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Distribution of Yeast Isolates from Invasive Infections and Their In Vitro Susceptibility to Antifungal Agents: Evidence from 299 Cases in a 3-Year (2010 to 2012) Surveillance Study

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Abstract Invasive yeast infections cause significant morbidity and mortality. Surveillance for the infection is necessary to detect trends in species distribution and antifungal resistance. We performed this retrospective study of yeast infection at Jinling Hospital, Nanjing in China, from year of 2010 to 2012. A total of 341 yeast isolates were obtained from patients with invasive infections in the period. Among these isolates, *Candida* spp. comprised of the highest percentage of yeast strains (91.8 %), followed by *Cryptococcus neoformans* (5.9 %) and other non-*Candida* yeast strains (2.3 %). Bloodstream isolates made up 41.3 % of yeast strains and the isolates from CVC made up 17.3 %. Among *Candida* spp., *C. albicans* was the most common species identified from non-blood clinical specimens (42.9 %), but appeared in only 20.8 % of blood isolates ($P < 0.001$). *C. tropicalis* was the most prevalent *Candida* species in the blood samples (28.5 %). *Candida* spp. was mainly isolated from specimens of the ICU patients, while *C. neoformans* was mainly isolated from specimens in medical wards. Resistance to FLC occurred in 3.7 % of *C. albicans*, 9.9 % of *C. tropicalis*, 74.0 % of *C. glabrata,* and 4.4 % of *C. parapsilosis*. Most (>92 %) isolates of *C. albicans*, *C. tropicalis*, *C. parapsilosis,* and *C. neoformans* strains were susceptible to VRC; However, 26.7 % of isolates of *C. glabrata* were VRC resistant.

Keywords Yeast infection · Distribution of species · Antifungal susceptibility

Introduction

The incidence of invasive yeast infections has increased during the past two decades [1], especially in the critically ill or immunosuppressive patients in the intensive care unit (ICU) [2]. Invasive candidiasis (IC), including candidemia, remains the commonest yeast infection [3], followed by *Cryptococcus neoformans*, and previously uncommon pathogens such as *Kodamaea ohmeri* [4] and *Rhodotorula* [5], have emerged. With the increasing usage of intensive care in the hospitals, it has become clearly evident that a population of critically ill patients is more susceptible to candidal infections. The increased susceptibility of these patients is largely because of the severe underlying illness, utilization of invasive medical devices, impaired immunological status, and long-term and

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broad-spectrum antibiotics abuse [6]. These infectious fungi can invade the bloodstream (fungemia, mainly Candidemia) and may be disseminated to internal organs [7]. Among *Candida* species, *C. albicans* is the predominant causative species in many countries in the world [8]. However, infections caused by non-*albicans Candida* species have been increasing in prevalence [9], such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* [7]. A broad utilization of azoles antifungal agents in preventative therapy or empiric treatment may have contributed to this progressive shift of the epidemiology of candidemia because of a possibility of development of drug resistance. Distribution of these species is also highly influenced by ages of the patients and anatomic sites of the infection [5].

Azole antifungals have been the mainstream of antifungal therapy so far. FLC has been widely used to treat infections with the fungal pathogen *C. albicans* because of its favorable pharmacokinetic profile for more than 20 years [10]. However, long-term exposure to FLC, as well as low doses of FLC during the empirical treatment, leads to an increased drug resistance. In order to overcome the shortcomings of first-generation triazoles, including FLC and itroconazole (ITC), the second-generation triazoles (VRC) have been developed and are available. VRC is more potent and possesses higher activity against resistant and emerging pathogens. Nevertheless, many species are cross-resistant to all azoles [11]. For example, *C. glabrata* showed the greater cross-resistance to azoles, followed by *C. tropicalis* and *C. parapsilosis* [12]. Therefore, susceptibility data of antifungal drugs are often required for definitive decisions of antifungal treatments.

In order to assess the epidemical trend in the distribution of infectious yeast species and susceptibility patterns of antifungal agents, especially in the cases of the *Candida* species, we conducted this surveillance investigation in a tertiary care hospital in China from 2010 to 2012.

