime), carbapenems (imipenem, meropenem, ertapenem), quinolones (ofloxacin, ciprofloxacin, levofloxacin), aminoglycosides (netilmicin, gentamicin, amikacin), glycopeptide (vancomycin and teicoplanin).

**Results.** Forty-four yeasts isolated from 16 ICUs patients (9 women and 7 men, median age 75 years) were included in this study. Intensive Care Unit patients were hospitalised for a mean of 101 (5-326) days. The identification results of 44 yeasts and the distribution of clinical specimens are shown in **Table 2**. The most common isolated yeast was *C. albicans* 23(54%); the other isolated yeasts were *C. tropicalis* 12 (27%), *C. glabrata* 5 (11%), *C. parapsilosis* 1 (2%), *C. lusitaniae* 1 (2%), *C. sake* 1 (2%), and *G. capitatum* 1 (2%). The rate of colonisation was 3.8% (16/421 patients). Five of the 16 patients with colonisation died. The candidemia ratio has been found to be low during this period (3 patients; 0.7%). One of the 3 candidemia patients was infected by *C. albicans* and non-*albicans Candida spp.*; in the second patient, we isolated *C. albicans*, and in the third patient, we identified non-*albicans Candida*. All of the patients with candidemia died. In our study, *Candida* species, which were identified by CHROMagar and corn meal agar, were confirmed by outputs from the ID 32C system. CHROMagar *Candida* correctly identified all of *C. albicans*, *C. tropicalis*, and *C. glabrata*, but for the other *Candida* species, for correct

identification, other tools of identification for example, the ID 32C system along with CHROMagar *Candida* (CHROMagar *Candida*, France) should be used. We found a mixture culture of yeast in one colonised patient (*C. albicans* and *C. sake*) and one patient with candidemia (*C. albicans* and *C. tropicalis*).

The MIC results for 9 antifungal agents are summarised in **Table 3**. Low MIC ratios for anidulafungin, micafungin, caspofungin, 5-flucytosine, and amphotericin B were found; MIC 50 and MIC 90 values for *Candida* strains were: 0.02 µg/ml-0.02 µg/ml; 0.008 µg/ml-0.01 µg/ml; 0.02µg/ml-0.03µg/ml; 0.06µg/ml-0.06µg/ml; and 0.50µg/ml-0.67µg/ml.

**Discussion.** In recent years, invasive candidiasis has been on the rise as a cause of life-threatening infections in critically ill and ICU patients. *Candida* is the most common fungal pathogen in human beings. Colonisation of skin and mucous membranes is a critical step in the pathogenesis of invasive candidiasis. Most ICU patients become colonised with *Candida* species, but only 5-30% of the patients develop invasive candidiasis. The presence of a central venous catheter, use of broad-spectrum antibiotics, prolonged length of stay, mechanical ventilation, colonisation, parenteral nutrition, dialysis, immunodeficiency, antifungal prophylaxis, and diabetes mellitus constitute the major risk factors for invasive candidiasis. The hands of medical personnel can be colonised with *Candida* species and are important evidence for the role of nosocomial transmission from healthcare workers in the hospital. In addition, transmission from common sources has been demonstrated to occur, for example, from contaminated intravenous fluids, hospital food, and medical devices.[13-17] Fungal infections have become a major problem in elderly patients, since age is a predisposing factor with increased impact on mortality.[13-20] In our study, the yeast colonisation rate of ICU patients was low (16/421; 3.8%). The sputum, wounds, urine, and endotracheal aspiration fluid of the patients were found to be colonised by yeasts. However, 5 of the 16 colonised patients died. *Candida albicans* was the yeast isolated from most of the colonised patients (7/16; 43.7%). We found 2 species of yeasts in one colonised patient (*C. albicans* and *C. sake*) and he died. Three of the 16 colonised patients received antibiotics before an operation, but not antifungal agents. Two of the 16 patients did not receive antibiotics or antifungals. The other patients received fluconazole prophylaxis and antibiotics (aminoglycosides and/or cephalosporins or glycopeptide). One patient was colonised with *C. albicans* before hospitalisation. Colonised patients were hospitalised in the ICU for a mean of 96 days. All colonised patients had central venous and other catheters. Thirteen patients were mechanically ventilated. We thought that developing colonisation due to use of long term broad-spectrum antibiotics, prolonged length of stay (average, 96 days), mechanical ventilation and presence of central venous catheter in our ICU patients.

