JCM
Journals.ASM.org

In Vitro Susceptibilities of Yeast Species to Fluconazole and Voriconazole as Determined by the 2010 National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) Study

He Wang,[a] Meng Xiao,[a,b] Sharon C.-A. Chen,[c] Fanrong Kong,[c] Zi-Yong Sun,[d] Kang Liao,[e] Juan Lu,[f] Hai-Feng Shao,[g] Yan Yan,[h] Hong Fan,[i] Zhi-Dong Hu,[j] Yun-Zhuo Chu,[k] Tie-Shi Hu,[l] Yu-Xing Ni,[m] Gui-Ling Zou,[n] and Ying-Chun Xu[a]

Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China[a]; Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China[b]; Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Darcy Road, Westmead, New South Wales, Australia[c]; Department of Clinical Laboratory, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China[d]; Department of Clinical Laboratory, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China[e]; Department of Clinical Laboratory, The First Affiliated Hospital of Harbin Medical University, Harbin, China[f]; Institute of Clinical Laboratory Medicine of PLA, Nanjing General Hospital, Nanjing, China[g]; Department of Clinical Laboratory, Peking University First Hospital, Beijing, China[h]; Laboratory of Clinical Microbiology, West China Hospital, Sichuan University, Chengdu, China[i]; Department of Clinical Laboratory, Tianjin Medical University General Hospital, Tianjin, China[j]; Department of Clinical Laboratory, The First Hospital of China Medical University, Shenyang, China[k]; Department of Clinical Laboratory, The People's Hospital of Liaoning Province, Shenyang, China[l]; Department of Clinical Microbiology and Infection Control, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China[m]; and Department of Clinical Laboratory, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, China[n]

We conducted active, laboratory-based surveillance for isolates from patients with invasive infections across China from August 2009 to July 2010. DNA sequencing methods were used to define species, and susceptibility to fluconazole and voriconazole was determined by the Clinical and Laboratory Standards Institute M44-A2 disk diffusion method but using up-to-date clinical breakpoints or epidemiological cutoff values. *Candida* spp. made up 90.5% of the 814 yeast strains isolated, followed by *Cryptococcus neoformans* (7.7%) and other non-*Candida* yeast strains (1.7%). Bloodstream isolates made up 42.9% of the strains, isolates from ascitic fluid made up 22.1%, but pus/tissue specimens yielded yeast strains in <5% of the cases. Among the *Candida* isolates, *Candida albicans* was the most common species from specimens other than blood (50.1%) but made up only 23% of the bloodstream isolates ($P < 0.001$). *C. parapsilosis* complex species were the most common *Candida* isolates from blood (33.2%). Uncommon bloodstream yeast strains included *Trichosporon* spp., *C. pelliculosa*, and the novel species *C. quercitrusa*, reported for the first time as a cause of candidemia. Most (>94%) of the isolates of *C. albicans*, *C. tropicalis*, and the *C. parapsilosis* complex were susceptible to fluconazole and voriconazole, as were all of the *Trichosporon* strains; however, 12.2% of the *C. glabrata sensu stricto* isolates were fluconazole resistant and 17.8% had non-wild-type susceptibility to voriconazole. Seven *C. tropicalis* strains were cross-resistant to fluconazole and voriconazole; six were from patients in the same institution. Resistance to fluconazole and voriconazole was seen in 31.9% and 13.3% of the uncommon *Candida* and non-*Candida* yeast strains, respectively. Causative species and azole susceptibility varied with the geographic region. This study provided clinically useful data on yeast strains and their antifungal susceptibilities in China.

Invasive yeast infections are a major threat to the health of hospitalized patients, particularly the critically ill, causing substantial morbidity and hospital costs (3, 12, 23, 30). Although invasive candidiasis (IC), including candidemia, remains the most common yeast infection, previously uncommon pathogens such as *Trichosporon* and *Geotrichum* species, with reduced susceptibility to antifungal agents, have emerged (23). In addition, there have been important changes in the epidemiology of IC, with an overall shift toward *Candida* spp. other than *Candida albicans*, particularly *Candida glabrata*, with reduced susceptibility to the azole antifungals (1, 42).

