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In Vitro Antidrug Susceptibility Testing of *Candida* Species Isolated from Aseptic Body Fluids

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Abstract

Background: *Candida* species are opportunistic pathogenic fungi that colonize in the human body. They may cause diseases ranging from non-life-threatening mucosal *Candida* infections to life-threatening invasive candidiasis among people with the aggressive use of immunosuppressive agents, cytotoxic therapies, treatment with broad-spectrum antifungal agents, prolonged central venous catheterization, total parenteral nutrition, AIDS, diabetes, and drug abuse.
Objectives: The aim of this study was to describe the distribution and antifungal susceptibility of clinical *Candida* isolates obtained from sterile fluids of patients who suffered from candidiasis from 2008 to 2010.
Methods: Vitek2 YST, CHROMagar *Candida* medium, and multiple PCR were used to identify the *Candida* species. The susceptibility testing to seven common antifungal agents, including amphotericin B, flucytosine, fluconazole, itraconazole, ketoconazole, clotrimazole, and nystatin, was performed using the methodology recommended in the M27-A3 document of the clinical and laboratory standards institute (CLSI).
Results: A total of 149 clinical *Candida* isolates were obtained from sterile fluids at a hospital in China. Within these isolates, *Candida albicans* was the most predominant species (47.7%), followed by *C. glabrata* (26.8%) and *C. tropicalis* (13.4%). The sources of fungal isolates were urine (75.8%), blood (16.8%), drainage liquid (4%), hydrothorax and ascites (2%), cerebrospinal fluid (0.7%), and succus prostaticus (0.7%). All of the *Candida* isolates were susceptible to amphotericin B. In addition, 27.5% of the isolates were resistant to ketoconazole, 22.1% to itraconazole, and 17.4% to fluconazole. Furthermore, 16.8% (25/149) of the isolates exhibited a cross-resistance to azoles. Interestingly, we found one flucytosine-resistant *C. albicans* isolated from urine.
Conclusions: Our findings indicate that a better preventive management and limited use of azole drugs are needed for *Candida* infections and further research is indispensable to identify cross-resistance mechanisms of azoles.

Keywords: Aseptic Body Fluids, Antidrug Susceptibility Testing, Cross-Resistance, *Candida* Species

1. Background

Candida species are opportunistic pathogenic fungi that colonize in human body surfaces and the mucous membrane of the oral cavity and digestive tract. They may cause diseases ranging from non-life-threatening mucosal *Candida* infections to life-threatening invasive candidiasis, when the host possesses certain risk factors, such as the aggressive use of immunosuppressive agents, cytotoxic therapies, treatment with broad-spectrum antifungal agents, prolonged central venous catheterization, total parenteral nutrition, AIDS, diabetes, and drug abuse. Candidiasis is one of the most common invasive fungal nosocomial infections associated with a high attributable mortality (1). Over the past several decades, the frequency of *Candida* infections has risen progressively, especially among immunocompromised patients (2, 3).

The ARTEMIS DISK global antifungal surveillance project found that 90% of fungal infections were caused by *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (4). Furthermore, the proportions of them in *Candida* infections are significantly associated with geographical differences. Retrospective studies in intensive care units (ICUs) showed the prevalence rate of *C. albicans* was be-

tween 29% and 67% of *Candida* infections in China (5, 6), while it accounted for 50 - 70% in the US (7, 8).

Candida albicans has traditionally been the leading cause of candidemia worldwide. However, the non- *albicans* species have been increasing in frequency to become the major pathogens in parts of Asia (including China), South America, and southern Europe in recent years (4, 9, 10) although *C. albicans* is still dominant. The increased incidence of the acquired resistance to azoles and the episode shift from *C. albicans* to non-*albicans* species may pose serious problems in the management of infections by such *Candida* spp., and it is necessary to identify the *Candida* species quickly in the clinical settings and monitor the antifungal resistance for providing appropriate treatments for candidiasis.

2. Objectives

In this study, we retrospectively investigated 149 isolates (from over 3,000 *Candida*) of the clinical *Candida* species from sterile fluids over a 3-year period at a tertiary hospital. The results would not only help us monitor the distribution and anti-drug susceptibility of the invasive *Candida* infections but also provide sufficient evidence for *Candida* treatment.

3. Methods

3.1. Ethics Statement

This study was approved by the ethics committee of the study hospital (code number: 2014036).