In this study, candidemia rate of ICU patients was low (3/421; 0.7%). However, all patients with candidemia died. We isolated 2 species of yeast in one candidemia patient (*C. albicans* and *C. tropicalis*), identified *C. albicans* in a second patient, and isolated *C. tropicalis* in a third. We detected yeasts from the blood cultures of all candidemia patients. All patients with candidemia received antibiotics (third-generation cephalosporins+aminoglycosides, or carbapenem, or quinolones, or glycopeptide, and fluconazole prophylaxis). All candidemia patients had central venous catheter and other catheters, and were

**Table 2 -** Number of *Candida* strains isolated from different clinical specimens of patients hospitalized in the intensive care units.

| Specimens | N=44 | % | Blood | Endotracheal aspiration fluid | Sputum | Urine | Wound |
|---|---|---|---|---|---|---|---|
| *C. albicans* | 23 | 54 | 6 | 7 | 4 | 3 | 3 |
| *C. tropicalis* | 12 | 27 | 5 | 5 | - | 2 | - |
| *C. glabrata* | 5 | 11 | - | - | 1 | 4 | - |
| *C. parapsilosis* | 1 | 2 | - | - | 1 | - | - |
| *C. lusiteniae* | 1 | 2 | - | - | 1 | - | - |
| *C. sake* | 1 | 2 | - | - | 1 | - | - |
| *G. capitatum* | 1 | 2 | - | - | 1 | - | - |
| **Total** | **44** | **100** | **11** | **12** | **9** | **9** | **3** |

C - *Candida*, G - Geotrichum

mechanically ventilated. The patients did not receive antibiotics and antifungal agents before hospitalization. The average duration of stay in the ICU was 244 days. In this study, the most common risk factor associated with candidemia was long-term antibiotic therapy (87.5%). The most common antibiotic used was the beta-lactams (75%), followed by other antibiotics like aminoglycosides and vancomycin. Long-term antibiotic therapy, especially with multiple antibiotics, has been found to be an independent risk factor in the development of candidemia in many studies.[17-21]

In recent years, rare antifungal drug resistance has been a cause for concern in the treatment of invasive fungal infections. Resistance can be primary or innate drug resistance and acquired resistance. Mechanisms of resistance to antifungal agents differ in various groups of drugs. Reduced drug absorption and accumulation decreased the affinity of the drug to its target; alteration in metabolic pathways to disturb the drug concentrations in a cell are some of the reasons for antifungal drug resistance. Studies have also elaborated on the molecular mechanisms of drug resistance in fungi. Biofilm formation has also been recognized as a potential factor for fungal resistance due to the production of a exopolymeric material that inhibits or restricts penetrations of antifungal drugs.[3,22] Resistance pattern to azole compounds among *Candida* isolates has been studied across the world. Many potential mechanisms of azole resistance have been proposed. Alteration of drug efflux, reduced intracellular accumulation of fluconazole due to changes in *Candida* drug resistance (CDR) genes, and increased expression of ATP-binding cassette transporter gene are some of the mechanisms responsible for the development of azole resistance in *Candida* species.[3,4,18]

Reports of bloodstream infections due to *C. glabrata* resistant to multiple triazoles and echinocandins have increased in recent years. In a review of data from population-based and lab-based surveillance programs in the USA, Pfaller et al[29] noted that 9.7% of *C. glabrata* isolates were resistant to fluconazole, of which 99% were cross-resistant to voriconazole and 8-9% were also cross-resistant to anidulafungin, micafungin, and caspofungin.[29] Their data suggested that while the majority of *C. glabrata* remains susceptible to echinocandins, the recent increase in multidrug resistant strains may represent an anonymous trend justifying continued surveillance and antimicrobial susceptibility testing.[4,23] Although the prompt administration of effective systemic antifungal therapy can significantly reduce the morbidity and mortality associated with invasive candidiasis, increasing rates of antifungal resistance, particularly among *C. glabrata*, are threatening to diminish the efficacy of current frontline agents for invasive candidiasis.[4,23,24]

We evaluated the antifungal susceptibility and resistance pattern of all *Candida* species with Sensititre YeastOne colorimetric microdilution panel. All *Candida* isolates were susceptible to 5-flucytosine and amphotericin B. *Candida parapsilosis* was found to be susceptible to all the studied antifungals. With the exception of *G. capitatum*, the other isolates were susceptible to anidulafungin, micafungin, and caspofungin. All of *C. albicans* isolates was found to be susceptible to anidulafungin, micafungin, caspofungin, 5-flucytosine, and amphotericin B. However, 11 of *C. albicans* isolates were resistant to voriconazole (MIC ≥8 μg/ml), itraconazole (MIC ≥16 μg/ml), fluconazole (MIC ≥256 μg/ml), and posaconazole (MIC ≥8 μg/ml). In addition, 3 of the *C. albicans* isolates was resistant to itraconazole (MIC ≥2 μg/ml) and susceptible-dose dependent (SDD) to fluconazole (MIC =16-32 μg/ml). All of C. tropicalis were found to be susceptible anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, fluconazole, and amphotericin B. On the other hand, 3 of *C. tropicalis* were resistant to itraconazole (MIC ≥1 μg/ml), and 2 *C. tropicalis* were SDD to itraconazole (MIC ≥0.25-0.5 μg/ml).