Materials and Methods

Study Design and Subjects

This study is a retrospectively observational study. The data were obtained from electronic records of the laboratory from January 2010 to December 2012 in Jinling Hospital (Nanjing, China), a tertiary care hospital which had about 55,000–60,000 admissions per year. All patients were treated according to a routine standard of care. The study enrolled a total of 299 patients which had positive results in culture of yeast species from 341 clinical specimens, including blood, central venous catheter (CVC) tip, and other sterile body fluids (Table 1).

Organism Identification

Species were identified using chromogenic culture media (CHROMagar, Paris, France) and the API 20C AUX yeast identification kit (BioMérieux, France). When necessary, sequencing of the rDNA was performed. Quality control isolates included *C. albicans* ATCC90028, *C. parapsilosis* ATCC22019, and *C. krusei* ATCC6258.

Susceptibility Test

Susceptibility to FLC and VRC was performed using the Clinical Laboratory Standards Institute (CLSI) M44-A disk diffusion method [13]. Agar plates containing Mueller–Hinton agar (obtained locally at all sites) supplemented with 2 % glucose and 0.5 μg of methylene blue per ml at a depth of 4.0 mm were used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. FLC (25 μg) and VRC (1 μg) disks (Becton–Dickinson, Sparks, MD) were placed onto the surfaces of the inoculated plates, and the plates were incubated in air at 35–37 °C and read at 18–24 h. Slowly growing isolates, primarily members of the genus Cryptococcus, were read after 48 h of incubation. Zone diameter endpoints were read at 80 % growth inhibition by using a Biomic image analysis plate reader system (Giles Scientific) [14, 15].

The interpretive criteria for the FLC and VRC disk diffusion tests were those of the CLSI [16–18]: susceptible (S), zone diameters of ≥19 mm (FLC), and ≥17 mm (VRC); susceptible dose dependent (SDD), zone diameters of 15–18 mm (FLC), and 14–16 mm (VRC); and resistant (R), zone diameters of ≤14 mm (FLC), and ≤13 mm (VRC). The corresponding MIC breakpoints [16, 17] are as follows: S, MICs of ≤8 μg/ml (FLC) and ≤1 μg/ml (VRC); SDD, MICs of 16–32 μg/ml (FLC) and 2 μg/ml (VRC); and R, MICs of ≥64 μg/ml (FLC) and ≥4 μg/ml (VRC).

Table 1 Prevalence of 341 yeast isolates in various clinical specimens

Clinical specimens	No. of isolates (%) with different species from various clinical specimens													Total
	CAL	CTR	CGL	CPA	CKR	CGU	CLU	CPE	CHA	CFA	CNE	RHO	Others[a]	
Blood	27 (19.1)	37 (26.2)	10 (7.1)	29 (20.6)	2 (1.4)	6 (4.3)		5 (3.5)	5 (3.5)	8 (5.7)	5 (3.5)	5 (3.5)	2 (1.4)	141
CVC	11 (18.6)	17 (28.8)	5 (8.5)	12 (20.3)		2 (3.4)		4 (6.8)	1 (1.7)	5 (8.5)	1 (1.7)		1 (1.7)	59
Pus	25 (59.5)	7 (16.7)	2 (4.8)	2 (4.8)						4 (9.5)	2 (4.8)			42
Ascites	25 (73.5)	4 (11.8)	1 (2.9)	1 (2.9)		1 (2.9)				2 (5.9)				34
Drainage fluid	6 (33.3)	7 (38.9)	2 (11.1)							3 (16.7)				18
Bile	7 (38.9)	5 (27.8)	3 (16.7)	1 (5.6)		1 (5.6)				1 (5.6)				18
CF	1 (8.3)										11 (91.7)			12
Joint fluid	2 (100)													2
Interstitial fluid	1 (25)						1 (25)			1 (25)	1 (25)			4
Pleural effusion	1 (14.3)	2 (28.6)			1 (14.3)			1 (14.3)		1 (14.3)			1 (14.3)	7
PDF		2 (50)								2 (50)				4
Total	106	81	23	45	3	10	1	10	6	27	20	5	4	341

