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Trends of Antifungal Agent Susceptibility of *Candida* Strains Isolated from Blood Cultures in 2009–2018

Yu-Yean Hwang[1], On-Kyun Kang[1], Chang-Eun Park[2], Moo-Sik Lee[3], Young-Kwon Kim[3], Hee-Jae Huh[4], Nam-Yong Lee[4]*

[1]Department of Laboratory Medicine, Samsung Medical Center, Seoul, Republic of Korea

[2]Department of Biomedical Laboratory Science, Molecular Diagnostics Research Institute, Namseoul University, Cheonan, Republic of Korea

[3]Department of Health Sciences, The Graduate School of Konyang University, Daejeon, Republic of Korea

[4]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

***Corresponding author:** Nam-Yong Lee, micro.lee@samsung.com

Abstract

Candida is one of the most common causes of bloodstream infections and a leading cause of morbidity and mortality among hospitalized patients. The purpose of this study was to provide important information for formulating empirical treatment plans for candidemia by investigating the antifungal resistance rate of *Candida*. Among the *Candida* strains (973 cases) isolated from blood culture tests at Samsung Medical Center in 2009–2018, 4.7% (n = 44) comprising the *Candida* spp. (932 strains) showed resistance to fluconazole. The resistant strains included *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*. In addition, *Candida* spp. (947 strains) showed resistance to amphotericin B (n = 6, 0.6%), flucytosine (n = 23, 2.4%) and voriconazole (n = 24, 3.1%). *C. albicans* was resistant to fluconazole (n = 23, 6.9%) and voriconazole (n = 21, 6.0%), The statistical analysis showed that *C. albicans* and non-albicans *Candida* species were resistant to fluconazole (P = 0.039) and voriconazole (P < 0.001). A monitoring system to understand the rate of candidiasis infections in a hospital setting is required. It is also important to make the right choice of antifungal agent based on drug susceptibility patterns. Therefore, an infection surveillance policy that tracks *Candida* resistance through regular antifungal susceptibility tests is necessary.

Keywords

Antifungal agent susceptibility
Blood culture
Candidemia
Candida bloodstream infections
Candida species

1. Introduction

Sepsis and septic shock due to bloodstream infection (BSI) are common syndromes among patients admitted to intensive care units (ICUs) and are caused by infections of microorganisms such as bacteria, fungi, viruses, and parasites in the blood [1]. According to

research results from North America and Europe, BSI occurs in more than 2 million cases annually and is one of the seven leading causes of death worldwide, causing 250,000 deaths and a mortality rate of 13% to 20% [2]. Research on BSI has primarily focused on bacteria, which are dominant pathogens compared to other types of microorganisms. Epidemiologic studies on BSI caused by fungi have received relatively less attention, possibly because they are challenging to detect in clinical samples [3]. Nevertheless, fungi are one of the major causative agents of BSI, and fungal BSI and invasive candidiasis, caused by fungi, remain a significant threat to hospitalized patients, resulting in up to a 71% mortality rate and increased healthcare costs [4,5]. In the United States, fungal BSIs account for 9% of cases and are the fourth most commonly isolated pathogen from the blood [6]. In South Korea, the prevalence of fungal BSIs varies depending on the time and region, with recent research showing that fungal BSIs account for 6.8% [7].

Candida is responsible for more than 90% of fungal BSI cases [8], and *Candida* species (spp.) are the most frequently isolated pathogens, especially in hospitalized patients [9]. Patients with *Candida* fungal BSI in ICU have a higher mortality rate and longer hospital stays compared to patients with Gram-positive and Gram-negative bacterial BSIs [10]. Despite significant advances in antifungal therapy, candidemia is still associated with a mortality rate of 35% to 71%, and it can worsen when empirical antifungal therapy is delayed [11]. The increasing incidence of fungal infections is attributed to various factors, including the rise in immunocompromised patients due to cancer treatments, organ transplantation, and acquired immunodeficiency, as well as increased use of catheters, devices, immunosuppressive therapy, and antibiotics, along with improved survival in intensive care.

The use of fluconazole has increased the treatment success of candidemia [12], and it is widely used worldwide due to its low toxicity, treatment effectiveness, and ease of administration. As a result, fluconazole resistance has emerged among *Candida* spp., including those causing invasive candidiasis. The distribution and antimicrobial susceptibility patterns of *Candida* spp. as causative agents of invasive candidiasis vary by institution, region, and country, and these differences are believed to be associated with variations in antifungal use and infection control policies at different hospitals or in different regions [13]. Therefore, periodic surveillance of the epidemiology of fungal infections, along with the distribution of species and antibiotic susceptibility patterns, is required to establish appropriate diagnostic and treatment policies. This study was conducted not only to enhance understanding of the epidemiology of fungal infections but also to provide essential information for establishing antifungal treatment guidelines for patients suspected of fungal infections.

