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ORIGINAL RESEARCH

Species Distribution and Antifungal Susceptibility of Invasive Candidiasis: A 2016-2017 Multicenter Surveillance Study in Beijing, China

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Objective: Invasive candidiasis (IC), a life-threatening fungal infection prevalent among hospitalized patients, has highly variable regional epidemiology. We conducted a multicenter surveillance study to investigate recent trends in species distribution and antifungal suscept-ibility patterns among IC-associated *Candida* spp. in Beijing, China, from 2016 to 2017.
Materials and Methods: A total of 1496 non-duplicate *Candida* isolates, recovered from blood and other sterile body fluids of IC patients, were identified using matrix-assisted laser desorption/ionization time of flight mass spectrometry combined with ribosomal DNA internal transcribed spacer (ITS) region sequencing. Broth microdilution-based susceptibility testing using six antifungal agents was also conducted.
Results: *Candida albicans* was the most frequently isolated species (49.9%), followed by *Candida tropicalis* (15.5%), *Candida glabrata* (14.7%) and *Candida parapsilosis* (14.2%). No significant differences in species distribution were observed when compared with a 2012–2013 dataset. Overall, the rates of susceptibility to fluconazole and voriconazole were high among *C. albicans* (98% and 97.2%, respectively) and *C. parapsilosis* species complex (91.1% and 92%, respectively) isolates but low among *C. tropicalis* (81.5% and 81.1%, respectively) isolates. In addition, the rate of azole resistance among *C. tropicalis* isolates increased significantly (1.8-fold, $P<0.05$) compared with that observed in 2012–2013, while micafungin resistance rates were <5% for all tested *Candida* species.
Conclusion: Our results suggest that species distribution has remained stable among IC-associated *Candida* isolates in Beijing. Resistance to micafungin was rare, but increased azole resistance among *C. tropicalis* isolates was noted. Our study provides information on local epidemiology that will be important for the selection of empirical antifungal agents and contributes to global assessments of antifungal resistance.
Keywords: invasive fungal infections, *Candida*, species distribution, antifungal resistance, surveillance, Beijing

Introduction

Invasive candidiasis (IC) has emerged as the most frequent fungal infection among immunocompromised patients and those hospitalized with serious underlying con-ditions. IC describes candidemia and other deep-seated candidiasis, with candide-mia being the most common and life-threatening presentation. The global incidence of candidemia has increased notably over the past two decades, rising from 2 cases to 14 cases per 100,000 persons based on population-based studies.[1–3] In addition, candidemia is associated with high mortality rates and long periods of hospitaliza-tion. For example, a PATH Alliance study in North America indicated that the crude

12-week mortality rate of candidemia varied from 23.7% to 52.9%, depending on the *Candida* species.[4]

The epidemiology of IC has significantly evolved in recent years. A progressive shift in the most common cause of infections from *Candida albicans* to non-*albicans Candida* spp. has been observed globally.[5–7] Antifungal resistance is also an increasingly difficult challenge for the implementation of effective empirical and prophylactic strategies,[8,9] with emerging species exhibiting resistance to multiple classes of antifungal agents.[10] Both nationwide and local epidemiological surveillance studies are very important for detecting emerging resistance, and provide information for proper antifungal stewardship. In the present study, we investigated species distribution and antifungal susceptibility patterns from 2016 to 2017 in Beijing, China, and compared our findings with trends in IC epidemiology and resistance from a 2012–2013 dataset.[11]

Materials and Methods
Study Design
A surveillance study was carried out from January 2016 to December 2017 and included 37 centers (24 university hospitals, six military hospitals, four tertiary general hospitals, two cancer hospitals, and one children's hospital) distributed across 12 districts in Beijing, China. The districts included Dongcheng (seven hospitals), Chaoyang (six hospitals), Haidian (six hospitals), Fengtai (five hospitals), Xicheng (four hospitals), Changping (two hospitals), Shijingshan (two hospitals), Fangshan (one hospital), Huairou (one hospital), Mentougou (one hospital), Pinggu (one hospital), and Tongzhou (one hospital). Consecutive non-duplicated *Candida* isolates retrieved from blood and other normally sterile body fluids were sent to the Department of Clinical Laboratory, Peking Union Medical College Hospital, for species confirmation and susceptibility testing. The isolates were stored at −80°C until use. The study was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (No. S-K524).