3.2. Isolates

A total of 149 clinical isolates taken from aseptic body fluids were collected by the clinical laboratory of the first affiliated hospital of Nanchang university between August 2008 and July 2010. Each positive isolate was recovered from a unique clinical specimen, purified with thrice streaking on Sabouraud dextrose agar (SDA), and incubated at 35°C for 48 hours.

3.3. Identification

Two traditional clinical identification methods involving CHROMagar *Candida* medium (CHROMagar, Paris, France) and Vitek2 YST (bioMe'rieux) were performed in combination for individual isolates. Multiple PCR, a method based on nucleic acid molecule identification, was used, as well (11). PCR reaction was performed with primers Its1 (5'-TCCGTAGGTGAACCTGCGG-3'), Its4 (5'-TCCTCCGCTTATTGATATGC-3'), Ca1 (5'-GGTTTGCTTGAAAGACGGTAG-3'), Ca2 (5'-AGTTTGAAGATATACGTGGTAG-3'), Ct1 (5'-CAATCCTACCGCCAGAGGTTAT-3'), and Ct2 (5'-TGGCCACTAGCAAAATAAGCGT-3') (11-13). PCR reactions were carried out in a GeneAmp PCR system 2400 (PerkinElmer, US) and performed in 0.2 mL microcentrifuge tubes with a final reaction mixture containing 2 μL of the prepared template, 12.5 μL of 2 $\times$ Taq PCR master mix (Tiangen Biotech (Beijing) Co., Ltd., China), 0.5 μL of each primer (10 mM)(BGI, China), and 7.5 μL ddH_2O. Amplification conditions were as follows: 5 minutes initial denaturation at 96°C, repeated for 40 cycles of 30 seconds denaturation at 94°C, 30 seconds of primer annealing at 53°C, and elongation at 72°C for 1 minute. The final elongation at 72°C was extended to last 10 minutes.

For each experiment, the size of the DNA fragments amplified by Multi-PCR was determined by direct comparison with the DNA marker. Control tubes without template were included in each run, and reproducibility was verified for each reaction. The PCR products were separated using electrophoresis in agarose gels (1.5%) at 100 V for approximately 45 min at room temperature in the 1 $\times$ TAE buffer. Reaction products were detected by ethidium bromide and visualized in UV light. *Candida albicans* included two bands, 150 bp and 550 bp (faint). Bands approximately at 900 bp, 350 bp, and 550 bp represented *C. glabrata*, *C. tropicalis*, and *C. krusei*, respectively.

3.4. Antifungal Susceptibility Testing

Broth microdilution testing was performed in accordance with the guidelines of CLSI document M27-A3 (14) using RPMI 1640 medium with 0.2% glucose an inoculum of 5 $\times 10^2$ to 2.5 $\times 10^3$ cells/mL, followed by incubation at 35°C. The minimal inhibitory concentration (MIC) values were determined visually after 24 hour of incubation (CLSI document M27-A3) as the lowest concentration of drug that caused a significant diminution ($\geq$ 50% or 90% inhibition) in growth relative to the growth of the control (14). In all instances, MIC trays were prepared using reagent-grade powder, as directed by CLSI. All of the drugs (including amphotericin B flucytosine, fluconazole, itraconazole, and ketoconazole) were bought from Sigma Aldrich, Germany.

Two quality control (QC) isolates, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, were used for testing, as recommended by CLSI (14, 15). Only those consequences for which QC MICs were within the established reference range were used in the study.

3.5. Interpretive Criteria for Susceptibility to Antifungal Agents

We adopted the clinical breakpoints (CBPs) that have been newly revised to provide species-specific interpretive