2. Materials and methods

2.1. Study subjects

This study retrospectively examined clinical bacterial isolates recovered from blood cultures of patients who were referred for BSI diagnosis at Samsung Medical Center (1,979 beds), a tertiary hospital in Seoul, South Korea, over a ten-year period from January 2009 to December 2018. Fungal identification and antibiotic susceptibility testing were performed as part of routine patient care, and electronic health records (EHR) were used for the retrospective analysis of the study. This study was conducted under a review exemption from the institutional review board (SMC 2019-07-74).

2.2. Study methods

2.2.1. Blood culture

A total of 717,996 samples were obtained from inpatients and outpatients suspected of having bacteremia and were cultured using the BacT/ALERT® 3D (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions, with a positive result

or cultured for up to five days. Aerobic and anaerobic media (BacT/ALERT FA Plus, BacT/Alert FN Plus, and BacT/ALERT PF Plus; bioMérieux) were used. For culture-positive blood cultures, a portion of the culture was taken for gram staining, while an appropriate amount was collected and subcultured onto blood agar plate (BAP, SHINYANG Diagnostics, Seoul, Korea), MacConkey agar (KORMED, Seongnam, Korea), chocolate agar (KORMED), and brucella agar (KORMED) using aseptic technique. Subcultured plates were then incubated at 35°C in a CO_2 incubator (Thermo Fisher Scientific, Massachusetts, USA) for 18 to 24 hours. Brucella agar plates were placed in an anaerobe chamber (BACTRON, Sheldon Manufacturing Inc., OR, USA) for 48 hours.

2.2.2. Fungal identification

From 2009 to 2015, fungal identification was performed using the VITEK® 2 system (bioMérieux) YST ID card according to the manufacturer's instructions. For quality control, standard strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used and tested following Clinical and Laboratory Standards Institute (CLSI) guidelines. From 2016 to 2018, VITEK-MS (bioMérieux) was used for fungal identification following the manufacturer's instructions. For quality control, the standard strain *C. glabrata* ATCC 2950 was used and tested according to CLSI guidelines.

2.2.3. Antifungal susceptibility testing

Antifungal susceptibility testing was conducted using the VITEK® 2 system (bioMérieux) YS07 card according to the manufacturer's instructions. The criteria for antifungal susceptibility testing were based on the CLSI M60 guideline. Interpretation of susceptibility (S), intermediate (I), susceptible dose-dependent (SDD), and resistance (R) was performed according to the cut-off values recommended in the CLSI M27-S3 guideline for fluconazole and voriconazole (reference method for broth dilution antifungal susceptibility testing of yeast; CLSI M27-S3: 3ED 2008) and referencing U.S. Food and Drug Administration (FDA) guidelines. For flucytosine, as CLSI did not provide cut-off values, FDA guideline cut-off values were used. Amphotericin B cut-off values were referenced from the manufacturer's (bioMérieux) literature, as neither CLSI nor FDA provided cut-off values for it. The breakpoints for each strain are shown in **Table 1**. For quality control, standard strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used and tested according to CLSI guidelines.

JCM-wild-type MIC distributions and epidemiological cut-off values for amphotericin B, flucytosine, and itraconazole. *Candida* species as determined by CLSI broth microdilution. Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible dose-dependent; JCM, Japan Collection of Microorganisms; CLSI, Clinical and Laboratory Standards Institute; FDA, U.S. Food and Drug Administration.

2.2.4. Statistical analysis

Percentages of culture results and antifungal resistance rates were analyzed using Microsoft Office Excel. IBM SPSS Statistics VER 27.0 (SPSS Inc. 233 S. Chicago, IL, USA) software was used for comparative analysis of antifungal resistance rates determined through susceptibility testing. A significance level of $P < 0.05$ was considered statistically significant. Chi-squared tests were used to confirm whether there were significant differences in resistance rates. In this analysis, only susceptibility (S) and resistance (R) results were compared, and intermediate (I) results were excluded.

3. Result

Over the ten-year period from 2009 to 2018, there were 717,996 blood culture referrals, with 54,739 (7.6%) of them yielding positive results. Fungal isolates were observed in 3,693 cases (6.7%) of the positive cultures. Among the fungal isolates, excluding mold

Table 1. Interpretation guidelines for antibiotic susceptibility tests of *Candida* species