Species Identification
Isolates were identified using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; Vitek MS, bioMérieux, Marcy l'Etoile, France) analysis in combination with ribosomal DNA internal transcribed spacer (ITS) region sequencing, as previously described.[12,13]

Antifungal Susceptibility Testing
In vitro susceptibilities to fluconazole (FLC), voriconazole (VRC), itraconazole (ITC), 5-flucytosine (5-FC), amphotericin B (AMB), and micafungin (MCA) were tested according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (M27-A3).[14] The minimum inhibitory concentration (MIC) of each reagent for each isolate was assessed after 24 h of incubation. Endpoints for 5-FC, MCA, and the azoles were set at a 50% reduction in growth relative to the drug-free control. For AMB, the MIC was defined as the lowest concentration at which there was no discernible growth. Quality control strains *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were included in each assay. Current species-specific clinical breakpoints (CBPs) or epidemiological cut-off values (ECVs) were used for interpretation of results.[15,16] ECVs were used to define wild-type (WT) and non-wild-type (non-WT) isolates if no CBPs were available from the CLSI.

Statistical Analysis
All statistical analyses were performed using SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA). Comparisons of continuous variables were performed using the chi-squared test or Fisher's exact test, as appropriate. A *P*-value of <0.05 was considered significant.

Results
Species Distribution and Patient Characteristics
From 2016 to 2017, 1496 non-duplicated *Candida* isolates comprising 26 *Candida* species were recovered from blood and other sterile body fluids from IC patients in Beijing (Table 1). *Candida albicans* was the most frequently isolated species (747 isolates, 49.9%), followed by *Candida tropicalis* (15.5%), *Candida glabrata* species complex (14.7%), and *Candida parapsilosis* species complex (14.2%). The median patient age was 57.8 years (range, 0–98 years), and the proportions of male and female patients were 59.3% and 40.7%, respectively. Notably, isolation rates of most *Candida* species increased gradually with increasing age, with the highest isolation rates (41.5%, 621/1496) observed among patients at the extreme end of the age spectrum (>65 years). *C. albicans* was the predominant species across all age groups >15 years, whereas *C. parapsilosis* species complex were most common in infants (0–1 year). *C albicans* accounted for approximately 39.4% (218/554) of

Table 1 Species Distribution of 1496 Invasive *Candida* Isolates Based on Clinical Characteristics

Characteristics	No. of Isolates or Patients							
	Total	*C. albicans*	*C. tropicalis*	*C. glabrata* complex[c]	*C. parapsilosis* Complex[d]	*C. lusitaniae*	*C. krusei*	Other *Candida*[e]
Number of isolates	1496	747	233	221	213	25	22	35
Patient age (years)								
0–1	72	27	3	1	29	12	0	0
2–14	37	13	13	1	9	1	0	0
15–49	281	134	60	33	38	5	3	8
50–65	485	261	68	74	58	3	10	11
>65	621	312	89	112	79	4	9	16
Gender								
Male	887	412	143	114	169	14	17	18
Female	609	335	90	107	44	11	5	17
Isolation site								
Blood	554	218	96	92	119	14	5	10
Ascitic fluid	402	233	63	65	20	2	10	9
Pus	133	70	20	12	26	1	0	4
Bile	120	72	18	12	10	2	4	2
Bronchoalveolar lavage fluid	102	62	12	16	3	4	2	3
Catheter	82	37	11	12	18	1	1	2
Pleural fluid	42	25	6	4	6	0	0	1
Tissue	22	11	0	3	5	1	0	2
Cerebrospinal fluid	15	8	4	0	3	0	0	0
Other specimens[a]	24	11	3	5	3	0	0	2
Clinical department								
ICU	471	240	85	80	44	8	6	8
Surgery	411	215	52	63	59	2	8	12
Medicine	341	171	58	50	46	3	3	10
Emergency	120	53	20	19	24	0	3	1
Other departments[b]	153	68	18	9	40	12	2	4