Two of *C. glabrata* isolates were resistant to itraconazole (MIC ≥2 μg/ml), and SDD to fluconazole (MIC =16-32 μg/ml). Three of *C. glabrata* were resistant to itraconazole (MIC ≥16 μg/ml) and fluconazole (MIC ≥128 μg/ml), and these *C. glabrata* isolates were also susceptible to the other antifungals.

*Candida lusitaniae* was SDD to itraconazole (MIC =0.25 μg/ml) and susceptible to the other studied antifungals. *Candida sake* was SDD to fluconazole (MIC =16 μg/ml) and susceptible to the other antifungals. The *G. capitatum* was nonsusceptible (NS) to anidulafungin (MIC ≥8 μg/ml), micafungin (MIC ≥8 μg/ml), and caspofungin (MIC ≥8 μg/ml). Also, this isolate was SDD to voriconazole (MIC ≥2 μg/ml), itraconazole (MIC ≥0.5 μg/ml), and fluconazole (MIC =16 μg/ml), and susceptible to the other antifungals (**Table 3**). CHROMagar *Candida* is a useful method for identifications of yeasts. Colors were developed in most of the yeasts, such as *C. albicans, C. tropicalis*, and *C. glabrata* after 24 hours of incubation, whereas some strains required 48 hours of incubation.

Amphotericin B resistance in *Candida* species is generally assumed to be rare, although many broth-based methods used for detecting resistance in *Candida* isolates may lack the sensitivity to reliably

**Table 3 -** Cumulative percentages of susceptibility to anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole, and amphotericin B of 44 *Candida (C)* strains (Sensititre YeastOne colorimetric antifungal microdilution panel).