criteria in this study (16). Considering the absence of the CBPs for some species, we applied the epidemiological cutoff values (ECVs) instead to detect the emergence of potential resistance to antifungal agents (reviewed by Pfaller et al. (16)). The strains with a MIC $\geq$ CBP were considered resistant (R). We also considered MIC $\leq$ ECV as the wild-type strains (WT) and MIC > ECV as the non-WT strains. Interpretive criteria for susceptibility to antifungal agents were as follow: for amphotericin B, the ECV was 2 μg/mL for each species of *Candida*. For flucytosine, the ECV was 0.5 μg/mL for *Candida* species except for 32 μg/mL for *C. krusei* and 1 μg/mL for *C. guilliermondii*. For fluconazole, MICs $\leq$ 2 μg/mL were considered S for *C. albicans*, *C. parapsilosis* and *C. tropicalis*, MICs $\geq$ 8 μg/mL were R, and MICs = 4 μg/mL were susceptible dose-dependent (SDD). For *C. glabrata*, MIC $\leq$ 32 μg/mL was considered SDD while MIC $\geq$ 64 μg/mL was considered R. For *C. krusei* and *C. guilliermondii*, the ECV was 64 μg/mL and 8 μg/mL, respectively. For itraconazole, the MIC $\leq$ 0.125 μg/mL was considered S for *C. albicans*, and MIC $\geq$ 1 μg/mL was R, 0.25 - 0.5 μg/mL was SDD, while the ECV was 2 μg/mL for *C. glabrata*, 0.5 μg/mL for *C. parapsilosis*, and *C. tropicalis*, and 1 μg/mL for *C. krusei* and *C. guilliermondii*. For ketoconazole, referring to M27-A2 and based on Sanguinetti et al. (17) and White et al. (18), MICs $\leq$ 0.125 μg/mL, 0.25 - 0.5 μg/mL, and $\geq$ 1 μg/mL were considered S, SDD, and R, respectively for each species of *Candida*.

4. Results

Distributions of *Candida* strains: baseline characteristics in the study population were analyzed (Table 1). Briefly, we obtained 149 *Candida* strains, mainly containing *C. albicans* (71, 47.7%), *C. glabrata* (40, 26.8%), *C. parapsilosis* (13, 8.7%), *C. tropicalis* (20, 13.4%), and *C. krusei* (3, 2.0%). Most *Candida* strains were isolated from urine (113, 75.8%), followed by blood (25, 16.8%). The remaining 7.4% of the strains were isolated from drainage (6, 4.0%), hydrothorax and ascites (3, 2.0%), succus prostaticus (1, 0.7%), and cerebrospinal fluid (1, 0.7%). The highest infection rate was found in ICU (52, 34.9%), followed by the departments of Urology (45, 30.2%) and Burn (15, 10.1%). Other departments, including chest surgery, gastroenterology, orthopedics, and gynaecology, separately represented a small proportion of the isolates. *C. albicans* was most predominant in ICU (53.8%), urology (46.7%), and burn (53.3%) departments. *C. glabrata* was the second one, with 19.2%, 31.1%, and 26.7% rates at the above three departments, respectively.

In Table 1, we analyzed the constituent ratio of the *Candida* species in each year from 2008 to 2010. The percentage of *C. albicans* or *C. glabrata* changed slightly, while the increasing trend of *C. tropicalis* was distinct as the second major species in non-albicans *Candida*.

Antifungal susceptibility testing: The frequency of resistance to some antifungal agents for the five most common *Candida* species is shown in Table 2. We counted the percentages of strains that were susceptible or resistant to each agent, except the two agents that have no interpretive criteria. Generally, all isolates were susceptible to amphotericin B and flucytosine, except one flucytosine-resistant *C. albicans* isolate. This is while azoles resistance was evidently uncommon with 27.5% to ketoconazole (32.4% of *C. albicans*, 15.0% of *C. glabrata*, 30.0% of *C. tropicalis* and 38.5% of *C. parapsilosis*), 22.1% to itraconazole (36.6% of *C. albicans*, 2.5% of *C. glabrata*, 20.0% of *C. tropicalis*, and 30.8% of *C. parapsilosis*) and 17.4% to fluconazole (28.2% of *C. albicans*, 15.0% of *C. tropicalis*, and 23.1% of *C. parapsilosis*). Notably, 16.1% (24/149) of the isolates exhibited a cross-resistance to fluconazole, itraconazole, and ketoconazole. For clotrimazole and nystatin, we analyzed the MIC ranges as listed in Table 2.