Notes: [a]Includes joint fluid, vitreous humor, peritoneal dialysate fluid. [b]Includes pediatrics, dermatology, gynecology and obstetrics, endocrinology, otorhinolaryngology. [c]Includes *Candida glabrata sensu stricto* (215) and *Candida nivariensis* (6). [d]Includes *Candida parapsilosis sensu stricto* (192), *Candida metapsilosis* (7), *Candida orthopsilosis* (11) and *Lodderomyces elongisporus* (3). [e]Include *Candida guilliermondii* (13), *Candida pelliculosa* (4), *Candida lipolytica* (3), *Candida norvegensis* (2), *Candida kefyr* (3), *Candida inconspicua* (3), *Candida haemulonii* (2), *Candida rugosa* (1), *Candida stellatoidea* (1), *Candida famata* (1), *Candida freyschussii* (1), *Candida fabianii* (1).

bloodstream *Candida* isolates. *C. parapsilosis* species complex were the second most common bloodstream isolates, responsible for 21.5% of all cases. Of the 1496 IC cases, 31.5% (471/1496) occurred in intensive care units (ICUs) and 27.5% (411/1496) in surgical wards. However, *C. albicans* was the most frequently isolated species across the different clinical departments (Table 1).

Antifungal Susceptibility

Table 2 summarizes the results of in vitro susceptibility testing of the invasive *Candida* isolates recovered in this study. FLC and VRC were most active against *C. albicans* (98% and 97.2% susceptible, respectively) and

C. parapsilosis species complex (91.1% and 92.0% susceptible, respectively). Overall, 94.1% of *C. glabrata* species complex isolates showed dose-dependent susceptibility to FLC, while decreased FLC susceptibility was observed among *C. tropicalis* (81.5% susceptible), *C. guilliermondii* (46.2% non-WT) and *C. lusitaniae* (88% non-WT) isolates. VRC was active against FLC-resistant *C. krusei* isolates (90.9% susceptible), but showed reduced activity against FLC-resistant *C. tropicalis* isolates (81.1% susceptible), with 37/40 (92.5%) FLC-resistant *C. tropicalis* isolates also showing VRC resistance. Notably though, all of the FLC-resistant *C. tropicalis* isolates were susceptible to MCA based on the

Table 2 In vitro Susceptibilities of 1496 *Candida* Isolates to Six Antifungal Agents as Determined by the Broth Microdilution Method According to the Guidelines of the Clinical and Laboratory Standards Institute

Organism and Agent	MIC (mg/L)[b]			% of Isolates in Each Indicated Susceptibility Category by CBP[c]				% of Isolates by ECV[d]	
	Range	50%	90%	S	S-DD	I	R	WT	non-WT
C. albicans (n = 747)									
Fluconazole	0.25–64	0.5	2	98	1.7		0.3		
Voriconazole	0.008–2	0.016	0.064	97.2		2.5	0.3		
Itraconazole	0.016–1	0.125	0.5						
Amphotericin B	0.016–2	0.5	1					100	0
5-flucytosine	0.064–128	0.064	0.125						
Micafungin	0.008–2	0.064	0.125	99.7		0	0.3		
C. tropicalis (n = 233)									
Fluconazole	0.125–512	0.5	64	81.5	1.3		17.2		
Voriconazole	0.008–32	0.032	2	81.1		3	15.9		
Itraconazole	0.016–32	0.25	0.5					96.6	3.4
Amphotericin B	0.25–2	1	1					100	0
5-Flucytosine	0.064–0.125	0.064	0.064						
Micafungin	0.008–2	0.032	2	97.9		0.9	1.3		
C. glabrata species complex (n = 221)									
Fluconazole	0.25–256	8	32		94.1		5.9		
Voriconazole	0.016–16	0.125	0.5					82.4	17.6
Itraconazole	0.016–16	0.5	1					96.8	3.2
Amphotericin B	0.008–2	1	1					100	0
5-flucytosine	0.064–8	0.064	0.064						
Micafungin	0.008–4	0.032	0.064	91.9		5.9	2.3		
C. parapsilosis species complex (n = 213)									
Fluconazole	0.25–128	1	2	91.1	4.2		4.7		
Voriconazole	0.008–8	0.032	0.125	92		4.7	3.3		
Itraconazole	0.016–2	0.25	0.5						
Amphotericin B	0.008–2	0.5	1					100	0
5-flucytosine	0.064–64	0.064	0.125						
Micafungin	0.008–4	1	2	99.5		0.5	0		
C. lusitaniae (n = 25)									
Fluconazole	0.25–16	0.5	2					88	12
Voriconazole	0.016–0.25	0.016	0.064						
Itraconazole	0.064–0.5	0.25	0.5					100	0
Amphotericin B	0.016–1	0.5	1						
5-flucytosine	0.064–2	0.064	0.25						
Micafungin	0.008–1	0.25	0.5					92	8
C. krusei (n = 22)									
Fluconazole	8–512	16	32						
Voriconazole	0.016–4	0.125	0.5	90.9		4.5	4.5		
Itraconazole	0.032–2	0.5	0.5					95.5	4.5
Amphotericin B	0.125–2	1	1					100	0
5-flucytosine	0.064–8	2	4						
Micafungin	0.008–0.5	0.125	0.5	90.9		9.1			
C. guilliermondii (n=13)									
Fluconazole	2–64	16	32					46.2	53.8
Voriconazole	0.064–2	0.25	1						

