Abstract. – OBJECTIVE: Recent research has suggested that fungemia may demonstrate an epidemiologic shift in etiologic agents. This study focuses on the agents causing fungemia and antifungal resistance in a tertiary hospital.

PATIENTS AND METHODS: We evaluated all-age fungemia cases admitted to Balikesir Ataturk City Hospital in 2017-2021. Blood cultures (BC) were studied using BacT/Alert® 3D (bioMérieux, Marcy’Etoile, France) and Render BC128 System (Render Biotech Co. Ltd., Shenzhen, China). On the data, we explored only the first fungal positive samples or the first isolates in different episodes of the same patients. Upon The Clinical and Laboratory Standards Institute (CLSI) disk diffusion guidelines, conventional methods and the Phoenix™ 100 System (Becton Dickinson, Franklin Lakes, NJ, USA) were utilized for antifungal susceptibility identifications.

RESULTS: The findings showed that 325 (0.84%) of 38,682 BC sets were positive for fungal growth. Except for four cases (1.2%) [Saprochaete capitata (n = 2); Trichosporon asahii (n = 1), and Saccharomyces cerevisiae (n = 1)], all positive cases yielded Candida spp. (98.8%) growth. In these patients, the following Candida spp. were isolated: Candida albicans complex (n = 155; 47.7%), Candida parapsilosis complex (n = 127; 39.1%), Candida glabrata complex (n = 19; 5.85%), Candida tropicalis (n = 12; 3.7%), Candida kefyr (n = 5; 1.54%), Candida krusei (n = 2; 0.62%), and Candida guilliermondii complex (n = 1; 0.31%). We also realized that while none of the Candida spp. had echinocandin resistance, 8 C. parapsilosis complex isolates were resistant to fluconazole, and 17 C. parapsilosis complex and 2 C. tropicalis isolates were susceptible dose-dependent to fluconazole.

CONCLUSIONS: In brief, antifungal resistance is more likely to restrict therapeutic options, albeit it is, fortunately, not prevalent in Turkey despite a few recent reports. Yet, a robust detection or management of antifungal resistance requires species-level identification and strict compliance with relevant management guidelines. Besides, challenges in research may be compensated with a national data set built with data from local laboratories.

Key Words: Invasive fungal infections, Bloodstream infections, Fungi, Candida.

Introduction

Invasive fungal infections (IFIs) are often characterized by high mortality rates with a loss of nearly two million annually. Although IFIs are associated with several predisposing factors (e.g., infections), there are also cases without such underlying conditions. In general, etiologic agents of IFIs are mainly predicted by geographic location, clinical status, and underlying disorders; nevertheless, the most prevalent causative agents are known to be Candida spp – top five causative species are Candida albicans complex, Candida glabrata complex, Candida parapsilosis complex, Candida tropicalis, and Candida krusei. Recently, the literature has reported an epidemiologic shift in the alignment of causatives in IFIs. Among the deadliest IFIs, the microbiological diagnosis of fungemia mainly depends on blood culture (BC) findings. In the last decade, relevant authorities [e.g., the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)] have released several guidelines in the diagnosis and management of fungemia cases. The recommendations on these guidelines primarily hinge upon the clinical status, infection types, and molecular structures of fungi, as well as their in vitro susceptibility to antifungals. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and The Clinical and Laboratory Standards Institute (CLSI) have also integratively published standard procedures, epidemiological cut-off values (ECOFFs), and clinical breakpoints (CBPs) in antifungal susceptibility testing (AFST) to optimize antimicrobial therapy. Accordingly,
species-level identification and AFST are routinely recommended in fungemia cases since proper early treatment directly affects its prognosis. However, the reference broth microdilution (BMD) as an antimicrobial susceptibility testing method is known to be expensive and requires experienced staff to be performed. Moreover, limited data exist for only particular species and antifungals in CLSI and EUCAST standards, further restraining laboratories from leading clinicians in fungemia cases. On the other hand, CLSI disk diffusion is also a reference but a more convenient testing method with a recently widened spectrum, but it is still not possible to assess any species except “Top 5”.

Epidemiologic alterations in etiologic agents and antifungal resistance (AFR) may be a hot but undermentioned issue within infectious diseases. Besides, as in bacterial infections, surveillance data of fungi is deemed essential for tracking the local/national status of fungal infections to lead to national guidelines. Thus, the present study attempted to address fungemia agents in a five-year period in a tertiary hospital and their antifungal resistance in the last two years.