5. Discussion

In this study, we described the distribution of species and determined *in vitro* antifungal susceptibility of *Candida* strains isolated from aseptic body fluids in our hospital. Consequently, we found the number of *Candida* infections was increasing from 2008 to 2010. It indicated that we should pay more attention to monitoring the candidiasis frequently because it is an intractable disease, especially candidemia. Compelling evidence has demonstrated that more than 90% of *Candida* infections were caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*, and these four *Candida* held 96.6% of all isolates in our study. Traditionally, *C. albicans* was considered the predominant pathogen in candidiasis worldwide. In the current study, the most frequent isolates were still *C. albicans*. The proportion concurs with recent reports in ICUs in China (6, 19), but is considerably lower than that in the ARTEMIS DISK global antifungal surveillance study between 2001 and 2007 (64%~ 67%) (4). The reasons for this discrepancy may include different specimen types and locations.

In addition, it is noteworthy that more than 50% of the *Candida* isolates in the current study were non-albicans. In the current study, *C. glabrata* (26.8%) was the most prevalent non-albicans *Candida*, which is in agreement with the results of the studies conducted by Hazen et al. (20) and Schmalreck et al. (21). In contrast, *C. parapsilosis* was the foremost non-albicans *Candida* species in Latin America (25%), Canada (16%), and Europe (17%) (22) and *C. tropicalis* was the most prevalent in Taiwan (23). It indicated that the distribution of species is significantly associated with the

Table 1. Distributions of the Isolates in the Current Study According to *Candida* Species in Nanchang, China (2008 - 2010)[a]

Characteristic	*Candida* species							
	All Isolates (n = 149)	***C. albicans* (n = 71)**	***C. glabrata* (n = 40)**	***C. tropicalis* (n = 20)**	***C. parapsilosis* (n = 13)**	***C. krusei* (n = 3)**	***C. guilliermondii* (n = 1)**	***C. intermedia* (n = 1)**
Departments								
Intensive care unit	52 (34.9)	28 (53.8)	10 (19.2)	6 (11.5)	6 (11.5)	2 (3.8)	Δ	
Urology	45 (30.2)	21 (46.7)	14 (31.1)	6 (13.3)	4 (8.9)	Δ	Δ	Δ
Burn	15 (10.1)	8 (53.3)	4 (26.7)	2 (13.3)	Δ	1 (2.2)	Δ	Δ
Cerebral surgery	9 (6.0)	3 (33.3)	3 (33.3)	2 (22.2)	Δ	Δ	Δ	1 (11.1)
Emergency	11 (7.4)	5 (45.5)	3 (27.3)	Δ	3 (27.3)	Δ	Δ	Δ
Others	17 (11.4)	6 (35.3)	6 (35.3)	4 (23.5)	Δ	Δ	1 (5.9)	Δ
Sources								
Urine	113 (75.8)	55 (48.7)	29 (25.7)	16 (14.2)	10 (8.8)	1 (0.9)	1 (0.9)	1 (0.9)
Blood	25 (16.8)	7 (28.0)	10 (40.0)	3 (12.0)	3 (12.0)	2 (8.0)	Δ	Δ
Drainage liquid	6 (4.0)	4 (66.7)	1 (16.7)	1 (16.7)	Δ	Δ	Δ	Δ
Hydrothorax and ascite	3 (2.0)	3 (100.0)	Δ	Δ	Δ	Δ	Δ	Δ
Cerebrospinal fluid	1 (0.7)	1 (100.0)	Δ	Δ	Δ	Δ	Δ	Δ
Succus prostaticus	1 (0.7)	1 (100.0)	Δ	Δ	Δ	Δ	Δ	Δ
Years								
2008	34	17 (50.0)	10 (29.4)	3 (8.8)	3 (8.8)	1 (2.9)	Δ	Δ
2009	47	19 (40.4)	13 (27.7)	8 (17.0)	4 (8.5)	2 (4.3)	Δ	1 (2.1)
2010	68	35 (51.5)	17 (25.0)	9 (13.2)	6 (8.8)	Δ	1 (1.5)	Δ

[a] Δ, data unavailable.

geographical area. Furthermore, our data showed that the prevalence of non-albicans *Candida*, especially *C. glabrata* and *C. tropicalis*, has been increasing recently. Owing to the non-albicans treatment that is slightly different from *C. albicans* treatment, the results remind us that we should be more concerned about the non-albicans infections.