(*Continued*)

Table 2 (Continued).

Organism and Agent	MIC (mg/L)[b]			% of Isolates in Each Indicated Susceptibility Category by CBP[c]				% of Isolates by ECV[d]	
	Range	50%	90%	S	S-DD	I	R	WT	non-WT
Itraconazole	0.064–2	1	2						
Amphotericin B	0.25–2	0.75	1						
5-flucytosine	0.064–128	0.064	4						
Micafungin	0.5–2	1	2	100		0	0		
Other *Candida* species (n = 22)[a]									
Fluconazole	1–512	8	32						
Voriconazole	0.016–16	0.125	0.5						
Itraconazole	0.032–32	0.5	1						
Amphotericin B	0.25–16	0.5	1						
5-flucytosine	0.064–128	0.125	8						
Micafungin	0.008–2	0.125	0.5						

Notes: [a]Includes *Candida pelliculosa* (n = 4), *Candida lipolytica* (n = 3), *Candida norvegensis* (n = 2), *Candida kefyr* (n = 3), *Candida inconspicua* (n = 3), *Candida haemulonii* (n = 2), *Candida rugosa* (n = 1), *Candida stellatoidea* (n = 1), *Candida famata* (n = 1), *Candida freyschussii* (n = 1), *Candida fabianii* (n = 1). [b]50% and 90%, MICs at which 50% and 90% of isolates are inhibited, respectively. [c]CBP, clinical breakpoint; S, susceptible; I, intermediate; S-DD, susceptible-dose dependent; R, resistant. [d]ECV, epidemiological cutoff values; WT, wild-type; non-WT, non-wild-type.

revised CBPs. As expected, MCA resistance was not prevalent, with only 0.3% (2/747) of *C. albicans*, 1.3% (3/233) of *C. tropicalis*, 2.3% (5/221) of *C. glabrata* species complex, and 4.5% (1/22) of *C. krusei* isolates demonstrating MCA resistance. For all *Candida* isolates, the MICs of AMB were ≤2 mg/L, except for one *C. haemulonii* isolate for which the MIC was 16 mg/L.

Geographic Variation in Species Distribution and Susceptibility Profiles

The four most prevalent species of *Candida* (*C. albicans, C. glabrata* species complex, *C. tropicalis*, and *C. parapsilosis* species complex) accounted for 94.5% (1414/1496) of all isolates in the current study. Although these four species were predominant at each of the study hospitals, their frequencies and rank orders varied considerably among different hospitals in Beijing, as shown in Figure 1. For example, although *C. albicans* was the most prevalent species in samples from 27/37 (73.0%) hospitals, the percentage at which it was isolated varied widely (from 0% to 100%) among the different centers. Among the 10 hospitals where *C. albicans* was not the dominant species, *C. parapsilosis* species complex, *C. glabrata* species complex, *or C. tropicalis* was the most common species. Notably, 25 (1.67%) isolates were identified as *C. lusitaniae* in the current study, representing 2.5% (14/554) of all bloodstream isolates. Ten of the 14 bloodstream *C. lusitaniae* isolates were recovered from a single hospital,