Early and appropriate antifungal therapy is essential for optimum patient outcomes in invasive yeast infections (13, 26). However, initiation of culture-directed therapy is often delayed since standard diagnostic methods are slow (2 to 3 days) and instead, antifungal strategies such as preemptive or empirical approaches are often practiced. These are reliant on robust epidemiological data to inform the selection of the initial antifungal therapy. Knowledge of local epidemiological patterns is important since epidemiological data may not be generalizable between countries because of geographic variation in epidemiology (30, 32). Thus, accurate identification of yeast pathogens and determination of their antifungal susceptibility profiles, together with surveillance data, are essential in guiding clinical decisions (31, 32).

Fluconazole is an inexpensive, effective antifungal agent often used as first-line therapy of yeast infections (25, 28). Since it does not reliably cover an increasing number of *Candida* species and other yeast strains, the major consideration influencing the decision to use fluconazole or other antifungal—depending on species identification and susceptibility results—is the likelihood of a fluconazole-resistant species. Voriconazole, a broad-spectrum

Received 29 April 2012 **Returned for modification** 5 June 2012
Accepted 18 September 2012
Published ahead of print 3 October 2012
Address correspondence to Ying-Chun Xu, xycpumch@yahoo.com.cn.
H.W. and M.X. contributed equally to this work.
Supplemental material for this article may be found at http://jcm.asm.org/.

doi:10.1128/JCM.01130-12

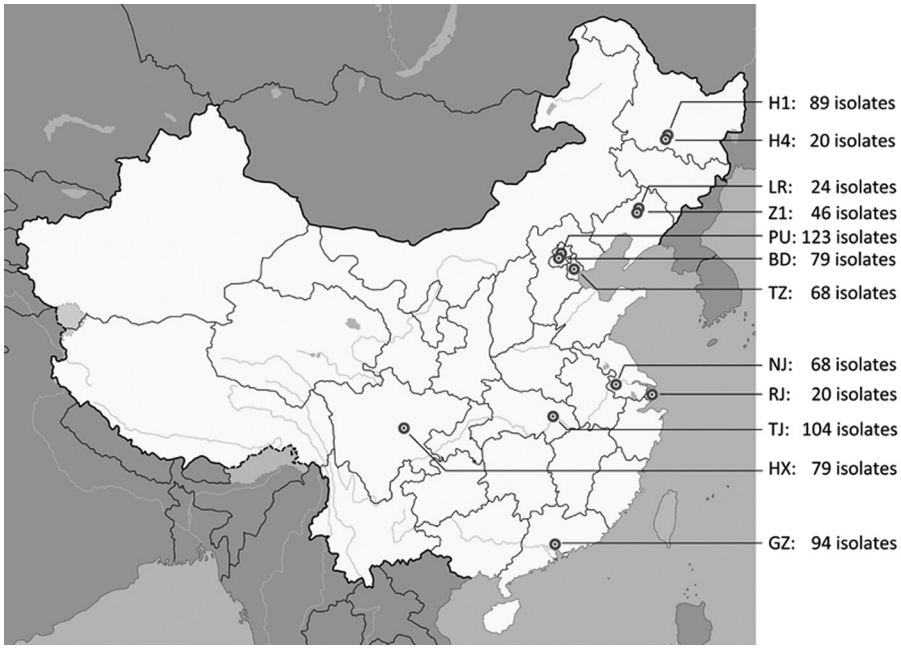

FIG 1 Distribution of the 12 surveillance sites that participated in CHIF-NET 2010 in China. Hospital codes: BD, Peking University First Hospital; GZ, The First Affiliated Hospital of Sun Yat-Sen University; H1, The First Affiliated Hospital of Harbin Medical University; H4, the Fourth Affiliated Hospital of Harbin Medical University; HX, West China Hospital; LR, The People's Hospital of Liaoning Province; NJ, Nanjing General Hospital of PLA; PU, Peking Union Medical College Hospital; RJ, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University; TJ, Tianjin Medical University General Hospital; Tongji Hospital; TZ, Tianjin Medical University General Hospital; Z1, The First Hospital of China Medical University.

azole, may be used in place of fluconazole for initial therapy, yet many yeast strains are cross-resistant to all azoles (27, 34). Hence, data on susceptibility to antifungals are often required for definitive treatment decisions.