**Patients and Methods**

**Sample**

We considered the findings of routine blood cultures (BCs) obtained from patients in all age groups at Balikesir Ataturk City Hospital in 2017-2021. BCs were studied using BacT/Alert® 3D (bioMérieux, Marcyl’Etoile, France) and Render BC128 System (Render Biotech Co. Ltd., Shenzhen, China). On the data, we explored only the first fungal positive samples or the first isolates in different episodes of the same patients.

**Methods**

All positive BC vials were Gram stained and subcultured onto 5% sheep blood agar, eosin-methylene blue agar, chocolate agar, Sabouraud dextrose agar (SDA) with chloramphenicol and gentamicin (RTA Laboratories, Kocaeli, Turkey), and ROSACHROM Candida Agar (Gül Biology Laboratories, Istanbul, Turkey). Plates were incubated at 35-37°C in a 5% CO₂ atmosphere for at least 48 hours. Conventional methods and the Phoenix™ 100 automated system (Becton Dickinson, Franklin Lakes, NJ, USA) with cornmeal tween 80 agar (RTA Laboratories, Kocaeli, Turkey) were utilized for antifungal susceptibility identifications.

**AFST**

AFSTs were applied using the disk diffusion method (Fluconazole 25 µg, Voriconazole 1 µg, Caspofungin 5 µg; Bioanalyse, Ankara, Turkey) upon the CLSI-M60 guidelines. Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used for quality control purposes. The testing could be applied for only the samples obtained within two years since previous isolates were not stockpiled, and regular AFST was not possible at the center. Any test with suspicious results was re-performed.

It should also be noted that AFSTs could not be applied for C. glabrata complex, C. tropicalis, Candida kefyr, Saprochaeta capitata, Trichosporon asahii, and Saccharomyces cerevisiae due to lack of zone diameter breakpoints and interpretative categories of tested antifungals. While fluconazole was not tested (intrinsic resistance-IR) for C. krusei, Candida guillermondii complex was only tested against caspofungin (no data on azoles). Finally, T. asahii was reported as IR for echinocandins. The findings were categorized as susceptible (S), intermediate (I), susceptible dose-dependent (SDD), and resistant (R).

**Statistical Analysis**

Since we designed the present research as a retrospective descriptive study, we share the ratios of fungemia-caused patient loss (FCPL) by services given the patient data. In addition, we statically analyzed the research data using the SPSS 22.0 (IBM Corp., Armonk, NY, USA) program. Categorical variables are denoted as numbers and percentages, and we performed a Chi-square test to compare the data between the independent groups. A *p*-value < 0.05 was considered statistically significant.

**Results**

The findings showed that 325 (0.84%) of 38,682 BC sets were positive for fungal growth. Except for four cases (1.2%) [Saprochaeta capitata, (2), Trichosporon asahii, (1), and Saccharomyces cerevisiae, (1)], all positive cases yielded Candida spp. (98.8%) growth. In these patients, the following Candida spp. were isolated: Candida albicans complex (n = 155; 47.7%), Candida parapsilosis complex (n = 127; 39.1%), Candida glabrata complex (n = 19; 5.85%), Candida tropicalis (n = 12; 3.7%), Candida kefyr (n = 5; 1.54%), Candida krusei (n = 2; 0.62%),
and Candida guilliermondii complex (n = 1; 0.31%). When it comes to species distributions by service, while non-albicans Candida cases were significantly predominant in intensive care units (ICUs) and surgical services, the rate of C. albicans complex isolations was found to be significantly higher in internal medicine services (p < 0.001 for both; Table I).

Table II presents all AFST findings along with the FCPL rates. Accordingly, all caspofungin-tested Candida isolates were susceptible, while fluconazole resistance was only observed in C. parapsilosis complex isolates (n = 8), three of which were also categorized as I for voriconazole. Besides, 17 C. parapsilosis complex and 2 C. tropicalis isolates were SDD to fluconazole. The difference between FCPL rates of C. albicans complex (38.0%) and non-albicans Candida (45.4%) was not statistically significant (p = 352).