Among the 149 clinical isolates, *Candida* was frequently isolated from urine specimens (75.8%), followed by blood (16.8%). Similar results were observed in Taiwan (urine, 45.2%; blood, 19.7%) as a survey reported in 2010 (23). In urine, *C. albicans* had the largest proportion (41.4%), followed by *C. glabrata* (21.8%) and *C. tropicalis* (12.0%). On the one hand, it may be developed from the *Candida* species that colonize on the mucous membrane of the urinary tract; on the other hand, it was more likely to be due to surgery and intubation. Notably, *C. glabrata* surpassed *C. albicans* to be the most common species isolated from the bloodstream, accounting for 40.0% of the isolates. In contrast, *C. albicans* was the most common cause of candidemia (35.9%) and *C. glabrata* was reported third (13.0%) in a retrospective analysis of 270 cases of candidemia occurring from 2000 to 2009 at a teaching hospital in China (24). Furthermore, clinical works should pay more attention to *C. albicans* and *C. parapsilosis* in urine because approximately 70.0% of them (39/55 of *C. albicans* and 7/10 of *C. parapsilosis*) were resistant (data not shown). In blood, however, *C. albicans* should be focused on because of its high resistance rate (57.1%, 4/7) (data not shown).

Candida infection rates in the departments of ICU and Urology were most predominant (34.9% and 30.2%, respectively), which is consistent with the distributions reported in the US (25). This is possible because the patients in ICUs may be immunocompromised or critically ill, and they were more likely to be infected with *Candida*. In addition,

Table 2. Susceptibilities of 149 Candida Isolates to Antifungal Agents.

Antifungal Agent	Isolates, No. (%)	MIC (mg/L)[a]			CBP[b]			ECV[c]	
		Range	50%	90%	S, No. (%)	SDD, No. (%)	R, No. (%)	WT, No. (%)	NWT, No. (%)
C. albicans	71 (47.7)								
Amphotericin B		0.25 - 1	1	1				71 (100.0)	0 (0.0)
Flucytosine		0.125 - 1	0.125	0.5				70 (98.6)	1 (1.4)
Fluconazole		0.125 - 64	2	16	39 (54.9)	12 (16.9)	20 (28.2)	16 (22.5)	55 (77.5)
Itraconazole		0.03125 - 16	0.5	16	18 (25.4)	27 (38.0)	26 (36.6)	18 (25.4)	53 (75.6)
C. glabrata	40 (26.8)								
Amphotericin B		0.25 - 1	0.5	1				40 (100.0)	0 (0.0)
Flucytosine		0.125 - 0.5	0.125	0.5				40 (100.0)	0 (0.0)
Fluconazole		0.125 - 16	1	8	NA[d]	40 (100.0)	0 (0.0)	40 (100.0)	0 (0.0)
Itraconazole		0.03125 - 4	0.5	1				39 (97.5)	1 (2.5)
C. tropicalis	20 (13.4)								
Amphotericin B		0.25 - 1	0.5	1				20 (100.0)	0 (0.0)
Flucytosine		0.125 - 0.5	0.25	0.5				20 (100.0)	0 (0.0)
Fluconazole		0.125-32	2	8	12 (60.0)	5 (25.0)	3 (15.0)	12 (60.0)	8 (40.0)
Itraconazole		0.03125 - 16	0.25	1				16 (80.0)	4 (20.0)
C. parapsilosis	13 (8.7)								
Amphotericin B		0.25 - 1	0.5	1				13 (100.0)	0 (0.0)
Flucytosine		0.125 - 0.5	0.125	0.25				13 (100.0)	0 (0.0)
Fluconazole		0.25 - 32	2	8	8 (61.5)	2 (15.4)	3 (23.1)	8 (61.5)	5 (38.5)
Itraconazole		0.03125 - 16	0.25	4				9 (69.2)	4 (30.8)
C. krusei	3 (2.0)								
Amphotericin B		0.5 - 1	1	1				3 (100.0)	0 (0.0)
Flucytosine		0.125 - 0.25	0.125	0.25				3 (100.0)	0 (0.0)
Fluconazole		1 - > 64	2	> 64				2 (66.7)	1 (33.3)
Itraconazole		1 - 2	1	2				2 (66.7)	1 (33.3)
C. guilliermondii	1 (0.7)								
Amphotericin B		0.5	NA	NA				1 (100.0)	0 (0.0)
Flucytosine		0.125	NA	NA				1 (100.0)	0 (0.0)
Fluconazole		4	NA	NA				1 (100.0)	0 (0.0)
Itraconazole		0.0625	NA	NA				1 (100.0)	0 (0.0)
C. intermedia	1 (0.7)								
Amphotericin B		0.5	NA	NA	NA		NA		
Flucytosine		0.125	NA	NA	NA		NA		
Fluconazole		2	NA	NA	NA		NA		
Itraconazole		0.5	NA	NA	NA		NA		