clustered in samples from the neonatal ICUs (NICUs). In addition, significant variation in the detection rate of invasive *Candida* isolates was observed among the participating hospitals. Overall, 15/37 (40.5%) medical centers, each with 400–1200 hospital beds, produced fewer than 10 *Candida* isolates from routine clinical specimens (blood and other sterile body fluids) during the study period, which may be partly associated with the patient population and laboratory capacity. FLC susceptibility among *C. tropicalis* isolates was also highly variable, with some institutions reporting no azole resistance and others (JM, YD, and TR) reporting FLC resistance rates as high as 60–100%. This may result from differences in clinician prescribing patterns associated with treatment of and prophylaxis against invasive candidiasis.

Discussion

A better understanding of the local epidemiology of IC and rates of antifungal resistance is critical for clinicians to make informed therapeutic decisions regarding antifungal prophylaxis for their patients while awaiting culture-based drug susceptibility data.[17,18] To this end, we undertook a retrospective, laboratory-based, 2-year continuous IC surveillance study in Beijing.

IC has emerged as an important nosocomial infection, especially among critically ill patients hospitalized in the ICU.[19] ICU patients may be particularly susceptible to such infections because they are exposed to broad-spectrum
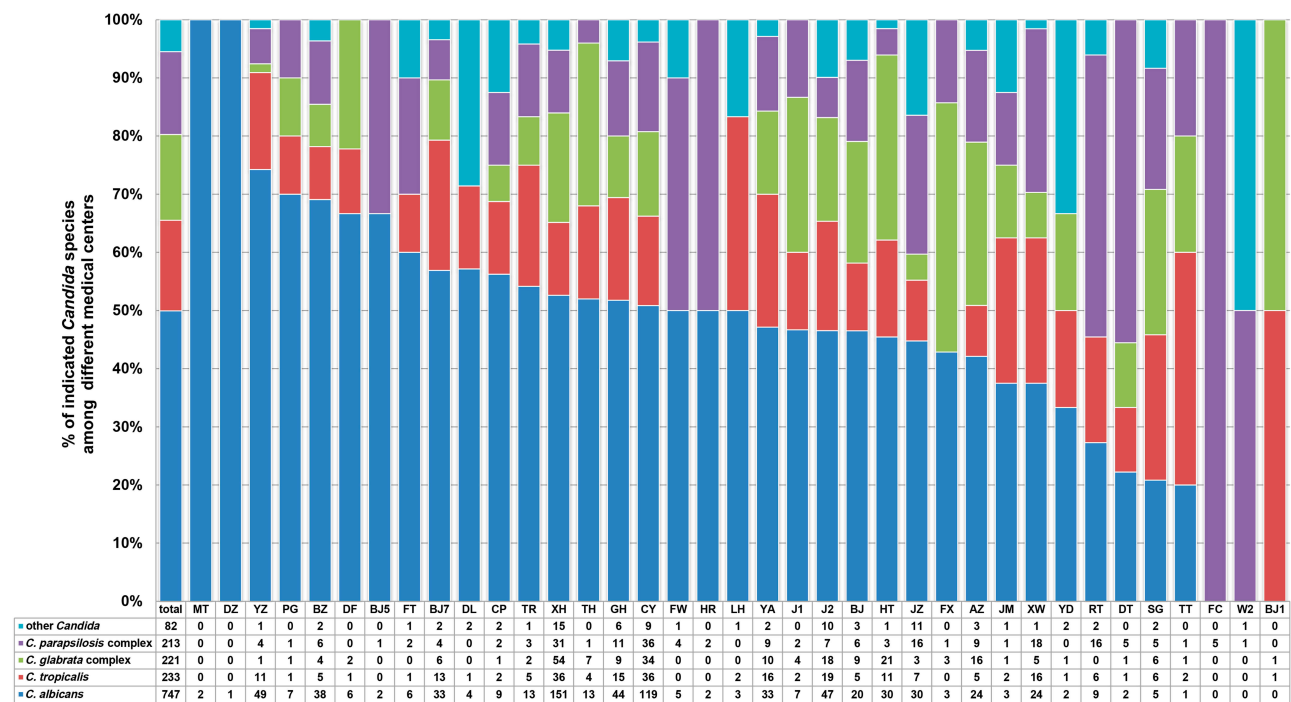
	total	MT	DZ	YZ	PG	BZ	DF	BJ5	FT	BJ7	DL	CP	TR	XH	TH	GH	CY	FW	HR	LH	YA	J1	J2	BJ	HT	JZ	FX	AZ	JM	XW	YD	RT	DT	SG	TT	FC	W2	BJ1
other *Candida*	82	0	0	1	0	2	0	0	1	2	2	2	1	15	0	6	9	1	0	1	2	0	10	3	1	11	0	3	1	1	2	2	0	2	0	0	1	0
C. parapsilosis complex	213	0	0	4	1	6	0	1	2	4	0	2	3	31	1	11	36	4	2	0	9	2	7	6	3	16	1	9	1	18	0	16	5	5	1	5	1	0
C. glabrata complex	221	0	1	1	4	2	0	0	6	0	1	2	1	54	7	9	34	0	0	0	10	4	18	9	21	3	3	16	1	5	1	0	1	6	1	0	0	1
C. tropicalis	233	0	0	11	1	5	1	0	1	13	1	2	5	36	4	15	36	0	0	2	16	2	19	5	11	7	0	5	2	16	1	6	1	6	2	0	0	1
C. albicans	747	2	1	49	7	38	6	2	6	33	4	9	13	151	13	44	119	5	2	3	33	7	47	20	30	30	3	24	3	24	2	9	2	5	1	0	0	0