Antifungal agent	Organisms	MIC range (μg/mL)				Source
		S	SDD	I	R	
Amphotericin B	*Candida* species	≤ 1		2	≥ 4	JCM
Fluconazole	*C. albicans*	≤ 2		4	≥ 8	CLSI
	C. glabrata		≤ 32		≥ 64	CLSI
	C. krusei	-	-	-	-	
	C. parapsilosis	≤ 2	4		≥ 8	CLSI
	C. tropicalis	≤ 2	4		≥ 8	CLSI
Voriconazole	*C. albicans*	≤ 0.12	0.25–0.5		≥ 1	CLSI
	C. glabrata	-	-	-	-	
	C. krusei	≤ 0.5	1		≥ 2	CLSI
	C. parapsilosis	≤ 0.12	0.25–0.5		≥ 1	CLSI
	C. tropicalis	≤ 0.12	0.25–0.5		≥ 1	CLSI
Flucytosine	*Candida* species	≤ 4			≥ 32	FDA

and *Cryptococcus*, *Candida* was found in 3,533 cases, with a 6.5% isolation rate. After eliminating duplicate isolates and categorizing them by patients, there were a total of 1,036 patients. *Candida* species were isolated as follows: *C. albicans* (33.8%), *C. tropicalis* (28.6%), *C. glabrata* (19.8%), *C. parapsilosis* (7.8%), and *C. krusei* (4.0%).

Antifungal susceptibility testing was performed on 973 isolates that excluded unidentified strains and strains without established interpretive criteria. The results are as follows (**Table 2** and **Figure 1**):

(1) For amphotericin B (AMB), 96.7% (*n* = 941) showed susceptibility (S), 2.7% (*n* = 26) exhibited intermediate (I), and 0.6% (*n* = 6) were resistant (R).

(2) For fluconazole, among 932 isolates (excluding naturally resistant *C. krusei*), 73.2% (*n* = 682) were susceptible (S), 22.1% (*n* = 206) were susceptible dose-dependent (SDD) or intermediate (I), and 4.7% (*n* = 44) were resistant (R).

(3) For flucytosine, 95.0% (*n* = 924) were susceptible (S), 2.7% (*n* = 26) were weakly dose-dependent (SDD), and 2.4% (*n* = 23) were resistant (R).

(4) For voriconazole, among 723 isolates (excluding *C. glabrata* without interpretive criteria), 95.9% (*n* = 693) were susceptible (S), 0.8% (*n* = 6) were intermediate (I), and 3.3% (*n* = 24) were resistant (R).

Further analysis of specific *Candida* species revealed the following (**Figure 2**):

(1) For *C. albicans* (*n* = 350), 0.3% (*n* = 1) was resistant to amphotericin B, and 6.6% (*n* = 23) exhibited resistance to fluconazole, with the highest resistance rate of 24.4% observed in 2017. Resistance to flucytosine remained stable at 4.9% (*n* = 17), and resistance to voriconazole was 6.0% (*n* = 21), with the highest rate of 24.4% also observed in 2017.

(2) *C. glabrata* (*n* = 205) exhibited 2.0% (*n* = 4) resistance to amphotericin B and 2.0% (*n* = 4) resistance to flucytosine, with sporadic resistance patterns. In this study, 3.9% (*n* = 8) of *C. glabrata* showed inherent resistance to fluconazole, while voriconazole was excluded due to the lack of interpretive criteria.

(3) *C. krusei* (*n* = 41) did not exhibit resistance to amphotericin B, voriconazole, or flucytosine. However, intermediate resistance to amphotericin B (14.6%), flucytosine (63.4%), and voriconazole (2.4%) was identified, suggesting impending