CAL, *C. albicans*; CTR, *C. tropicalis*; CGL, *C. glabrata*; CPA, *C. parapsilosis*; CKR, *C. krucei*; CGU, *C. guilliermondii*; CLU, *C. lusitaniae*; CPE, *C. pelliculosa*; CHA, *C. haemulonii*; CFA, *C. famata*; CNE, *Cryptococuss neoforman*; RHO, *Rhodotorula*; CVC, central venous catheter tip; CF, cerebrospinal fluid; and PDF, peritoneal dialysis fluid

[a] Includes one strain of *K. ohmeri* and one *C. pulcherrima* from blood, one isolate of *H. polymorpha* from CVC, and one isolate of *C. utilis* from pleural effusion

Statistical Analyses

Statistical analysis was performed using SPSS 13.0 for Windows (SPSS13.0, USA). When necessary, Chi-squared test was used to test for significance. Tests were two-tailed, and a level of significance with $P < 0.05$ was considered as statistical significance.

Results

A total of 341 strains of yeasts were isolated from various clinical specimens, including 141 specimens of blood (41.3 %), 59 ones of CVCs (17.3 %), 42 ones of pus (12.3 %), 34 ones of ascitic fluid (10.0 %), and the others (Table 1). Most of the isolates ($n = 337$) were identified by API 20C AUX yeast identification kit, and the rest ($n = 4$) were confirmed by analysis of rDNA sequencing. *Candida* species were the most common species in numbers out of the isolates (314/341, 92.1 %), followed by *C. neoformans* ($n = 20$, 5.9 %) and the others ($n = 7$, 2.1 %).

The distribution of fungi in different sample varied. Isolation of *Candida* spp. was broadly distributed in these specimens, comprising the majority of blood culture isolates (130/141, 92.2 %, Table 1). *Cryptococci*, *Rhodotorula,* and the other yeasts accounted for 7.8 % of strains from blood culture. The majority of yeasts were *Candida* species from CVC (57/59, 96.6 %), pus (40/42, 95.2 %), and interstitial fluid (3/4, 75 %),while *Candida* spp. was the only isolate from drainage fluid, ascites, bile, pleural effusion, joint fluid, and peritoneal dialysis solution. *C. tropicalis* held the highest proportions in the blood, CVC, and drainage fluid. However, *C. albicans* was predominated in the pus, ascites, and bile. Conversely, 91.7 % of species from cerebrospinal fluid (CSF) were *C. neoformans*.

Among *Candida* spp., *C. albicans* was significantly more likely to be recovered from non-blood sites (79/184, 42.9 % of all *Candida* isolates) compared with blood cultures (27/130, 20.8 %; $P < 0.001$). In contrast, *C. parapsilosis* ($n = 29$ isolates)—the second commonest cause of candidemia, was disproportionately represented in blood cultures compared with non-blood specimens (29/130 isolates, 22.3 % vs. 16/184 isolates, 8.7 %; $P = 0.001$). *C. tropicalis*—the most common cause of candidemia ($n = 37$ isolates), was also higher in blood (37/130, 28.5 %) than in non-blood samples (44/184, 23.9 %), although not significantly. Five of twenty *C. neoformans* isolates came from blood and the remaining 15 from CSF ($n = 11$), pus ($n = 2$), CVC, and interstitial fluid ($n = 1$, each). Other rare yeasts in blood were *Rhodotorula* ($n = 5$), *C. krusei* ($n = 2$), *C. pulcherrima* ($n = 1$), and *K. ohmeri* ($n = 1$).

Of all isolates from clinical specimens, 95.9 % (327/341) ones were isolated from inpatients (ICU 47.2 %, medical wards 23.8 %, surgical wards 22.6 %), and 4.1 % ones in the outpatient/emergency department (Table 2). The dominant location of infectious patients varied with pathogen group. Among inpatients, *Candida* spp. was more prevalent in those in ICU (149/300, 49.7 %); however, the frequency of *C. neoformans* was the highest in patients in medical wards (10/20 cases, 50 %) in comparison with *Candida* spp. (70/300, 23.3 %; $P = 0.008$).

The proportion of various yeast species changed appreciably between 2010 and 2012. In the three years, there was an upward trend for *C. albicans*; in the first year, it appeared for 27.8 %; in the second year, it was up to 33.1 %; and in the third year, it was 32.1 %.