Figure 1 Geographic variations of *C. albicans*, *C. tropicalis*, *C. glabrata* complex, *C. parapsilosis* complex and other *Candida* species at 37 surveillance sites.
Abbreviations: MT, China Meitan General Hospital; DZ, Dongzhimen Hospital of Beijing University of Chinese Medicine; YZ, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital; PG, Beijing Pinggu Hospital; BZ, Peking University Cancer Hospital and Institute; DF, Dongfang Hospital of Beijing University of Chinese Medicine; BJ5, Peking University Third Hospital; FT, Beijing Fengtai Hospital; BJ7, The 8th Medical Center of Chinese PLA General Hospital; DL, Beijing Electric Power Hospital; CP, Beijing Changping Hospital; TR, Beijing Tongren Hospital; XH, Peking Union Medical College Hospital; TH, Beijing Tsinghua Changgung hospital; GH, Chinese PLA General Hospital; CY, Beijing Chaoyang Hospital; FW, Fuwai hospital; HR, Beijing Huairou Hospital; LH, Beijing Luhe Hospital; YA, You'an Hospital; J1, The Fourth Medical Center of the Chinese PLA General Hospital; J2, The Fifth Medical Center of Chinese PLA General Hospital; BJ, Beijing Hospital; HT, Aerospace Center Hospital; JZ, The Seventh Medical Center of PLA General Hospital; FX, Fuxing Hospital; AZ, Anzhen Hospital; JM, Beijing Jingmei Group General Hospital; XW, Xuanwu Hospital; YD, Hebei Yanda Hospital; RT, Children's Hospital, Capital Institute of Pediatrics; DT, Beijing Ditan Hospital; SG, Peking University Shougang Hospital; TT, Beijing Tiantan Hospital; FC, Beijing Gynecology and Obstetrics Hospital; W2, Beijing Armed Police Corps Second Hospital; BJ1, Beijing Chaoyang Hospital (West).

antibacterial agents, often have central venous catheters, have undergone major surgery, and are more likely to be elderly (>65 years) or experiencing immunosuppression.[17] Importantly, a significant trend towards increased IC-associated mortality in ICU patients was observed. According to a large prospective hospital-based IC surveillance program in Paris,[19] ICU patients had a higher overall death rate (51%) than non-ICU patients (30.7%; *P*<0.001). Consistent with previous reports,[9] samples from ICU patients contained the highest proportion of *Candida* isolates in our study (31.5%). Furthermore, we noted that FLC resistance rates were much higher among *C. tropicalis* isolates from ICU patients than among isolates from other clinical wards (24.7% versus 17.1%, *P*=0.021), which may be an important consideration when developing guidelines for prophylaxis and treatment of IC patients.