[a] 50% and 90%, MIC value encompassing 50% and 90% of isolates tested, respectively.
[b] Categories of susceptible (S), susceptible-dose dependent (SDD), and resistant (R) according to CLSI (2012) and Pfaller and Diekema (2012).
[c] Categories of wild-type (WT) and non-wild type (NWT) according to Pfaller and Diekema (2012).
[d] NA, data not available or not applicable.

the high resistance rates of *C. albicans* in ICU and Urology departments reveal that more attention should be paid to ICU or urology patients to prevent them from contracting *Candida* (data not shown).

Of all the 149 isolates, 27.5% were resistant to ketoconazole. In contrast, only 7.7% of the *Candida* strains isolated from HIV/AIDS patients were identified to be resistant to ketoconazole in a study conducted by Mulu et al. (26). This contradiction may be because of the divergence of the methods and interpretive criteria to ketoconazole or due to the sources of strains. We found that a large percentage of *C. albicans* (32.4%), *C. glabrata* (15.0%), *C. parapsilosis* (38.5%), and *C. tropical* (30.0%) were ketoconazole-resistant. It was significantly higher than the results obtained by Badiee and Alborzi (9.4% of *C. albicans*, 15% of *C. glabrata*, and 3.5% of *C. parapsilosis*) (27). These may prompt the reconsideration of ketoconazole therapy.

With regard to itraconazole, 22.1% of the isolates were

resistant and the rate was slightly above a recent report in a tertiary care hospital in south India (14%) (28). 36.6% of *C. albicans* exhibited resistance to itraconazole, followed by *C. parapsilosis* (30.8%) and *C. tropicalis* (20.0%), unlike the results of the China-SCAN (16.0% of *C. albicans*, 39.8% of *C. parapsilosis*, and 31.3% of *C. tropicalis*) (19). For fluconazole, 18.1% of the isolates were resistant, and this rate was similar to the result of a population-based surveillance in India (14.8%) (28). However, the proportion of ketoconazole- or itraconazole-resistant isolates was significantly higher compared to fluconazole-resistant isolates. It may be because of prescribing habits in clinics; fluconazole was not commonly used to treat the infections of the bloodstream and other sterile sites.

Some surveillance had shown that resistance to fluconazole was highly predictive of resistance to voriconazole (25, 29). In this regard, we found that 16 out of 20 fluconazole-resistant *C. albicans* were cross-resistant to itraconazole. Furthermore, six of them were also resistant to ketoconazole. This phenomenon highlights the importance of cross-resistance among azole agents. One of the three *C. krusei* was resistant to itraconazole, ketoconazole, and fluconazole. However, *C. krusei* was considered inherently resistant to triazoles (16). The high prevalence of azoles resistance among *Candida* spp. may be correlated with the increased use of these drugs in the area. It appears to have a major impact in selecting azole-resistant *Candida* spp. when strains are continuously exposed to azoles (30).

Amphotericin B was reported to be the first systemic antifungal agent for the treatment of invasive fungal infection in many studies; however, it has limited use due to nephrotoxicity in up to 80% of the patients. In this study, its ECV value was 2 μg/mL for *Candida* spp. and the MICs of all isolates were below 2 μg/mL. Flucytosine was used to treat systemic severe *Candida* infections. With regard to flucytosine, all 149 isolates were susceptible except one *C. albicans* isolated from urine, contrary to the result (1.7%) of a study conducted by Pfaller et al. (31) whose strains came from nine cities across China. The findings of antifungal susceptibility of *Candida* spp. in the current study and previous reports revealed that the prevalence of antifungal resistance in *Candida* isolates differs from area to area and time to time.

5.1. Conclusions

In conclusion, we described the distributions and *in vitro* antifungal susceptibilities of invasive *Candida* strains isolated from sterile fluids. The trends of species distribution and increasing resistance of *Candida* spp. (especially the occurrence of cross-resistant isolates) confirm the importance of continuous epidemiological surveillance and the rational use of fungal agents. The mechanisms of the cross-resistance of antifungal agents in our study are not clear, while this problem may be interpreted in further studies.