Table 2 Distribution of yeast species in different clinical services

Species	No. of isolates (% of total)	Clinical services							
		Inpatient					Outpatient/emergency		
		Total	ICU	Medicine	Surgery	Other[a]	Total	Emergency	Outpatient
Candida spp.	314 (92.1)	300	149	70	73	8	14	13	1
Cryptococcus spp.	20 (5.9)	20	8	10	2				
Other yeast spp.	7 (2.1)	7	4	1	2				
Total	341 (100)	327	161	81	77	8	14	14	1

[a] Includes dermatology, obstetrics and gynecology, ophthalmology, stomatology, radiotherapy, and male infertility department

Table 3 Distribution of yeast species isolated from clinical samples over time

Species	No. (%) of isolates			Total
	2010	2011	2012	
C. abicans	30 (27.8)	42 (33.1)	34 (32.1)	106
C. tropicalis	22 (20.4)	38 (29.9)	21 (19.8)	81
C. parapsilosis	15 (13.9)	14 (11.0)	16 (15.1)	45
C. glabrata	5 (4.6)	10 (7.9)	8 (7.6)	23
C. guilliermondii	3 (2.8)	3 (2.4)	4 (3.8)	10
C. pelliculosa	2 (1.9)	3 (2.4)	5 (4.7)	10
C. haemulonii	0	4 (3.2)	2 (1.9)	6
C. krusei	3 (2.8)	0	0	3
C. lusitanaie	1 (0.9)	0	0	1
C. famata	13 (12.0)	7 (5.5)	7 (6.6)	27
Cryptococcus	11 (10.2)	3 (2.4)	6 (5.7)	20
Rhodotorula	2 (1.9)	1 (0.8)	2 (1.9)	5
Others[a]	1 (0.9)	2 (1.6)	1 (0.9)	4
Total	108	127	106	341

[a] *Kodamaea ohmeri* ($n = 1$), *H. polymorpha* ($n = 1$), *C. pulcherrima* ($n = 1$), and *C. utilis* ($n = 1$)

Table 4 Distribution of *Candida* spp. in bloodstream isolates in various age group

Species	% of isolates in indicated age group [year of age (no. of isolates found)]				
	0–14 (1)	15–24 (16)	25–44 (28)	45–64 (50)	≥65 (35)
C. abicans		12.5	32.1	12.0	28.6
C. tropicalis	100	37.5	21.4	30.0	25.7
C. glabrata		12.5	7.1	6.0	8.6
C. parapsilosis		31.3	17.9	26.0	17.1
Others[a]		6.3	21.4	26.0	20

[a] *C. famata* ($n = 8$), *C. guilliermondii* ($n = 6$), *C. pelliculosa* ($n = 5$), *C. haemulonii* ($n = 5$), *C. krusei* ($n = 2$), and *C. pulcherrima* ($n = 1$)

The trend of changes for *C. glabrata* was similar to that of *C. albicans,* while the trend of the frequency of occurrence for *C. tropicalis* was different with 20.4 % in 2010, a sharp rise to 29.9 % in 2011 and a down size to 19.8 % in 2012, although such a change was not statistically significant (Table 3).

The distribution of *Candida* spp. isolated from blood varied with ages of patients (Table 4). The rank order for the groups of the patients with 15–24 and 45–64 years old was as follows: *C. tropicalis* > *C. parapsilosis* > *C. albicans* > *C. glabrata*. The proportion of *C. tropicalis* was significantly higher than that of *C. albicans* (15/50, 30.0 % vs. 6/48, 12.0 %. $P = 0.027$) in the group with 45–64 years old. Meanwhile, the situation for those groups with 25–44 and ≥65 years old was quite different: *C. albicans* > *C. tropicalis* > *C. parapsilosis* > *C. glabrata*. Only one isolate was obtained in those with ≤14 years old.