Despite the global trend towards decreased frequency of *C. albicans* and increased frequency of non-*albicans Candida* species,[5,6] *C. albicans* was the most commonly isolated *Candida* species at most hospitals in our study,

accounting for 49.9% of all isolates. This prevalence rate is roughly consistent with data obtained in a study from Beijing in 2012–2013 (52.5%),[11] and is similar to the prevalence in Europe (52.5%; 1997–2016 SENTRY Program). However, it is higher than the prevalence rates reported in North America, Latin America, the Asia-Pacific region and nationwide China (CHIF-NET), which range from 40% to 45%.[6,9]

Despite its overall prevalence, *C. albicans* accounted for only 39.4% (218/554) of the candidemia isolates with *C. parapsilosis* species complex identified as the leading non-*albicans Candida* bloodstream pathogen. *C. parapsilosis* species complex accounted for 21.5% (119/554) of all bloodstream isolates, a prevalence rate that is similar to previous reports from China (CHIF-NET Program).[9] In contrast, *C. glabrata* species complex were more prevalent among candidemia cases in the United States[20] and some European countries.[21] Interestingly, the species distribution of IC-associated pathogens shows regionality in Asia. A multi-center study in the Asia-Pacific region

revealed that either *C. tropicalis* or *C. parapsilosis* species complex was the most prevalent non-*albicans* species isolated from bloodstream infections.[22] In Pakistan, the prevalence of *C. tropicalis* surpassed that of *C. albicans*, ranking it as the most common IC-associated *Candida* species, followed by *C. albicans* in neonates and *C. parapsilosis* species complex in adults.[23] In our study, *C. tropicalis* was the second and the third most prevalent cause of IC and candidaemia, respectively. It is thought that the trend towards increased prevalence of non-*albicans* species may be related in part to the increased use of FLC for treatment and prophylaxis.[24]

Of the five major *Candida* species associated with IC, *C. krusei* was the least frequently isolated, accounting for only 1–3% of isolates.[21] *C. krusei* is a prominent pathogen among patients with hematologic malignancies and others who have received prolonged azole prophylaxis.[17,25] Likewise, the frequency of *C. krusei*-associated IC infections was low in our study (1.5%). Notably, there was a cluster of *C. lusitaniae* candidemia cases from a single NICU, emphasizing the importance of epidemiological studies and further investigations of the role of *C. lusitaniae* in neonatal fungemia. Similarly, a retrospective study from Kuwait shed light on the emerging role of *C. lusitaniae* as a healthcare-associated pathogen capable of causing fungemia in preterm neonates.[26] *C. lusitaniae* is also recognized as a causative agent of breakthrough fungemia in cancer patients,[27] with a study from the United States showing that the incidence of candidemia caused by *C. lusitaniae* has increased significantly over the past 16 years.[28]

Candida auris, first reported in Japan in 2009, is an emerging pathogen that has quickly spread to multiple countries across several continents, becoming a significant clinical problem.[10,29] *C. auris* has been associated with nosocomial outbreaks in ICUs, which is particularly concerning given that the large majority of isolates exhibit FLC resistance and variable susceptibility to other azoles, AMB, and echinocandins.[10] In addition, *C. auris* can be misidentified by conventional commercial API-20C or Vitek-2 systems, necessitating the use of molecular methods or MALDI-TOF MS for identification.[29] In the current study, all *Candida* isolates were forwarded to the central laboratory of the Department of Clinical Laboratory, Peking Union Medical College Hospital, for species confirmation by MALDI-TOF MS (Vitek MS, IVD Knowledgebase v.3.0 (*C. auris* included); bioMérieux), which was confirmed using ITS region sequencing.