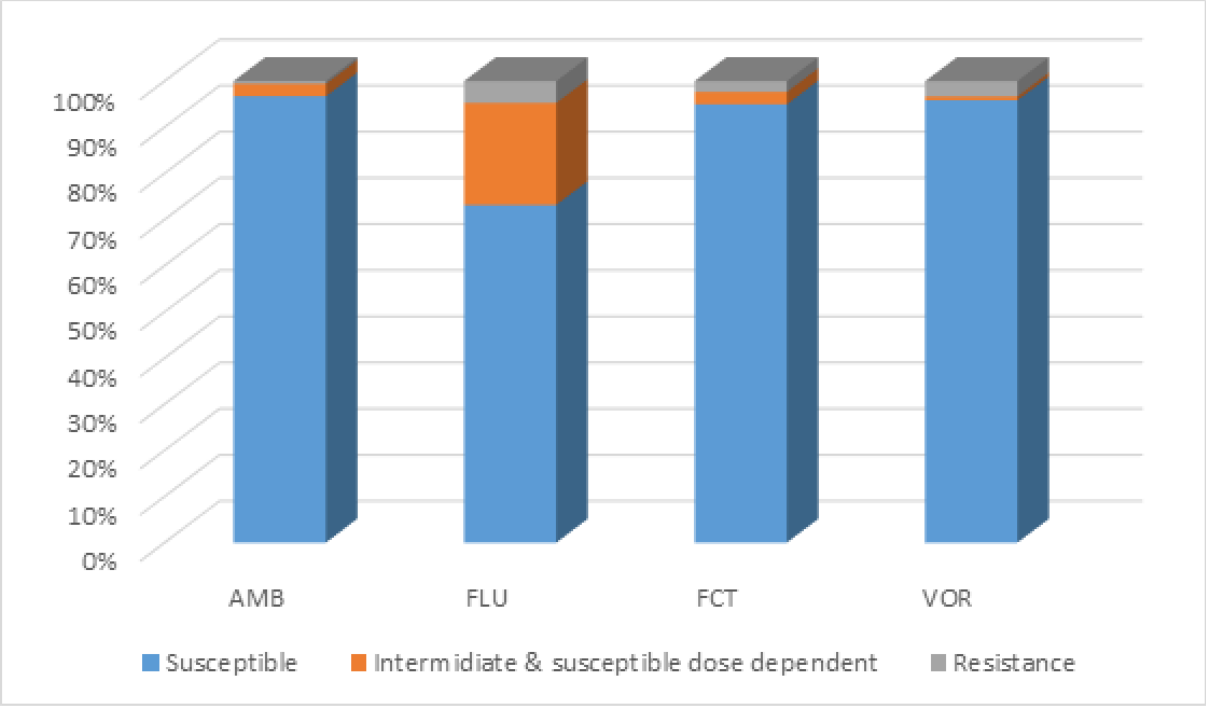

Figure 1. Antifungal resistance ratio of all *Candida* species isolated from blood cultures from 2009 to 2018. Abbreviations: See **Table 1**.

Table 2. Antifungal susceptibility frequency of all *Candida* species strains isolated from blood culture in 2009–2018

Interpretation of susceptibility to antifungal agents	Results of antifungal susceptibility test (%)			
	Amphotericin B	Fluconazole	Flucytosine	Voriconazole
Susceptible	941 (96.7)	682 (73.2)	924 (95.0)	693 (95.9)
Susceptible dose-dependent & intermediate	26 (2.7)	206 (22.1)	26 (2.7)	6 (0.8)
Resistant	6 (0.6)	44 (4.7)	23 (2.4)	24 (3.3)
Total	973 (100)	932 (100)	973 (100)	723 (100)

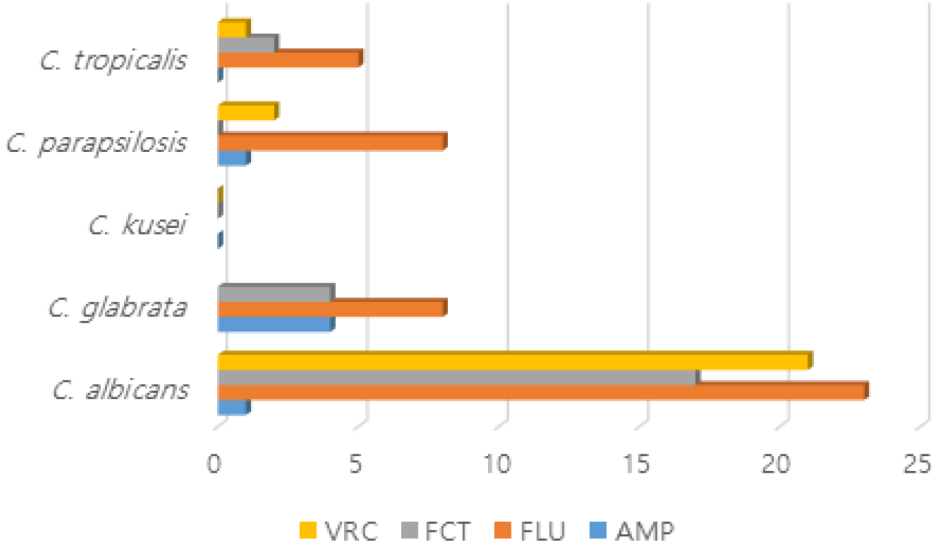

Figure 2. Antifungal resistance rates of *Candida* species isolated from blood cultures in 10 years. Abbreviations: VRC, voriconazole; FCT, flucytosine; FLU, fluconazole; AMB, amphotericin B.

resistance. Fluconazole was excluded for *C. krusei* as it is naturally resistant.

(4) *C. parapsilosis* (n = 81) showed 1.2% (n = 1) resistance to amphotericin B and 9.9% (n = 8) resistance to fluconazole. Flucytosine exhibited 100% (n = 81) susceptibility and voriconazole showed 2.5% (n = 2) resistance.

(5) *C. tropicalis* (n = 296) exhibited 100% susceptibility to amphotericin B, with 1.7% (n = 5) resistance to fluconazole. Flucytosine and voriconazole showed 0.7% (n = 2) and 0.3% (n = 1) resistance, respectively.