The results of in vitro susceptibility tests to FLC and VRC are summarized in Table 5 for yeast species. Some strains were not tested for VRC. Overall, 16.8 %(57/340)of isolates were resistant to FLC. Among them, 83.3 % of *C. haemulonii* was FLC resistant, which was the highest among all isolates, followed by *C. glabrata* (73.9 %) and *C. krusei* (66.7 %). The 72.6 % of *C. albicans* collected during the three-year surveillance program were inhibited by FLC, and only four isolates of them were resistant to FLC. VRC exhibited excellent antifungal activity against almost all species (95.2 % susceptibility) except for *C. haemulonii*, *C. glabrata,* and *Rhodotorula*. Overall, the rate for VRC resistance was low: Only 4 % of all fungal species isolates were resistant to VRC with no VRC resistance detected in isolates of *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, *C. pelliculosa*, *C. lucitaniae*, *C. krusei*, *C. utilis*, *C. famata*, *C. neoformans*, *Handenula polymorpha,* and *C. pulcherrima.* Seven out of 57 isolates with FLC resistant showed cross-resistances to VRC, including three cases of *Rhodotorula*, two *C. glabrata*, one *C. tropicalis*, and one *C. haemulonii*. 70 % (42/60) of the resistant isolates were from patients who were treated with two or more drugs, and 15 % (9/60) was from patients who had never been treated with antifungal drugs.

The results showed that from year 2010 to year 2012, there was a significant increase in FLC susceptibility with 39.3, 53.5, and 64.2 %, respectively ($P = 0.001$). However, the resistance to FLC was not changed significantly with 18.7, 15.0, and 17.0 %, respectively (Table 6). There was a slight decrease in VRC susceptibility from 97.2 % in 2010 to 92.5 % in 2012. There was a rise in VRC resistance from 1.9 % in 2010 to 6.6 % in 2012, although there was not yet a statistical significance in VRC case either.

Table 5 In vitro activities of agents against fungal species isolated from sterile samples

Species	FLC				VRC			
	No.	%S	SDD	%R	No.	%S	SDD	%R
C. albicans	106	77 (72.6)	25 (23.7)	4 (3.7)	79	79 (100)	0	0
C. tropicalis	81	36 (44.4)	37 (45.7)	8 (9.9)	50	46 (92)	2 (2)	2 (4)
C. parapsilosis	45	33 (73.3)	10 (22.3)	2 (4.4)	35	35 (100)	0	0
C. glabrata	23	1 (4.3)	5 (21.7)	17 (74)	15	11 (73.3)	0	4 (26.7)
C. guilliermondii	10	5 (50)	4 (40)	1 (10)	9	9 (100)	0	0
C. pelliculosa	10	5 (50)	2 (20)	3 (30.0)	8	8 (100)	0	0
C. haemulonii	6	1 (16.7)	0	5 (83.3)	3	2 (66.7)	0	1 (33.3)
C. krusei	3	0	1 (33.3)	2 (66.7)	3	3 (100)	0	0
C. lusitanaie	1	1 (100)	0	0	1	1 (100)	0	0
C. utilis	1	1 (100)	0	0	1	1 (100)	0	0
C. pulcherrima	1	1 (100)	0	0	1	1 (100)	0	0
C. famata	27	10 (37)	8 (30.7)	9 (33.3)	23	23 (100)	0	0
Cryptococcosis	19	7 (36.8)	10 (52.7)	2 (10.5)	16	16 (100)	0	0
Rhodotorula	5	0	1 (20)	4 (80)	4	1 (25)	0	3 (75)
KO	1	0	1 (100)	0	0	0	0	0
HP	1	0	1 (100)	0	1	1 (100)	0	0
Total	340	52.4	30.9	16.8	249	95.2	0.8	4

S, susceptible; SDD, susceptible dose dependent; R, resistant; *KO*, *Kodamaea ohmeri*; and *HP*, *Handenula polymorpha*

Table 6 Changes in FLC susceptibility and resistance to yeast species over the entire surveillance period

Susceptibility	No. (%) of isolates			*P* value
	2010	2011	2012	
Susceptible	42 (39.3)	68 (53.5)	68 (64.2)	0.001
SDD	45 (42.1)	40 (31.5)	20 (18.9)	0.001
Resistant	20 (18.7)	19 (15.0)	18 (17.0)	0.747
Total	107	127	106	340

SDD, susceptible dose dependent

Discussion

A total of 341 cases of yeasts, mainly *Candida* spp., are reported here for the course of this three-year surveillance program from 2010 to 2012 from Nanjing Jinling Hospital in China. This study has investigated the distribution of fungal species, the location of the patients where the specimens are collected, and the in vitro antifungal susceptibilities.

