Distribution and antifungal susceptibility patterns of Candida species at a university hospital in Northern Turkey

Mustafa Kerem Calgin¹, Yeliz Cetinkol¹

¹ Faculty of Medicine, Medical Microbiology Department, Ordu University, Ordu, Turkey

Abstract
Introduction: The aim of this study was to establish local resistance profiles of Candida species isolated from various clinical specimens by identifying isolates and determining their susceptibilities to commonly used antifungal agents.

Methodology: All isolates were identified to species level and amphotericin B, flucytosine, fluconazole, voriconazole, caspofungin and micafungin antifungal susceptibility testing was performed using Vitek 2 Compact Advanced Expert System (AES).

Results: The specimens consisted of 152 urines (69%), 49 blood (22%), 15 sputum (7%) and 4 wound (2%) samples. Of the 220 isolated Candida strains, the most prevalent species were Candida albicans (43.3%), Candida tropicalis (25%) and Candida parapsilosis (13.7%). In blood specimens C. parapsilosis was the dominant species (43%), followed by C. albicans (32.5%) and C. tropicalis (12.2%). Overall resistance to amphotericin B, flucytosine, fluconazole, voriconazole, caspofungin and micafungin was 7.3%, 10%, 9.4%, 7.3%, 2% and 6.5%, respectively.

Conclusions: Due to the increase in patient populations at risk of Candida infections, rational treatment planning and resistance rates should be checked along with antifungal susceptibility testing on C. albicans, C. tropicalis and C. parapsilosis isolates.

Key words: antifungal susceptibility; candida species; yeast.

Introduction
Infections caused by opportunistic pathogens, such as yeasts, are becoming important causes of morbidity and mortality. Variations in the immune system, a significant population of immunocompromised patients (requiring invasive techniques such as intravenous catheters) and the use of broad spectrum antibiotic therapy are factors that contribute to the rise of these infections [1,2]. Better control of the main diseases and improved antibiotic therapies result in prolonged survival, thus putting these patients at higher risk of acquiring an opportunistic fungal infection. The increase in risk factors for fungal infections will likely raise the incidence of nosocomial fungal infections in the coming decades [3].

Species of the genus Candida are commonly isolated as the cause of invasive fungal infections. The genus is highly variable, containing different Candida species identified as etiological agents of infections. Nevertheless, about 90% of invasive infections are caused by Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata and Candida krusei [4]. Investigations in Europe have noted an increase in the prevalence of fungal infections caused by Candida species other-than C. albicans such as C. parapsilosis, C. glabrata and C. krusei, however, C. albicans still causes about 60% of cases [5,6]. Furthermore, it seems that marked differences exist in species distributions and resistance to antifungal agents between different countries, emphasizing the need for continued investigation in each country to observe trends in pathogen distribution and drug susceptibilities [7].

In Turkey and globally, increasing incidence of Candida infections are becoming a significant health problem. It is important to monitor these infections and to determine the resistance profile of the causal agents. In particular, monitoring the incidence of Candida infections will help to identify resistance profiles and the management of empirical treatment. This study was completed on Candida strains isolated from clinical samples sent to Ordu University School of Medicine Education and Research Hospital Clinical Microbiology Department Laboratory with the aim of determining occurrence, evaluating the in vitro efficacy of currently used antimycotics and contributing to regional epidemiologic data.
Examples were cultivated on Sabouraud dextrose agar and blood agar and urine, wound and sputum specimens were incubated in BacTAlert (Biomerieux, Marcy l'Etoile, France) blood culture system. Positive blood cultures confirmed C. albicans ATCC 90028 (Salubris, Istanbul, Turkey) and incubated for 24-48 hours at 35 °C. At the end of incubation, 0.5 mm diameter colonies which looked like yeast strains were stained and examined microscopically for yeast morphology. Colony slides were analyzed and identified for each yeast species according to the data sheets of ATCC strains. The 220 yeast strains were isolated between the 1st of January 2015 and the 6th of May 2017 at Ordu University School of Medicine Education and Research Hospital in the Clinical Microbiology laboratory. Specimens sent from anesthesiology and surgery departments. When the same strains were isolated from the same patient, one of them were included in the study. Blood samples were incubated in BacTAlert (Biomerieux, Marcy l'Etoile, France) blood culture system. Positive blood cultures and urine, wound and sputum specimens were cultivated on Sabouraud dextrose agar and blood agar (Salubris, Istanbul, Turkey) and incubated for 24-48 hours at 35 °C. At the end of incubation, 0.5-1.0 mm diameter colonies which looked like yeast strains were Gram-stained and examined microscopically for yeast morphology.