**Discussion**

Bloodstream infections (BSIs) are known to be severe death-causing reasons with 13-20% fatality rates. When compared to bacteremiae, fungemiae are relatively rare but may end up with mortality over 70%. Although Candida spp. are mostly encountered organisms, recent reports indicate a rise of rare species too. In

| Table I. Distribution of Isolated Species by Service (2017-2021). |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Species/Service   | ICUs¹ | Internal medicine services² | Surgical services³ | Total |
|                   | N | % | N | % | N | % | N | % |
| Candida albicans complex | 129 | 41.1 | 15 | 4.6 | 6 | 1.8 | 155 | 47.6 |
| Candida parapsilosis complex | 113 | 34.7 | 4 | 1.2 | 10 | 3.1 | 127 | 39.1 |
| Candida glabrata complex | 14 | 4.3 | 1 | 0.3 | 4 | 1.2 | 19 | 5.8 |
| Candida tropicalis | 11 | 3.3 | 1 | 0.3 | N | N | 12 | 3.6 |
| Candida kefyr | 2 | 0.6 | 2 | 0.6 | 1 | 0.3 | 5 | 1.5 |
| Candida krusei | 2 | 0.6 | N | N | N | N | 2 | 0.6 |
| Candida guilliermondii complex | 1 | 0.3 | N | N | N | N | 1 | 0.3 |
| Saprochaete capitata | 1 | 0.3 | N | N | N | N | 1 | 0.3 |
| Trichosporon asahii | N | N | 1 | 0.3 | N | N | 2 | 0.6 |
| Saccharomyces cerevisiae | N | N | N | N | N | N | 325 | 100 |

¹General adult, cardiovascular, surgical, neurology, neonatal, and pediatric ICUs; ²Including pediatrics; ³Pediatrics and adult surgery services; ⁴Non-albicans Candida cases were significantly predominant in ICUs and surgical services, while C. albicans complex dominance was obvious in internal medicine services (p < 0.001).

| Table II. Antifungal Susceptibility Profiles (2020-2021). |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Species/Antifungal | Fluconazole (n, %) | Voriconazole (n, %) | Caspofungin (n, %) | FCPL rate (%) |
|                   | R | SDD | R | I | R | SDD | p = 0.352¹ |
| Candida albicans complex | N | N | N | N | N | N | 38 |
| Candida parapsilosis complex | 8 (6.3%) | 17 (13.4%) | N | 3 (2.4%) | N | N | 41 |
| Candida glabrata complex | NA | NA | NA | NA | NA | NA | 58 |
| Candida tropicalis | N | 2 (16.7%) | N | N | N | N | 83 |
| Candida kefyr | NA | NA | NA | NA | NA | NA | 20 |
| Candida krusei | IR | IR | N | N | N | N | 50 |
| Candida guilliermondii complex | NA | NA | NA | NA | N | N | N |
| Saprochaete capitata | NA | NA | NA | NA | NA | NA | N |
| Trichosporon asahii | NA | NA | NA | NA | IR | IR | N |
| Saccharomyces cerevisiae | NA | NA | NA | NA | NA | NA | N |

FCPL: Fungemia-Caused Patient Loss; SDD: Susceptible dose-dependent; R: Resistant; I: Intermediate; IR: Intrinsic resistance; NA: Not Applicable; N: None. ¹The difference between FCPL rates of C. albicans complex (38.0%) and non-albicans Candida (45.4%) was not statistically significant, but FCPL was higher in non-albicans Candida fungemia cases.
addition, the frequency alignment among *Candida* spp. is also in a change. Interestingly, the term “mixed fungemia” has recently been coined in the mycology literature, which implies the significance of culture- and species-level identification to be able to offer appropriate treatment15.