Fortunately, none of the samples examined in the current study contained *C. auris*. Accurate identification of *C. auris* by MALDI-TOF MS is only reliable when the species is present in the database. Thus, laboratories should confirm with the manufacturer that the *C. auris* reference strain spectrum is present in their database.[29]

In our study, acquired resistance to FLC and VRC among *C. albicans* and *C. parapsilosis* species complex isolates was rare (<5%). The rates of FLC resistance among *C. glabrata* species complex isolates decreased slightly between the study conducted in 2012–2013 (7.2%)[11] and the current study (5.9%) (P=0.598). This may be caused by differences between participating hospitals in the two surveillance periods, with rates of resistance known to vary depending on patient population characteristics and by region or even hospital.[1,2,5] However, rates of resistance to FLC among *C. tropicalis* isolates increased significantly, from 9.4% in 2012–2013[11] to 17.2% in the current study period (P<0.05). Thus, FLC resistance in *C. tropicalis* should be subject to careful monitoring. Furthermore, a high rate of cross-resistance (92.5%) between FLC and VRC was observed among *C. tropicalis* isolates. Overall, our findings are in agreement with the national CHIF-NET study from China, which showed that the susceptibility of *C. tropicalis* to both FLC and VRC decreased continuously over the study period, dropping from 94.3% (for both azoles) in year one to about 76.8% in year five.[9] High rates of FLC resistance among *C. tropicalis* isolates have also been recorded in the Asia-Pacific region (18.1%)[22] and Belgium (20%).[30] The association between increased resistance and widespread use of FLC prophylaxis and treatment in clinical practice has been widely debated and remains controversial.[8,31] Regardless, increasing azole resistance calls for optimal FLC dosing and appropriate selection of antifungal agents for IC patients. Currently, the European Society of Clinical Microbiology and Infectious Diseases guidelines recommend the use of echinocandins as a first-line empirical treatment for IC prior to species identification and susceptibility testing.[32] Worldwide, echinocandin resistance is rare and generally limited to *C. glabrata* isolates (resistance rates of 2–5%).[1,6] The overall rate of echinocandin resistance was also very low in our study (<5%); however, resistance rates of up to 12.3% have been noted in the United States.[33] Therefore, with the increased use of echinocandins, it is important that we monitor rates of resistance.

We observed a high degree of inter-laboratory variation in species distribution and detection rates of invasive *Candida* (Figure 1), which may be influenced by the patient population and laboratory diagnostic capacity of each center. A survey by the Asia Fungal Working Group[34] showed that the status of diagnostic mycology services in Asian countries is far from satisfactory. Approximately 31% of Chinese laboratories did not have a separate space for mycology diagnostics and most laboratories lacked continuing education and training in medical mycology. Furthermore, the National External Quality Assessment (EQA) program for mycology is still not available in China. With the increasing incidence of fungal disease, it is urgent that laboratories take steps to improve fungal diagnostics such as regular staff training and EQA program participation.

Our study had several limitations. First, this was a local epidemiological study, meaning that the results may not be generalizable to all patients with IC. However, data presented in our study may provide useful information for the treatment of fungal infections in areas outside of Beijing, as well as being used to establish better antifungal stewardship for IC patients in Beijing. Secondly, there were disparities among the numbers of isolates collected from different hospitals, which may influence the accuracy of the geographic picture of species distribution and antifungal resistance. Finally, we did not have access to FLC usage data, which would have allowed us to correlate FLC consumption and increased resistance rates.

Conclusion

Comparison of our results with data from 2012 to 2013 showed that species distribution of IC-associated *Candida* has remained stable in Beijing, and that acquired echinocandin resistance remains rare. Based on the increasing rate of azole resistance among *C. tropicalis* isolates, our study highlights the need for accurate species identification and azole susceptibility testing in clinical practice. In addition, our findings emphasize the importance of continued efforts to identify epidemiological changes associated with IC and determine the possible molecular mechanisms underlying azole resistance in *C. tropicalis*.

Acknowledgments

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Author Contributions

Y-C X, L-N G, and MX conceived the work. S-Y Y, L-N G, YY, and GZ carried out species identification and antifungal susceptibility testing. L-N G, C-X Y, C-M B, MC, L-Y Y, JL, G-W L, RM, YZ, HL, Y-L L, L-J L, and Y-J H

were responsible for data collection and analysis. L-N G, P-R H, and Y-C X drafted the manuscript. All authors contributed to data analysis, drafting, or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

All authors declare that they have no conflicts of interest.

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