### Identification and antifungal susceptibility of isolates

The Vitek 2 Compact (Biomerieux, Marcy l'Etoile, France) Advanced Expert System (AES) was used for identification and antifungal susceptibility tests for amphotericin B, fluconazole, voriconazole, caspofungin and miconafungin. The test was carried out according to the manufacturer’s instructions. About 2–3 colonies of 24-hours Candida cultures were inoculated into 5-mL glass tubes containing 3 mL of 10% saline, adjusted to 2.0 McFarland standards (acceptable range of 1.8 to 2.2) using the DensiChek (Biomerieux, Marcy l'Etoile, France) instrument. Inoculum suspension was placed into a Vitek 2-YST and Vitek 2-AST cards with 2-fold serial dilutions of antifungals for each organism. Loaded cassettes were then placed into the Vitek 2 instrument and incubated for 9 to 33 hours (the time of incubation varied based on the rate of growth in the drug-free control well) depending on the sample. Standard strains of C. albicans ATCC 90028 and C. parapsilosis ATCC 22019 were treated similarly for quality control. Intermediate susceptibility results were reported as resistant.

### Table 1. Distribution of the Candida isolates according to the specimen type.

<table>
<thead>
<tr>
<th>Species</th>
<th>Urine (n:152)</th>
<th>Blood (n:49)</th>
<th>Sputum (n:15)</th>
<th>Wound (n:4)</th>
<th>Total (n:220)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>C. albicans</td>
<td>42</td>
<td>64</td>
<td>32.5</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>31</td>
<td>47</td>
<td>12.2</td>
<td>6</td>
<td>13.3</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4.6</td>
<td>7</td>
<td>43</td>
<td>21</td>
<td>6.7</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>4</td>
<td>6</td>
<td>8.1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>C. famata</td>
<td>4.6</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>4</td>
<td>6</td>
<td>2.1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>4.6</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4.6</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. dubliensi</td>
<td>0.6</td>
<td>1</td>
<td>2.1</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

n: Number of isolates, %: Percentage of isolates.

### Methodology

#### Isolation/cultivation of Candida species

The 220 yeast strains were isolated between the 1st of January 2015 and the 6th of May 2017 at Ordu University School of Medicine Education and Research Hospital in the Clinical Microbiology laboratory. Specimens were sent from anesthesiology and surgery intensive care units (ICU), internal medicine, infectious diseases, urology, chest disease, general surgery, gynecology and obstetrics, pediatrics, plastic surgery, orthopedics and neurosurgery departments. When the same strains were isolated from the same patient, one of them were included in the study. Blood samples were incubated in BacTAlert (Biomerieux, Marcy l'Etoile, France) blood culture system. Positive blood cultures and urine, wound and sputum specimens were cultivated on Sabouraud dextrose agar and blood agar (Salubris, Istanbul, Turkey) and incubated for 24-48 hours at 35 °C. At the end of incubation, 0.5-1.0 mm diameter colonies which looked like yeast strains were Gram-stained and examined microscopically for yeast morphology.

### Table 2. Distribution of the Candida isolates according to different departments of the hospital.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>C. albicans</td>
<td>37</td>
<td>38</td>
<td>25</td>
<td>3</td>
<td>70</td>
<td>7</td>
<td>55.6</td>
<td>5</td>
<td>100</td>
<td>4</td>
<td>33.3</td>
<td>1</td>
<td>66.6</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>22.5</td>
<td>15</td>
<td>25</td>
<td>3</td>
<td>30</td>
<td>3</td>
<td>22.2</td>
<td>2</td>
<td>-</td>
<td>33.3</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>20.5</td>
<td>21</td>
<td>7.5</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>11.1</td>
<td>1</td>
<td>-</td>
<td>33.4</td>
<td>1</td>
<td>-</td>
<td>33.4</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>6</td>
<td>6</td>
<td>5.5</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>11.1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. famata</td>
<td>3</td>
<td>3</td>
<td>8.3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>2</td>
<td>2</td>
<td>8.3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>3</td>
<td>3</td>
<td>33.4</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4</td>
<td>4</td>
<td>3.7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33.3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. dubliensi</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n: Number of isolates, %: Percentage of isolates, *: Total number of isolates according to department.
Results

Yeasts were isolated from 220 clinical specimens. The mean age of the patients was 71 years, ranging from 1 month to 96 years. The gender distribution of patients was 120 (54.5%) females and 100 (45.5%) males.