Invasive *Candida* (IC) is the most common (91.8 %) invasive fungal infections. The predominant *Candida* species, *C. albicans*, accounts for 33.8 % of *Candida* species, which is lower than that reported in other large surveillance studies: 38.3 % in the study of 2010 National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) [19] and 62.6 % in the multicentre ARTEMIS study (1997–2007) [20]. Instead, we have found that the frequency of occurrence for *C. tropicalis* (25.8 % of all *Candida)* is higher than that in CHIF-NET (16.9 %) and in ARTEMIS (7.2 %).

C. albicans has been the most common species of *Candida* isolated from bloodstream infections (BSI) worldwide, ranging from a low of 37 % in Latin America to a high of 70 % in Norway [21]. However, a decreasing trend in the isolation of *C. albicans* and increasing trend *of* non-*albicans Candida* have been observed in the past a few years. We have found that *C. tropicalis* is the most prevalent species in blood cultures (26.2; versus 19.2 % for *C. albicans*), which is similar to some studies by Shivaprakasha et al. [22] (35.6 %) and Adhikary and Joshi [23] (39.7 %) and Xess et al. [24] (35.3 %) from India. This result may be due to selection of less susceptible species by the pressure of antifungal agent such as FLC. It has been reported that *C. tropicalis* candidemia is associated with cancer and neutropenia [25]. The relationship between *C. tropicalis* and hematologic malignancies

has been found [26]. In our study, *C. parapsilosis* candidemia (20.6 vs. 19.2 % for *C. albicans*) is less prevalent than that reported in CHIF-NET (33.2 %) [19], but higher than that in the SENTRY Antimicrobial Surveillance Program (17.1 %) for 2008–2009 [26]. *C. parapsilosis* fungemia has been found to be significantly associated with intravascular catheter, malignity, and age [27]. In reality, *C. parapsilosis* is notorious for its capability of growing in hyperalimentation solutions with high concentrations of glucose and of forming biofilms on catheters and other implanted devices, for its nosocomial spread by the hands of healthcare workers, and for its persistence in the hospital environment [28]. In addition, six cases of *C. guilliermodii*, five ones of *C. pelliculosa*, five ones of *C. haemulonii*, eight ones of *C. famata*, five ones of *C. neoformans,* and five ones of *Rhodotorula* were recovered in blood culture in our surveillance. Although many of these species are uncommon, their appearance in this survey underscores an increased effort by clinical laboratories worldwide to identify isolates of *Candida* to the species level.

In the case of non-blood samples, *C. albicans* was the most commonly isolated organism (39.5 %), followed by *C. tropicalis* (22 %), *C. parapsilosis* (8 %), and *C. glabrata* (6.5 %). The rank order of these species is in accordance with the reports by Li et al. [29] and Wang et al. [19].

Rarer species identified in this study are *C. lusitaniae*, *C. pulcherrima*, *C. utilis*, *K. ohmer,* and *Hansenula polymorpha*, some of which are seldom reported in China. For example, to our best knowledge, this may be the first *C. utilis* infection reported in humans in China. The patient was a 64-year-old male with infectious cardiothoracic inflammation with bilateral pleural effusion in whom ICI (invasive *Candida* infections) was diagnosed by pleural effusion culture 6 days after hospitalization. Initial IVC treatment for this patient was switched to FLC, but he chose to discontinue treatment and left the hospital. In another example, *H. polymorpha* infection was diagnosed by CVC tips culture 8 days after hospital admission in ICU in an 18-year-old man with abdominal stab wound with hemorrhagic shock. The patient received treatment with FLC. After having a successful surgery, he recovered and was discharged. *H. polymorpha* infection in humans has been reported by McGinnis et al. [30].

The association of age of patients with the sequence of *Candida* spp. producing BSI has been previously reported [31–33]. In this study, the frequency of occurrence of *C. glabrata* increased with age of patients, and in patients ≥80 years old, approximately 1/3 of all episodes were due to this species. In our surveillance, such a rule has not been noted and this is probably due to the small (eight) number of isolates for this strain. In the groups with ages of 15–24 and of 45–64 years old, *C. tropicalis* and *C. parapsilosis* account for the largest proportion (68.8, and 58.3 %, respectively), with *C. tropicalis* being significantly higher than that of *C. albicans* in group with 45–64 years old. The reasons for such situation are currently unclear.