Antifungal resistance surveillance programs such as the ARTEMIS Global Antifungal Surveillance Program (1997 to 2007) have been instrumental in monitoring trends in causative species and susceptibility to antifungal agents among pathogenic yeast strains (31, 32). The ARTEMIS initiative further resulted in the development of standardized Clinical and Laboratory Standards Institute (CLSI) criteria for resistance or susceptibility to fluconazole and voriconazole using disk diffusion methodology as an alternative to broth microdilution (7, 8, 27, 28, 34, 35).

Although five Chinese hospitals participated in the ARTEMIS program, contemporary data on yeast susceptibility are lacking, with many clinicians relying on earlier data to guide treatment choices, with possible adverse patient outcomes (H. Wang, personal communication). Epidemiological data on invasive yeast infections in China have been restricted either to studies in single centers or to a limited number of yeast species (20, 41). To address these deficiencies, the multicenter nationwide China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study was initiated in July 2009 to prospectively monitor trends in the epidemiology of yeast infections and provide up-to-date data on susceptibility to antifungal drugs. Here we report the results of the first year (August 2009 to July 2010) of the study, focusing on the *in vitro* susceptibilities of yeast strains to fluconazole and voriconazole.

MATERIALS AND METHODS

Study design. This study was a prospective, laboratory-based, multicenter study of invasive yeast infections with its inception in August 2009. A total of 12 "rank-A tertiary" hospitals (900 to 4,300 hospital beds with 1.09 to 3.15 million outpatient visits per year), serviced by a regional referral mycology laboratory, in nine major cities in China participated, i.e., two hospitals each from Beijing, Harbin, and Shenyang and one each from Chengdu, Guangzhou, Nanjing, Shanghai, Tianjin, and Wuhan (Fig. 1).

For each episode of yeast isolation (see the criteria for study inclusion below), data were entered into a standard case report form. The information collected included the patient's age and gender, the patient's classification (inpatient or outpatient), and the ward location (e.g., emergency department [ED], surgical, medical, intensive care unit [ICU]) of the patient at the time of collection of the sample. The date of sample collection, the specimen type, the body site of isolation, and the initial species identification made by the referring laboratory were also recorded. A unique study identifier was assigned to each episode of yeast isolation.

Criteria for study inclusion. All *Candida*, *Cryptococcus*, and other yeast strains recovered from blood; other sterile body fluids, including ascitic fluid and peritoneal dialysate fluid; pus; and tissue from patients with invasive yeast infections (11) were included in this study (Table 1). Yeast strains from bronchoalveolar lavage (BAL) fluid samples, central venous catheter (CVC) tips, and the gastrointestinal tracts (e.g., biliary tract fluid [aseptically collected]) of patients with invasive infections were tested; however, yeast strains from urine and the genital tract and others considered colonizers were excluded. Isolates of the same species and of the same susceptible or resistant biotype profile from the same site of a given patient that were recovered at a different time were considered duplicates and also excluded. All isolates were forwarded to a central laboratory (Department of Clinical Laboratory, Peking Union Medical College Hospital) for study.

Isolate identification. Yeast strains were identified at each study center by routine mycological methods. By growth on Brilliance *Candida* agar (Oxoid Ltd., Hampshire, United Kingdom), *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *C. parapsilosis* complex were identified according to the manufacturer's instructions. Isolates that were not identifiable underwent analysis with the Vitek 2 Compact YST (bioMérieux,

TABLE 1 Isolation of *Candida*, *Cryptococcus*, and other yeast species by specimen type, CHIF-NET study, 2010, China