The specimens comprised 152 urine, 49 blood, 15 sputum and 4 wound samples. Candida albicans (43.3%) was the most frequently isolated species. Candida tropicalis was the second most prevalent species from all sites (25%), followed by C. parapsilosis (13.7%), C. lusitaniae (4.6%), C. famata (3.1%), C. glabrata (3.1%), C. kefyr (3.1%), C. krusei (3.1%) and C. dubliniensis (1%). Distribution of the Candida isolates according to the specimen type is shown in Table 1.

These samples were received from anesthesiology and surgery intensive care units (ICU), internal medicine, infectious diseases, urology, chest disease, general surgery, gynecology and obstetrics, pediatrics, plastic surgery, orthopedics and neurosurgery departments. Table 2 summarizes distribution of the Candida species according to different departments in the hospital.

Resistance amongst the 220 isolates to amphotericin B, flucytosine, fluconazole, voriconazole, caspofungin and micafungin was 7.3%, 10%, 9.4%, 7.3%, 2% and 6.5%, respectively. Antifungal resistance distribution for Candida isolates are shown in Table 3.

Discussion

Fungal species commonly found in nature are also found in the gastrointestinal system and skin flora of organisms and under appropriate conditions may gain pathogenic traits and cause infections. Together with the increase in fungal infections, changes have begun to be observed in the variety of species causing these infections. Some fungal species exhibit intrinsic resistance to some antifungal agents, which highlights the importance of species identification for medication selection before treatment [8]. C. albicans is the most common species isolated from invasive fungal infections. Additionally, the incidence of non-albicans Candida is increasing [3].

Candida glabrata, C. krusei, C. tropicalis and C. parapsilosis are the leading causes of candidiasis after C. albicans. The distribution of these non-albicans Candida in infected patients varies throughout the world. The main causes underlying these differences are the medical state of patients, geographic distribution, age and gender [9,10]. In a recent study from the Czech Republic, Hrabovský et al. obtained 800 isolates from upper respiratory tract, stool, urine blood culture, peritoneal aspirate and wound specimens from intensive care unit patients and found the most prevalent species was C. albicans (58.9%), followed by C. glabrata (20.4%), C. krusei (8.6%), C. parapsilosis (3.6%), C. tropicalis (3.4%) and C. lusitaniae (1.1%) in all clinical specimens [11]. In Turkey, Ece et al. isolated yeast-like fungi from 337 varied clinical specimens (wound, urine, blood, respiratory specimen). The most commonly 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%) [12]. Consistently, C. albicans was the most commonly isolated species in our study (43.3%) followed by C. tropicalis (25%), C. parapsilosis (13.7%), C. glabrata (3.1%) and C. krusei (3.1%).

Fungal bloodstream infection (fungemia) is the second most common fungal infection in hospitalized patients after urinary tract infections. Candidemia also carries the highest related mortality (40%) of all nosocomial bloodstream infections. C. albicans used to be the predominant reason for candidemia, accounting for >80% of all candidal isolates recovered from nosocomial yeast infections in the 1980s, though in recent years the other Candida species have increased significantly [13]. In our study, it was predominant in 69% of urinary system samples and second most prevalent in 22% of blood culture samples and C. albicans was the most common fungus isolated from urine and sputum cultures, followed by C. tropicalis. In the United States of America (USA) and Canada, C.
**parapsilosis** is the most commonly isolated candidemia agent after *C. albicans*, while in other regions outside North America, *C. parapsilosis* is the second most common agent after *C. albicans* [14]. In European countries, a study revealed that more than half of the cases of candidemia were caused by *C. albicans*, and the rates for non-albicans candidemia infections were 14% each for *C. parapsilosis* and *C. glabrata*, 7% for *C. tropicalis* and 2% for *C. krusei* [15]. In our study the most isolated yeast strain in blood specimens was *C. parapsilosis* (43%). *C. albicans* (32.5%) was second, followed by *C. tropicalis* (12.2%). Similarly, Ece et al. from Turkey indicated *C. parapsilosis* was the most isolated strain from blood cultures [12,16]. However, in some studies in Turkey, *C. albicans* was the dominant species and *C. parapsilosis* was second or third [17,18].