| *Candida* specimens | Antifungal agents | <0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. albicans* | Anidulafungin | | 5 | 15 | 3 | | | | | | | | | | | | |
| n=23 (54%) | Micafungin | 20 | 3 | | | | | | | | | | | | | | |
| | Caspofungin | | 9 | 14 | | | | | | | | | | | | | |
| | 5-flucytosine | | | | 20 | 3 | | | | | | | | | | | |
| | Posaconazole | 3 | 9 | | | | | | | | | 11 | | | | | |
| | Voriconazole | 12 | | | | | | | | | | 11 | | | | | |
| | Itraconazole | | 5 | 4 | | | | | | 3 | | | 11 | | | | |
| | Fluconazole | | | | | | 9 | | | | | | 2 | 1 | | 3 | 8 |
| | Amphotericin B | | | | | | | 23 | | | | | | | | | |
| *C. tropicalis* | Anidulafungin | | 7 | 5 | | | | | | | | | | | | | |
| n=12 (27%) | Micafungin | 5 | 7 | | | | | | | | | | | | | | |
| | Caspofungin | | 3 | 9 | | | | | | | | | | | | | |
| | 5-flucytosine | | | | 12 | | | | | | | | | | | | |
| | Posaconazole | | 5 | | | | 4 | 3 | | | | | | | | | |
| | Voriconazole | 5 | | | | 4 | 3 | | | | | | | | | | |
| | Itraconazole | | | 7 | | | 1 | 1 | 3 | | | | | | | | |
| | Fluconazole | | | | | | 5 | | | 7 | | | | | | | |
| | Amphotericin B | | | | | | | | 12 | | | | | | | | |
| *C. glabrata* | Anidulafungin | | | 5 | | | | | | | | | | | | | |
| n=5 (11%) | Micafungin | | 5 | | | | | | | | | | | | | | |
| | Caspofungin | | | 1 | 3 | 1 | | | | | | | | | | | |
| | 5-flucytosine | | | | 5 | | | | | | | | | | | | |
| | Posaconazole | | | | | | | | 5 | | | | | | | | |
| | Voriconazole | | | | | | 5 | | | | | | | | | | |
| | Itraconazole | | | | | | | | | 4 | | | 1 | | | | |
| | Fluconazole | | | | | | | | | | | | 1 | 1 | 2 | 1 | |
| | Amphotericin B | | | | | | | | 5 | | | | | | | | |
| *C. parapsilosis* | Anidulafungin | | | | | | | 1 | | | | | | | | | |
| n=1 (2 %) | Micafungin | | | | | | | 1 | | | | | | | | | |
| | Caspofungin | | | | | | 1 | | | | | | | | | | |
| | 5-flucytosine | | | | 1 | | | | | | | | | | | | |
| | Posaconazole | | | 1 | | | | | | | | | | | | | |
| | Voriconazole | | 1 | | | | | | | | | | | | | | |
| | Itraconazole | | | | 1 | | | | | | | | | | | | |
| | Fluconazole | | | | | | | | 1 | | | | | | | | |
| | Amphotericin B | | | | | | | 1 | | | | | | | | | |
| *C. lusiteniae* | Anidulafungin | | | | | 1 | | | | | | | | | | | |
| n=1 (2 %) | Micafungin | | | | 1 | | | | | | | | | | | | |
| | Caspofungin | | | | | | 1 | | | | | | | | | | |
| | 5-flucytosine | | | | | | | | | | 1 | | | | | | |
| | Posaconazole | | | 1 | | | | | | | | | | | | | |
| | Voriconazole | 1 | | | | | | | | | | | | | | | |
| | Itraconazole | | | | | 1 | | | | | | | | | | | |
| | Fluconazole | | | | | | | | 1 | | | | | | | | |
| | Amphotericin B | | | | | | | 1 | | | | | | | | | |
| *C. sake* | Anidulafungin | | | | | | | | | 1 | | | | | | | |
| n=1 (2%) | Micafungin | | | | | | | | | 1 | | | | | | | |
| | Caspofungin | | | | | | | | 1 | | | | | | | | |
| | 5-flucytosine | | | | 1 | | | | | | | | | | | | |
| | Posaconazole | | | | | | | | 1 | | | | | | | | |
| | Voriconazole | | | | 1 | | | | | | | | | | | | |
| | Itraconazole | | | | | 1 | | | | | | | | | | | |
| | Fluconazole | | | | | | | | | | | | 1 | | | | |
| | Amphotericin B | | | | | | | | 1 | | | | | | | | |
| *G. capitatum* | Anidulafungin | | | | | | | | | | | 1 | | | | | |
| n=1 (2%) | Micafungin | | | | | | | | | | | 1 | | | | | |
| | Caspofungin | | | | | | | | | | | 1 | | | | | |
| | 5-flucytosine | | | 1 | | | | | | | | | | | | | |
| | Posaconazole | | | | | | | 1 | | | | | | | | | |
| | Voriconazole | | | | | | | | | 1 | | | | | | | |
| | Itraconazole | | | | | | | 1 | | | | | | | | | |
| | Fluconazole | | | | | | | | | | | | 1 | | | | |
| | Amphotericin B | | | | | | | 1 | | | | | | | | | |

detect resistance invitro. *Candida* species for which MICs >1 mg/L are unusual, may at the very least require higher doses of amphotericin B for optimal treatment.[4,25] Although amphotericin B has a rapid cidal action against most strains of *Candida spp.*, it is not the first choice for treatment with candidemia patients due to nephrotoxicity associated with it. The lipid formulation of amphotericin B has a better side-effect profile.[18] We did not detect amphotericin B-resistant *Candida* strains in our study. Echinocandins (anidulafungin, micafungin, and caspofungin) are among the most widely prescribed antifungals in patients with invasive candidiasis. Despite some pharmacokinetic differences, all 3 echinocandins act by inhibiting 1,3 ß-D-glucan synthase, thereby distrupting glucan biosynthesis in the cell wall. In some echinocandin-resistant strains, which may be an important limiting factor for the emergence of some resistant subpopulations during treatment,[4,27] *G. capitatum* excepted, the other isolates were susceptible anidulafungin, micafungin, and caspofungin in our study. Antifungal resistance for *Candida* strains varies according to regions in Turkey.[28-31]

We used for a long time fluconazole prophylaxis and treatment in our ICU patients. Previously, it was taken good response to fluconazole. But, recently, we became aware due to the treatment of failure to fluconazole and then we performed to this study. The limitation of our study is that we used small sized samples.

In conclusion, we found the yeast colonisation and/or infection rates of patients treated in our ICUs are very low and *C. albicans* is still the most common species. CHROMagar *Candida* has the advantage of rapid identification of *Candida* species, technically simple, rapid, and economical compared with other methods. Thus, after identification of *Candida* species, preliminary antifungal treatment can be administrated with confidently. We also found decreasing susceptibility to azole compounds of *Candida* species. Therefore, we suggest that all 3 echinocandins should be used to *Candida* species because of good activity.

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