The results of the statistical analysis indicated that the resistance rate to fluconazole in *C. albicans* was significantly higher when compared to the resistance rate in non-albicans *Candida* species (P = 0.039). Furthermore, the resistance rate of voriconazole in *C. albicans* was significantly higher than that in non-albicans *Candida* species (P < 0.001). However, the resistance rate to fluconazole in *C. albicans* did not show a significant difference when compared to the resistance rates for other antifungal agents (P = 0.067). Similarly, the resistance rate to voriconazole in *C. albicans* did not exhibit a significant difference compared to the resistance rates for other antifungal agents (P < 0.099) (**Tables 3** and **4**).

4. Discussion

Accurately identifying the incidence of fungal diseases is challenging because fungal infections often present with nonspecific symptoms and are difficult to diagnose [14]. Diagnosis typically requires invasive

Table 3. Significant difference between fluconazole and voriconazole resistance patterns in *C. albicans* and non-albicans *Candida* species

Antifungal drug	*Candida albicans*			Non-albicans *Candida* species			*P*-value
	Organism	*n*	Resistance number (%)	Organism	*n*	Resistance number (%)	
Fluconazole	*C. albicans*	350	23 (6.6)	*C. glabrata*	205	8 (3.9)	
				C. parapsilosi	81	8 (9.9)	
				C. tropicalis	296	5 (1.7)	
Total		350	23 (6.6)		582	21 (3.6)	0.039
Voriconazole	*C. albicans*	350	21 (6.0)	*C. krusei*	41	1 (2.4)	
				C. parapsilosi	81	2 (2.5)	
				C. tropicalis	296	1 (0.3)	
Total		350	21 (6.0)		418	4 (0.72)	0.001

Table 4. Significant difference between fluconazole and voriconazole resistance patterns in *C. albicans* compared to other antifungal agents

Organism	Antifungal drug	*n*	Resistance number (%)	Antifungal drug	*n*	Resistance number (%)	*P*-value
C. albicans	Fluconazole	350	23 (6.6)	Amphotericin B	350	1 (0.3)	
				Voriconazole	350	21 (6.0)	
				Flucytosine	350	17 (4.9)	
	Total	350	23 (6.6)		1,050	39 (3.7)	0.067
	Voriconazole	350	21 (6.0)	Fluconazole	350	23 (6.6)	
				Amphotericin B	350	1 (0.3)	
				Flucytosine	350	17 (4.9)	
	Total	350	21 (6.0)		1,050	41 (3.9)	0.099

tissue sampling, and fungal cultures do not always yield positive results. Additionally, histopathologic differentiation is challenging, and cross-reactivity in fungal antibody tests can occur. Skin tests for latent infections are generally not applicable. Therefore, surveillance activities can provide valuable information on the trends of fungal diseases. However, routine surveillance for most fungal infections is lacking, making the availability of data for trend analysis quite limited. Consequently, empirical antimicrobial therapy is often used in clinical practice. The causative fungal species and antifungal susceptibility results for candidemia can vary by region, study period, and other factors, and thus, periodic analysis is necessary.

The incidence of candidemia in patients admitted to hospitals and ICUs has been reported in many countries, including South Korea. The frequency of *Candida* isolates and trends in antifungal resistance rates can vary greatly within the same country and over time [15]. This variation may be due to various factors, including the basic ecology of *Candida* species, differences in patient populations, and available resources. It can also result from variations in medical and training programs, hospital infection control programs, and surveillance methods. The *Candida* species isolated from blood and the results of antifungal susceptibility testing can provide important information for empirical treatment strategies and antifungal therapy. Although many single-center studies have been reported, there are very few countries that have conducted widespread surveillance [16]. To understand the global incidence of candidemia, international collaboration between countries is essential [17].

Antifungal agents used to treat *Candida* BSIs include polyenes, echinocandins, azoles, and the flucytosine class. Initially, polyene amphotericin B was primarily used for the treatment of *Candida* BSIs due to its low toxicity [18]. However, it was later replaced by echinocandins, caspofungin acetate powder, voriconazole, and fluconazole. Fluconazole is relatively inexpensive, has excellent efficacy, and is easy to administer orally, making it widely used in clinical practice. The issue of fluconazole resistance in *Candida* species is a concerning situation, as it may also signify resistance to other azole antifungals. In this study, the resistance rate to fluconazole was 4.7%, which is relatively high among the studied antifungal agents.