Footnotes

Authors' Contribution: Guoshi Xiang and Lingbing Zeng contributed equally to this work.

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References

1. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. *Clin Infect Dis*. 2003;**37**(9):1172–7. doi: 10.1086/378745. [PubMed: 14557960].
2. Arendrup MC. Epidemiology of invasive candidiasis. *Curr Opin Crit Care*. 2010;**16**(5):445–52. doi: 10.1097/MCC.0b013e32833e84d2. [PubMed: 20711075].
3. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. *Crit Rev Microbiol*. 2010;**36**(1):1–53. doi: 10.3109/10408410903241444. [PubMed: 20088682].
4. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol*. 2010;**48**(4):1366–77. doi: 10.1128/JCM.02117-09. [PubMed: 20164282]. [PubMed Central: PMC2849609].
5. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009;**302**(21):2323–9. doi: 10.1001/jama.2009.1754. [PubMed: 19952319].
6. Li S, An YZ. [Retrospective analysis of invasive fungal infection in surgical intensive care unit]. *Zhonghua Yi Xue Za Zhi*. 2010;**90**(6):382–5. [PubMed: 20367934].
7. Kett DH, Azoulay E, Echeverria PM, Vincent JL; Extended Prevalence of Infection in ICU Study (EPIC II) Group of Investigators. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. *Crit Care Med*. 2011;**39**(4):665–70. doi: 10.1097/CCM.0b013e318206c1ca. [PubMed: 21169817].
8. Kriengkauykiat J, Ito JI, Dadwal SS. Epidemiology and treatment approaches in management of invasive fungal infections. *Clin Epidemiol*. 2011;**3**:175–91. doi: 10.2147/CLEP.S12502. [PubMed: 21750627]. [PubMed Central: PMC3130903].
9. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans Candida spp among candidemia isolates from inpatients in various parts of the world: a systematic review. *Int J Infect Dis*. 2010;**14**(11):e954–66. doi: 10.1016/j.ijid.2010.04.006. [PubMed: 20797887].
10. Echeverria PM, Kett DH, Azoulay E. Candida prophylaxis and therapy in the ICU. *Semin Respir Crit Care Med*. 2011;**32**(2):159–73. doi: 10.1055/s-0031-1275528. [PubMed: 21506052].