Firstly, while a previous study4 at our center shared 3-year epidemiologic data (2017-2019), the present study attempted to depict 5-year data of fungal BSIs (2017-2021) and AFR findings of the last two years to contribute to local preemptive and empirical therapies. Regarding our findings, *C. albicans* complex was discovered to be the most prevalent organism (n = 155; 47.7%), as expected, albeit high rates of *C. parapsilosis* complex (n = 127; 39.1%) seem alarming. Our findings overlap with the results of a longitudinal study of a Turkey-based mycology laboratory (21.5%)3 and a nationwide study in Italy (26.2%)16. We also found that non-*albicans Candida* findings, C. albicans complex dominance was conspicuous in internal medicine services (p < 0.001 for both). On the other hand, our *C. parapsilosis* complex isolation rates were found to be higher than in other studies, which may be attributed to insufficient care of catheters since the majority of strains were isolated from ICUs and surgical services. Er et al17 exactly reported the same issue, even with a higher rate of isolation in their ICUs, since Montagna et al16 stated that parenteral nutrition in the ICUs may be a noteworthy risk factor for fungemia caused by non-*albicans Candida* species that exhibit a certainly higher mortality rate than *C. albicans* complex. *C. parapsilosis* complex was recently reported to be able to show resistance to fluconazole (>10%) and/or dwindling susceptibility, possibly due to clonal spreading18,19. In the first multicenter study2 on AFR in fungemia agents from Turkey, overall fluconazole resistance of this organism was reported to be 7.7%. In this study, our findings revealed this rate to be 6.3%, slightly lower than previous reports, but SDD rates may offer a clue of future perspective (13.4%). We may classify the “I” category of voriconazole susceptibility (2.4%) as another notable issue, totally compatible with the mentioned report (2.1%). In their study, Er et al17 documented significantly higher levels of AFR for both azoles, which might be because of methodological differences (i.e., utilizing the gradient strip test, non-reference method). Similarly, the literature hosts Turkish reports utilizing different methodologies and suggesting resistance rates in a wide spectrum (e.g., fluconazole R: 0-27%; voriconazole SDD: 0-2.1%)20-23. Nevertheless, further research may need to scrutinize high rates *C. parapsilosis* complex isolations with more “standardized” Turkish data on AFR.

FCPL rates of *C. albicans* complex (38.0%) and non-*albicans Candida* (45.4%) did not show a significant difference (p = 0.352; Table II). Nevertheless, while being higher in non-*albicans Candida* fungemia cases, this rate was the highest in *C. tropicalis* cases (83%). *C. tropicalis* is usually not that prevalent among fungemias, but it is particularly noteworthy that most of *C. tropicalis*-BSIs in lost patients were sourced by urinary tract (UTIs) and potentially nosocomial cases. This situation may indicate the same problem as in *C. parapsilosis* complex, and clonal spreading was also stated24. On the other hand, there is a paucity of data on the global AFR of *C. tropicalis*, but the rise of azole non-susceptibility (even pan-azole R) has become a concern recently25. Several Turkey-based studies2,20,21 did not observe R to azoles in fungemia cases, as in our study (only two isolates were SDD to fluconazole). However, relevant guidelines should compose and recommend a more dedicated pharmacological approach, as well as potential microbiological resistance, by infection site.

It is particularly interesting that we detected rare species, such as *S. capitata*, *T. asahii*, and *S. cerevisiae*, as causative agents along with *C. kefyr* and *C. guilliermondii* complex. Although the ESCMID9 published a management guideline for these rare yeasts, it is barely known of their AFR potential and therapeutic success against any antifungal agents. In their study, Alp et al26 obviously stated that fungemia by noncommon species is often underestimated; thus, its susceptibility patterns may show variations. Besides, it is deemed crucial for a laboratory to be aware of diagnostic insufficiency of conventional and automated methods (BD Phoenix™ 100, Becton Dickinson (Becton Dickinson, Franklin Lakes, NJ, USA), and VITEK 2, bioMérieux, Marcyl’Etoile, Paris, France) since there was evidence in previous research27-29 regarding misidentifications of uncommon species, including *Candida auris*: this may be how our study differs from the mentioned studies. Although the novel technology MALDI-TOF MS offers promising results to achieve this goal, joint and comparative us-
Microbiological analysis of fungemia

The present study has a few limitations. Since the CLSI disk diffusion method offers a rather limited spectrum of interpretation, we could not comment on particular species, including C. glabrata complex\(^\text{11}\). In addition, we could not make any further evaluations of these species (e.g., FKS mutation screening), which was already beyond the scope of this study. Besides, we could not categorize BSIs (e.g., catheter-associated, nosocomial, etc.) due to a lack of necessary data in our center’s information management system. Lastly, it was unavailable and also beyond the scope of this study to evaluate species-based mortality considering other risk factors, underlying disorders, and clinical findings.

Conclusions

The species spectrum of fungi-caused BSIs has been widened, and AFR has become a crucial parameter in the prognosis of these BSIs. Indeed, C. auris has recently reminded the importance of continuous screening for fungi and fungi-caused BSIs\(^\text{8}\). Since the population of “immunoproblematic” individuals is growing worldwide, laboratories should evaluate and optimize their diagnostic capacities, relevant authorities should regularly update their guidelines, and further research and clinicians should be locked on such infections.

Acknowledgements

A part of this study was presented orally in “6th International Congress on Medical Sciences and Multidisciplinary Approaches” conference, 11-12 March 2023, Istanbul, Turkey.

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