Given the increasing reports of resistance in non-albicans *Candida* species from many institutions and regions, this is a matter of great importance [19], and as reported by the World Health Organization (WHO), fluconazole resistance is more prevalent in non-albicans *Candida* species [20]. The fluconazole antifungal susceptibility results in this study showed that *C. parapsilosis* exhibited a relatively high resistance rate, with 9.9% in *C. parapsilosis*, 6.6% in *C. albicans*, 3.9% in *C. glabrata*, and 3.9% in *C. tropicalis*. As mentioned earlier, the predominant infecting non-albicans *Candida* species can vary by region, and azole resistance rates may differ from one institution to another. This can impact clinical prescription patterns for the treatment and prevention of invasive candidiasis [21]. Studies by St-Germain *et al.* reported fluconazole resistance rates in blood-isolated *Candida* species to be less than 1% for *C. albicans*, 0% for *C. parapsilosis*, and 0% for *C. tropicalis* [13]. Similar results have been reported in domestic research. Chae *et al*. reported that from 1994 to 2001, fluconazole minimum inhibitory concentrations (MICs) for *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were consistently below 0.5 μg/mL, and no resistant strains were observed [22]. However, since 2000, studies have shown an increasing trend in resistance rates. Khan *et al.* reported that among *C. albicans* isolates from 371 patients between 2006 and 2011, only 0.8% (3 isolates) showed fluconazole resistance. Over the next six years (2012–2017), out of 363 patients, 1.4% (5 isolates) exhibited fluconazole resistance [23]. For *C. parapsilosis*, resistance was observed in 3.2% (1 isolate) out of 31 isolates between 2006 and 2011, and 4.5% (2 isolates) out of 44 isolates between 2012 and 2017 [23].

In a study by Rodriguez *et al*., 4% of the isolated strains exhibited fluconazole resistance, and 17% showed susceptibility dose-dependent (SDD). In this study, fluconazole resistance was observed in 4.7% of the 932 patients [24]. However, it is important to note that in 22.1% of the 206 isolates, including those with inherent fluconazole resistance in *C. glabrata*, the resistance rates increased significantly to SDD & intermediate, indicating that resistance was imminent. According to the World Health Organization (WHO), fluconazole resistance in *C. albicans* is estimated to be between 0% and 5%, with the highest rates reported in South Africa [25]. For non-albicans *Candida* species, fluconazole resistance presents a more significant problem, with rates ranging from 5% to 65%, and the highest rates reported in Denmark [25].

In this study, a significant difference in fluconazole resistance rates was found between *C. albicans* and non-albicans *Candida* species ($P = 0.039$). However, when comparing fluconazole resistance rates in *C. albicans* to resistance rates with other antifungal agents (AMB, FCT, VRC), no significant difference was found ($P = 0.067$). Specifically, the fluconazole resistance rate in *C. albicans* did not appear notably higher than the resistance rates with other antifungal agents. Additionally, when comparing the resistance rates in *C. albicans* to those in non-albicans *Candida* species, the voriconazole resistance rate in *C. albicans* was significantly higher ($P < 0.001$), suggesting an increased risk of resistant strains in *C. albicans* compared to non-albicans *Candida* species. Voriconazole is as effective as fluconazole for treating invasive candidiasis in clinical studies and is useful for fluconazole- and itraconazole-resistant strains. Consequently, the rising use of voriconazole against *C. albicans* is believed to contribute to the increase in voriconazole resistance in this species. In this study, the voriconazole resistance rate for *C. albicans* was 6.0%, which is similar to the fluconazole resistance rate.

Recent large-scale surveillance studies in the United States have reported an increase in echinocandin-resistant *C. glabrata* [26]. The exact causes of azole and echinocandin core resistance in some *C. glabrata* isolates remain unknown, though exposure to antifungals in the past may play a role, especially since many patients with invasive candidiasis caused by *C. glabrata* have various comorbid conditions [27]. Echinocandin resistance has also been reported in some settings, with approximately 6% of *C. glabrata* isolates in the United States demonstrating echinocandin resistance [15].

This study did not investigate echinocandin resistance. However, amphotericin B resistance was observed in *C. glabrata* isolates, with 4 out of 14 amphotericin B-resistant *Candida* isolates being *C. glabrata*. While amphotericin B resistance is rare in *Candida* species isolated in clinical settings, induction of resistance after amphotericin B therapy has been reported [28]. Estimating reliable incidence and prevalence rates is challenging, given the lack of surveillance data, particularly in developing countries. Establishing infection rates and prevalence rates through surveillance systems and preparing for the threat of emerging fungal infections is crucial. Hence, developing a long-term and sustainable surveillance program for fungal infections is a priority.

The main factors driving the emergence of antifungal resistance appear to be the increasing use of systemic antifungal agents and inappropriate prescribing practices. The availability of antifungal agents without prescriptions has also contributed significantly to the rise in resistance. Therefore, careful use of antifungal agents, appropriate dosing, and the need for regular surveillance to monitor the actual frequency of antifungal resistance, including pathogen tracking, should be considered. Various data, including information on the route of infection, will be crucial not only for patient treatment but also for infection management. Many antifungal agents for research are under development, but there is a need for the development of new antifungal agents with new mechanisms of action that can overcome the limitations

and side effects of clinically available antifungal agents.