*Candida parapsilosis* has taken its place among important hospital-acquired pathogens in recent years, causing 7-10% of candidemia, with rates of up to 30-50% isolated in some centers. Isolated as a candidemia factor, *C. parapsilosis* has infection potential due to properties such as better proliferation in high glucose concentrations and better penetration of acrylic surfaces. It is stated that infection develops with direct administration of microorganisms into the veins. As it easily proliferates in glucose-rich solutions for hyperalimentation treatment, and commonly colonizes the skin, it has been identified to mix with blood flow during invasive interventions [10]. Our findings agree with other studies that indicate that *C. parapsilosis* is one of the most important species causing candidemia in Turkey [12,16].

*Candida* species found in normal body flora may pass natural barriers during invasive interventions like catheter or endotracheal tube insertion performed on patients in intensive care and cause infections [6,19]. *Candida albicans* is less frequently isolated in surgical and neonatal intensive care unit settings and currently accounts for about half of all nosocomial bloodstream *Candida* isolates in the United States [13]. In our study more than half of the *Candida* species isolated in our laboratory were isolated from intensive care units. Apart from two *C. parapsilosis* strains isolated from the neurosurgery service, *C. albicans* was the yeast species isolated most frequently in samples from all departments.

The increasing problems caused by fungal pathogens and observation of resistant strains have increased the need for in vitro susceptibility tests. When compared with antibacterial susceptibility tests, in vitro antifungal susceptibility tests are not fully standardized and clinical importance is not fully recognized [20]. In our study the Vitek2 Compact System was used [4,20,21]. The reason for choosing this method is that it provides more rapid results than broth micro-dilution, and identification may be completed at the same time as antifungal susceptibility tests.

There are differences in susceptibility to antifungals among *Candida* species. For example; *C. krusei* is naturally resistant to fluconazole and it is stated that *C. glabrata* strains exhibit lower sensitivity to fluconazole than other strains, whereas *C. lucitaniae* strains exhibit lower sensitivity to amphotericin B. Antifungal drugs such as caspofungin, voriconazole and posaconazole are adding treatment options for strains resistant to other antifungals. Amphotericin B is considered the standard medication for systemic treatment of candidemia. From various locations globally, different rates (2-20%) of resistance to amphotericin B are reported. In our study the amphotericin B resistance of 7.3% was in accordance with these rates. Few studies have reported amphotericin B resistance in *Candida* species [22]. Additionally, the use of azole group medications like fluconazole for prophylactic antifungal treatment have led to an increase in infections developing caused by fluconazole-resistant *Candida* species [23,24]. Among the non-albicans species, *C. tropicalis* and *C. parapsilosis* are both generally susceptible to azoles; however, *C. tropicalis* is less susceptible to fluconazole than *C. albicans* [25]. In our findings, *C. tropicalis* and *C. parapsilosis* are susceptible to azoles and *C. albicans* was 11% resistant to fluconazole and 11.8% resistant to voriconazole. As echinocandins have toxic effects on the liver, they are not used in routine antifungal treatment. In our study *Candida* species were observed to have least resistance to echinocandin class antifungals. Micafungin resistance was highest in *C. parapsilosis*. Additionally, in 7 micafungin-resistant strains, 5 actually had intermediate susceptibility.

**Conclusion**

Among *Candida* species isolated from clinical samples from a variety of services in our hospital, *C. albicans* was the most common, *C. tropicalis* the second most commonly isolated species. In blood cultures, *C. parapsilosis* was most prevalent, *C. albicans* the second most commonly isolated species. In conclusion, in parallel with the increase in patient populations at risk of *Candida* infections, rational treatment planning and resistance rates should be determined for *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates. In terms of hospital-acquired fungal infections, intensive care units are considered...
hospital environments requiring careful monitoring both in terms of location and health personnel conditions. In the future, a greater number of samples should be collected to identify and analyze Candida species, which will help us to monitor and choose appropriate antifungal treatment at our hospital.

References

Corresponding author
Mustafa Kerem Calgin, MD.
Ordu Universitesi Egitim ve Arastirma Hastanesi, Bucak Mh.
Nefsi Bucak Cd. Altinordu, Ordu, Turkey.
Postal code: 52200
Fax: +90 452 225 01 90
Phone: +90 505 495 17 66
email: mkcalgin@gmail.com

Conflict of interests: No conflict of interests is declared.