It has been reported in ARTEMIS study that the resistance rate to FLC for most of common *Candida*, including *C. albicans*, *C. tropicalis*, *C. glabrata,* and *C. parapsilosis*, is low (<10 %). Our data are similar to that of the surveillance except for *C. glabrata* that exhibits very high rate of resistance to FLC (17/23; 73.9 %). This finding is consistent with some studies in which the greatest resistance to FLC has been also observed in *C. glabrata* (36 %) [34]. The reason for such a high proportion of resistance is unclear. Some reports have demonstrated that *C. glabrata* FLC MICs are independently increased by CgSNQ2 over expression and the interaction between CgPDH1 gene copy number and CgPDH1 expression level in the multivariate analysis [35]. Nevertheless, over expression of the AKR gene has been associated with increased FLC and ITC resistance in *C. glabrata* [36]. In our study, many patients received empirical therapy and 52.2 % (156/299) patients were treated with FLC, followed by VRC (29.4 %, 88/299) and caspofungin (7.7 %, 23/299). The increasing resistance rate of *Candida* spp. may be associated with precious antifungal agent exposure. However, 123 yeast isolates, including nine cases of FLC resistance strains, were obtained from 113 patients without using any antifungal drugs. Our FLC resistance rate (7.3 %; 9/123) is close to the result by Wu et al. [37] from Hainan. In their study, 29 % of the isolates were resistant to at least one drug with 8 % FLC resistance rate, although none of the sampled hosts had taken any antifungal drugs at least 3 months before samples were taken. Selection pressure from the agricultural fungicide application, rising antifungal drug using in hospitals, and drug-resistance

yeasts from surrounding regions were considered as the potential source of resistance [37].

Like many reports in the literature, VRC has excellent in vitro activity against most of *Candida* species in our study. Only four cases of *C. glabrata*, two ones *of C. tropicalis*, and one ones of *C. haemulonii* exhibited resistance to VRC. Cross-resistance between azoles has also been observed in our surveillance. The seven VRC-resistant *Candida* isolates are also resistant to FLC. In ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002 [38], only 13 % FLC-resistant *C. glabrata* isolates (46) were susceptible to VRC, in comparison with 63.6 % (7/11) in our study. However, this remains to be brought to our attention because VRC-resistant *C. glabrata* emerging as a threat in people receiving VRC therapy has been raised in a report of breakthrough fungal infections [39].

Resistance to FLC was seen only in two *C. neoformans* isolates, and all were VRC susceptible in our study. The resistance rates to FLC may vary with geographic region (13.6 % in Latin America, 12.4 % in Africa/Middle East, 8.1 % in North America) and be probably in a increasing trend (with 7.3 % in 1997–2000, 10.9 % in 2001–2004, and 11.7 % in 2005–2007 in the ARTEMIS study) [40], in which 80 % (4/5) *Rhodotorula* spp. were resistant to FLC and 75 % (3/4) of it were resistant to VRC.

In conclusion, this study shows the epidemiology and antifungal resistance of invasive yeasts, mainly *Candida* spp., isolated from Jinling Hospital in Nanjing, China between 2010 and 2012. Non-*albicans Candida* is responsible for over half of the candidemia episodes, with *C. tropicalis* being the most common strain (28.5 %), while *C. albicans* (42.9 %) is the predominant species in the other samples. The proportion of various fungal species does not change appreciably. Most of the IC cases occur in the ICU, and *Cryptococcus* encephalitis is mainly in medical wards. *C. glabrata* exhibits high rate of FLC resistance. VRC demonstrates an excellent activity against *C. albicans*, *C. parapsilosis*, *C. tropicalis,* and *C. neoformans*, but not against *C. glabrata*. Cross-resistance to azoles is noted, especially for *C. glabrata.*

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Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standard Informed consent was obtained from patients or their representatives. The protocol of the study was approved by Ethics Committees of Jinling Hospital.

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