11. Luo G, Mitchell TG. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. *J Clin Microbiol*. 2002;**40**(8):2860–5. [PubMed: 12149343]. [PubMed Central: PMC120665].
12. Chang HC, Leaw SN, Huang AH, Wu TL, Chang TC. Rapid identification of yeasts in positive blood cultures by a multiplex PCR method. *J Clin Microbiol*. 2001;**39**(10):3466–71. doi: 10.1128/JCM.39.10.3466-3471.2001. [PubMed: 11574557]. [PubMed Central: PMC88373].
13. Chen YC, Eisner JD, Kattar MM, Rassoulian-Barrett SL, Lafe K, Bui U, et al. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J Clin Microbiol*. 2001;**39**(11):4042–51. doi: 10.1128/JCM.39.11.4042-4051.2001. [PubMed: 11682528]. [PubMed Central: PMC88485].
14. Rex JH. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard*. 2 ed. Clinical and Laboratory Standards Institute; 2008.
15. Alexander BD. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*. 4 ed. Clinical and Laboratory Standards Institute; 2017.
16. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol*. 2012;**50**(9):2846–56. doi: 10.1128/JCM.00937-12. [PubMed: 22740712]. [PubMed Central: PMC3421803].
17. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of Candida glabrata collected during a hospital survey of antifungal resistance. *Antimicrob Agents Chemother*. 2005;**49**(2):668–79. doi: 10.1128/AAC.49.2.668-679.2005. [PubMed: 15673750]. [PubMed Central: PMC547307].
18. White TC, Holleman S, Dy F, Mirels LF, Stevens DA. Resistance mechanisms in clinical isolates of Candida albicans. *Antimicrob Agents Chemother*. 2002;**46**(6):1704–13. [PubMed: 12019079]. [PubMed Central: PMC127245].
19. Liu W, Tan J, Sun J, Xu Z, Li M, Yang Q, et al. Invasive candidiasis in intensive care units in China: in vitro antifungal susceptibility in the China-SCAN study. *J Antimicrob Chemother*. 2014;**69**(1):162–7. doi: 10.1093/jac/dkt330. [PubMed: 24004860].
20. Hazen KC, Baron EJ, Colombo AL, Girmenia C, Sanchez-Sousa A, del Palacio A, et al. Comparison of the susceptibilities of Candida spp. to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. *J Clin Microbiol*. 2003;**41**(12):5623–32. [PubMed: 14662952]. [PubMed Central: PMC309015].
21. Schmalreck AF, Willinger B, Haase G, Blum G, Lass-Florl C, Fegeler W, et al. Species and susceptibility distribution of 1062 clinical yeast isolates to azoles, echinocandins, flucytosine and amphotericin B from a multi-centre study. *Mycoses*. 2012;**55**(3):e124–37. doi: 10.1111/j.1439-0507.2011.02165.x. [PubMed: 22233267].
22. Cuenca-Estrella M, Rodero L, Garcia-Effron G, Rodriguez-Tudela JL. Antifungal susceptibilities of Candida spp. isolated from blood in Spain and Argentina, 1996-1999. *J Antimicrob Chemother*. 2002;**49**(6):981–7. [PubMed: 12039890].
23. Yang YL, Chen HT, Lin CC, Chu WL, Lo HJ; Tsary Hospitals. Species distribution and drug susceptibilities of Candida isolates in TSARY 2010. *Diagn Microbiol Infect Dis*. 2013;**76**(2):182–6. doi: 10.1016/j.diagmicrobio.2013.03.003. [PubMed: 23574935].
24. Zhang XB, Yu SJ, Yu JX, Gong YL, Feng W, Sun FJ. Retrospective analysis of epidemiology and prognostic factors for candidemia at a hospital in China, 2000-2009. *Jpn J Infect Dis*. 2012;**65**(6):510–5. [PubMed: 23183203].
25. Li F, Wu L, Cao B, Zhang Y, Li X, Liu Y. Surveillance of the prevalence, antibiotic susceptibility, and genotypic characterization of invasive candidiasis in a teaching hospital in China between 2006 to 2011. *BMC Infect Dis*. 2013;**13**:353. doi: 10.1186/1471-2334-13-353. [PubMed: 23899244]. [PubMed Central: PMC3733982].
26. Mulu A, Kassu A, Anagaw B, Moges B, Gelaw A, Alemayehu M, et al. Frequent detection of 'azole' resistant Candida species among late presenting AIDS patients in northwest Ethiopia. *BMC Infect Dis*. 2013;**13**:82. doi: 10.1186/1471-2334-13-82. [PubMed: 23398783]. [PubMed Central: PMC3577436].
27. Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J Microbiol*. 2011;**3**(4):183–8. [PubMed: 22530086]. [PubMed Central: PMC3330181].
28. Jayachandran AL, Katragadda R, Thyagarajan R, Vajravelu L, Manikesi S, Kaliappan S, et al. Oral Candidiasis among Cancer Patients Attending a Tertiary Care Hospital in Chennai, South India: An Evaluation of Clinicomycological Association and Antifungal Susceptibility Pattern. *Can J Infect Dis Med Microbiol*. 2016;**2016**:8758461. doi: 10.1155/2016/8758461. [PubMed: 27403171]. [PubMed Central: PMC4923570].
29. Lyon GM, Karatela S, Sunay S, Adiri Y, Candida Surveillance Study I. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. *J Clin Microbiol*. 2010;**48**(4):1270–5. doi: 10.1128/JCM.02363-09. [PubMed: 20129963]. [PubMed Central: PMC2849617].
30. Kontoyiannis DP. Why prior fluconazole use is associated with an increased risk of invasive mold infections in immunosuppressed hosts: an alternative hypothesis. *Clin Infect Dis*. 2002;**34**(9):1281–3. author reply 1283. doi: 10.1086/339946. [PubMed: 11941561].
31. Pfaller MA, Castanheira M, Messer SA, Jones RN. In vitro antifungal susceptibilities of isolates of Candida spp. and Aspergillus spp. from China to nine systemically active antifungal agents: data from the SENTRY antifungal surveillance program, 2010 through 2012. *Mycoses*. 2015;**58**(4):209–14. doi: 10.1111/myc.12299. [PubMed: 25727853].