While many previous studies have been reported, this study is relatively rare in that it collected and analyzed a relatively large sample of 973 isolates over several years from a single institution. Due to variations in antifungal agent usage and infection management policies between hospitals or regions, the distribution and antifungal resistance patterns of *Candida* species can differ. Thus, this study, which investigated the distribution of *Candida* species and antifungal resistance patterns of causative *Candida* species in BSI, will provide valuable data for the appropriate initiation of patient treatment, patient management, and antifungal treatment guidelines. Nevertheless, this study has two major limitations: First, due to its retrospective nature, clinical details, including antifungal agent treatment, were not available. Second, it was not feasible to send all *Candida* isolates for identification and antifungal susceptibility testing to another reference laboratory for confirmation. Future studies could explore the molecular genetic and epidemiological characteristics of antifungal resistance strains through antifungal resistance gene analysis, considering the limitations of this study.

Disclosure statement

The authors declare no conflict of interest.

References

[1] López Dupla M, Martínez JA, Vidal F, et al., Clinical Characterization of Breakthrough Bacteraemia: A Survey of 392 Episodes. J Intern Med, 258(2): 172–180. https://doi.org/10.1111/j.1365-2796.2005.01513.x

[2] Goto M, Al-Hasan MN, 2013, Overall Burden of Bloodstream Infection and Nosocomial Bloodstream Infection in North America and Europe. Clin Microbiol Infect, 19(6): 501–509. https://doi.org/10.1111/1469-0691.12195

[3] Trecarichi EM, Pagano L, Candoni A, et al., 2015, Current Epidemiology and Antimicrobial Resistance Data for Bacterial Bloodstream Infections in Patients with Hematologic Malignancies: An Italian Multicentre Prospective Survey. Clin Microbiol Infect, 21(4): 337–343. https://doi.org/10.1016/j.cmi.2014.11.022

[4] Antinori S, Milazzo L, Sollima S, et al., 2016, Candidemia and Invasive Candidiasis in Adults: A Narrative Review. Eur J Intern Med, 34: 21–28. https://doi.org/10.1016/j.ejim.2016.06.029

[5] Bassetti M, Righi E, Ansaldi F, et al., 2014, A Multicenter Study of Septic Shock Due to Candidemia: Outcomes and Predictors of Mortality. Intensive Care Med, 40: 839–845. https://doi.org/10.1007/s00134-014-3310-z

[6] Wisplinghoff H, Bischoff T, Tallent SM, et al., 2004, Nosocomial Bloodstream Infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Surveillance Study. Clin Infect Dis, 39(3): 309–317. https://doi.org/10.1086/421946

[7] Kim JS, Gong SY, Kim JW, et al., 2019, Antimicrobial Susceptibility Patterns of Microorganisms Isolated from Blood Culture During the Last 8 Years: 2010~2017. Korean J Clin Lab Sci, 51(2): 155–163. https://doi.org/10.15324/kjcls.2019.51.2.155

[8] Pappas PG, Kauffman CA, Andes DR, et al., 2016, Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis, 62(4): e1–e50. https://doi.org/10.1093/cid/civ933

[9] Horn DL, Fishman JA, Steinbach WJ, et al., 2007, Presentation of the PATH Alliance® Registry for Prospective Data

Collection and Analysis of the Epidemiology, Therapy, and Outcomes of Invasive Fungal Infections. Diagn Microbiol Infect Dis, 59(4): 407–414. https://doi.org/10.1016/j.diagmicrobio.2007.06.008

[10] Kett DH, Azoulay E, Echeverria PM, et al., 2011, Candida Bloodstream Infections in Intensive Care Units: Analysis of the Extended Prevalence of Infection in Intensive Care Unit Study. Crit Care Med, 39(4): 665–670. https://doi.org/10.1097/CCM.0b013e318206c1ca

[11] Colombo AL, Guimaraes T, Sukienik T, et al., 2014, Prognostic Factors and Historical Trends in the Epidemiology of Candidemia in Critically Ill Patients: An Analysis of Five Multicenter Studies Sequentially Conducted Over a 9-Year Period. Intensive Care Med, 40: 1489–1498. https://doi.org/10.1007/s00134-014-3400-y

[12] Rex JH, Walsh TJ, Sobel JD, et al., 2000, Practice Guidelines for the Treatment of Candidiasis. Clin Infect Dis, 30(4): 662–678. https://doi.org/10.1086/313749

[13] St-Germain G, Laverdière M, Pelletier R, et al., 2001, Prevalence and Antifungal Susceptibility of 442 *Candida* Isolates from Blood and Other Normally Sterile Sites: Results of a 2-Year (1996 to 1998) Multicenter Surveillance Study in Quebec, Canada. J Clin Microbiol, 39(3): 949–953. https://doi.org/10.1128/JCM.39.3.949-953.2001