Specimen type(s)	No. (%) of isolates of:			
	All spp.	*Candida* spp.	*Cryptococcus* spp.	Other yeast species
Blood	349 (42.9)	322 (92.3)	20 (5.7)	7 (2.0)
Ascitic fluid	180 (22.1)	177 (98.3)	1 (0.6)	2 (1.1)
CVC tip	62 (7.6)	60 (96.8)	1 (1.6)	1 (1.6)
CSF	62 (7.6)	24 (38.7)	37 (59.7)	1 (1.6)
Pus (from abscesses)[a]	39 (4.8)	39 (100)		
BAL fluid	32 (3.9)	31 (96.9)		1 (3.1)
Bile	31 (3.8)	31 (100)		
Pleural fluid	23 (2.8)	22 (95.7)		1 (4.3)
Tissue[b]	21 (2.6)	16 (76.2)	4 (19.0)	1 (4.8)
Peritoneal dialysate fluid	11 (1.4)	11 (100)		
Bone marrow	2 (0.2)	2 (0.3)		
Eye	2 (0.2)	2 (0.3)		
All	814 (100)	737 (100)	63 (100)	14 (100)

[a] Includes 29 isolates from intra-abdominal infections.
[b] Includes 11 isolates from lung tissue, 6 from liver tissue, 1 from heart tissue, and 3 from other tissues.

Marcy l'Etoile, France) and/or API 20C AUX (bioMérieux) system. These initial species identifications and isolates were forwarded to the central laboratory for species identity confirmation by DNA sequencing of the fungal internal transcribed spacer (ITS) region following ITS amplification using the primer pair ITS1/ITS4 as previously described (14). Sequencing of the D1/D2 domain of the 28S rRNA gene (amplified by primer pair F63/R635) (14) was performed for those isolates where ITS sequence analysis failed to produce a result (14% of the strains) (data not shown).

Agreement of the initial species identification and molecular identification results was evaluated as follows. A minor error was considered to have occurred when the (i) initial identification correctly identified an isolate to the genus level but was unable to identify a species (e.g., *Candida catenulata* identified as *Candida* spp.) or (ii) the initial identification correctly identified an isolate to the species complex level but not to the species level (e.g., *Candida metapsilosis* or *Candida orthopsilosis* identified as *Candida parapsilosis* complex and *Candida nivariensis* as *C. glabrata* complex) (21, 22, 24). A major error was defined as other disagreements between the initial identification and molecular identification. Definitive identification to the species level was that done by DNA sequencing.

Antifungal susceptibility testing. Susceptibility to fluconazole and voriconazole was determined using the Clinical and Laboratory Standards Institute (CLSI) M44-A2 disk diffusion method (7). Briefly, agar plates containing BBL Mueller-Hinton II agar (BD, Sparks, MD) 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 a McFarland standard turbidity of 0.5 and standardized by the bioMérieux McFarland kit (bioMérieux). Fluconazole (25 μg) and voriconazole (1 μg) disks (BD) were placed onto the surfaces of the inoculated plates, and the plates were incubated in air at 37°C and read at 24 h. Slow-growing isolates, e.g., members of the genus *Cryptococcus*, were read after 48 h of incubation. Quality control was performed with each test run by using *C. albicans* ATCC 90029 and *C. parapsilosis* ATCC 22019.

The zone diameters of all isolates were recorded electronically. The species-specific interpretive criteria for fluconazole (susceptible [S], susceptible dose dependent [S-DD], and resistant [R]) and voriconazole (S, intermediate [I], and R) were applied to *C. albicans*, *C. tropicalis*, *C. parapsilosis* complex, and *C. krusei* isolates as described by Pfaller et al. (27, 28). *C. glabrata* complex isolates were categorized as S, S-DD, and R to fluconazole by species-specific clinical breakpoints (CBPs) (28), but with regard to susceptibility to voriconazole, they were categorized as wild type (WT) or non-WT (strains with acquired or mutational resistance mechanisms) by using species-specific epidemiologic cutoff values (ECVs) (27). For other yeast species, interpretation of fluconazole and voriconazole susceptibilities (S, S-DD, and R) was done in accordance with the CLSI M44-S3 guidelines (8).

Statistical analysis. All comparisons were performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL). Comparisons of continuous variables were performed by using the Mann-Whitney test, and comparisons of categorical variables were performed by using a chi-square test or Fisher's exact test, as appropriate. A P value of 0.05 was considered significant.

(He Wang presented results of the CHIF-NET10 study at the 22nd European Congress of Clinical Microbiology and Infectious Diseases, London, United Kingdom, 31 March to 3 April 2012.)