[14] Chakrabarti A, Sood P, Rudramurthy SM, et al., 2014, Incidence, Characteristics and Outcome of ICU-Acquired Candidemia in India. Intensive Care Med, 41: 285–295. https://doi.org/10.1007/s00134-014-3603-2

[15] Cleveland AA, Harrison LH, Farley MM, et al., 2015, Declining Incidence of Candidemia and the Shifting Epidemiology of *Candida* Resistance in Two US Metropolitan Areas, 2008–2013: Results from Population-Based Surveillance. PLoS One, 10(3): e0120452. https://doi.org/10.1371/journal.pone.0120452

[16] Hesstvedt L, Gaustad P, Andersen CT, et al., 2015, Twenty-Two Years of Candidaemia Surveillance: Results from a Norwegian National Study. Clin Microbiol Infect, 21(10): 938–945. https://doi.org/10.1016/j.cmi.2015.06.008

[17] Vallabhaneni S, Mody RK, Walker T, et al., 2016, The Global Burden of Fungal Diseases. Infect Dis Clin North Am, 30(1): 1–11. https://doi.org/10.1016/j.idc.2015.10.004

[18] Rex JH, Pappas PG, Karchmer AW, et al., 2003, A Randomized and Blinded Multicenter Trial of High-Dose Fluconazole plus Placebo versus Fluconazole plus Amphotericin B as Therapy for Candidemia and Its Consequences in Nonneutropenic Subjects. Clin Infect Dis, 36(10): 1221–1228. https://doi.org/10.1086/374850

[19] Lackner M, de Hoog GS, Verweij PE, et al., 2012, Species-Specific Antifungal Susceptibility Patterns of *Scedosporium* and *Pseudallescheria* Species. Antimicrob Agents Chemother, 56(5): 2635–2642. https://doi.org/10.1128/AAC.05910-11

[20] World Health Organization, 2014, Antimicrobial Resistance: Global Report on Surveillance, viewed January 22, 2022, https://iris.who.int/bitstream/handle/10665/112642/9789241564748_eng.pdf

[21] Pfaller MA, Diekema DJ, Jones RN, et al., 2001, International Surveillance of Bloodstream Infections Due to *Candida* Species: Frequency of Occurrence and *In Vitro* Susceptibilities to Fluconazole, Ravuconazole, and Voriconazole of Isolates Collected from 1997 through 1999 in the SENTRY Antimicrobial Surveillance Program. J Clin Microbiol, 39(9): 3254–3259. https://doi.org/10.1128/JCM.39.9.3254-3259.2001

[22] Chae MJ, Shin JH, Cho D, et al., 2003, Antifungal Susceptibilities and Distribution of *Candida* Species Recovered from Blood Cultures over an 8-Year Period. Korean J Lab Med, 23: 329–335.

[23] Khan Z, Ahmad S, Al-Sweih N, et al., 2019, Changing Trends in Epidemiology and Antifungal Susceptibility Patterns of Six Bloodstream *Candida* Species Isolates Over a 12-Year Period in Kuwait. PLoS One, 14(5): e0216250. https://doi.org/10.1371/journal.pone.0216250

[24] Rodriguez L, Bustamante B, Huaroto L, et al., 2017, A Multi-Centric Study of *Candida* Bloodstream Infection in Lima-Callao, Peru: Species Distribution, Antifungal Resistance and Clinical Outcomes. PLoS One 12(4): e0175172. https://doi.org/10.1371/journal.pone.0175172

[25] World Health Organization, 2014, Antimicrobial Resistance: Global Report on Surveillance 2014 Summary, viewed January 14, 2022, https://iris.who.int/bitstream/handle/10665/112647/WHO_HSE_PED_AIP_2014.2_eng.pdf

[26] Vallabhaneni S, Cleveland AA, Farley MM, et al., 2015, Epidemiology and Risk Factors for Echinocandin Nonsusceptible *Candida glabrata* Bloodstream Infections: Data from a Large Multisite Population-Based Candidemia Surveillance Program, 2008–2014. Open Forum Infect Dis, 2(4): ofv163. https://doi.org/10.1093/ofid/ofv163

[27] Healey KR, Zhao Y, Perez WB, et al., 2016, Prevalent Mutator Genotype Identified in Fungal Pathogen *Candida glabrata* Promotes Multi-Drug Resistance. Nat Commun, 7: 11128. https://doi.org/10.1038/ncomms11128

[28] Cho E-J, Shin JH, Kim SH, et al., 2015, Emergence of Multiple Resistance Profiles Involving Azoles, Echinocandins and Amphotericin B in *Candida glabrata* Isolates from a Neutropenia Patient with Prolonged Fungaemia. J Antimicrob Chemother, 70(4): 1268–1270. https://doi.org/10.1093/jac/dku518