RESULTS

Isolates. A total of 814 yeast isolates were studied (range, 20 to 123 isolates from each hospital; Fig. 1). *Candida* species were the most common yeast strains isolated (737/814; 90.5%), with *Cryptococcus neoformans* and non-*Candida* yeast strains comprising 7.7% ($n = 63$) and 1.7% ($n = 14$) of the isolates, respectively. Blood culture isolates made up 42.9% of the yeast strains, and ascitic fluid accounted for 22.1%, while pus from deep abscesses and tissue biopsy specimens yielded yeast strains uncommonly (<5%; Table 1).

Candida species isolates were broadly distributed and made up the majority of the blood culture isolates (92.3%; Table 1). Cryptococci and non-*Candida* yeast strains accounted for 7.7% of the yeast strains from blood cultures. The majority of the yeast strains from ascitic fluid (96.7%) and CVC tips (98.4%) were *Candida* spp., and *Candida* spp. were the only yeast isolates from pus. Conversely, 59.7% of the yeast strains from cerebrospinal fluid (CSF) samples were *C. neoformans*. There was a single isolate of *Trichosporon asahii* from a CSF sample (Table 1).

Patient location. Of the isolates recovered, 92.4% were from hospital inpatients (ICUs, 34.3%; medical wards, 27.8%; surgical wards, 30.3%) and 7.6% were from the outpatient/ED setting (outpatient clinics, 2.6%; EDs, 5.6%) (Table 2). The predominant patient location varied with the pathogen group. *Candida* and

TABLE 2 Distribution of *Candida*, *Cryptococcus*, and other yeast spp. by clinical service

Organism	No. of isolates tested (% of total)	No. of isolates							
		Inpatient					Outpatient/emergency		
		Total	ICU	Medicine	Surgery	Other[a]	Total	Emergency	Outpatient
Candida spp.	737 (90.5)	680	272	124	226	58	57	37	20
Cryptococcus spp.	63 (7.7)	58		44	10	4	5	4	1
Other yeast spp.	14 (1.7)	14	7	1	4	2			
Total	814 (100)	752	279	169	240	64	62	41	21

[a] Includes pediatrics, dermatology, obstetrics and gynecology, and ophthalmology services.

TABLE 3 Species distribution of 814 yeast strains isolates and comparison of molecular and initial identification results

Organism	No. of isolates (% of all yeast isolates)	Molecular vs initial identification results[a]			No. of blood/ nonblood isolates
		% agreement	% of identification errors[b]		
			Major	Minor	
Candida species	737 (90.5)	84.3	12.2	3.5	322/415
C. albicans	282 (34.6)	97.2	2.8		74/208
C. parapsilosis species complex					
C. parapsilosis sensu stricto	143 (17.6)	91.6	7	1.4	90/53
C. metapsilosis	22 (2.7)		36.4	63.6	11/11
C. orthopsilosis	4 (0.5)			100	4/0
L. elongisporus	3 (0.4)		100		2/1
C. tropicalis	123 (15.1)	86.2	11.4	2.4	48/75
C. glabrata species complex					
C. glabrata sensu stricto	90 (11.1)	86.7	12.2	1.1	50/40
C. nivariensis	2 (0.2)		50	50	1/1
C. krusei	18 (2.2)	83.3	16.7		5/13
C. guilliermondii	12 (1.5)	41.7	50	8.3	8/4
C. pelliculosa	12 (1.5)	25	75		8/4
C. lipolytica	10 (1.2)		100		9/1
C. lustitaniae	6 (0.7)	83.3	16.7		4/2
C. quercitrusa	3 (0.4)		100		3/0
C. catenulata	2 (0.2)	100			2/0
C. fabianii	1 (0.1)		100		1/0
C. famata	1 (0.1)		100		1/0
C. haemulonii	1 (0.1)	100			1/0
C. kefyr	1 (0.1)	100			0/1
C. norvegensis	1 (0.1)		100		0/1
C. neoformans	63 (7.7)	93.7	6.3		20/43
Other yeast species	14 (1.7)	57.1	35.7	7.1	7/7
Geotrichum capitum	2 (0.2)		100		1/1
Kodamaea ohmeri	2 (0.2)	100			1/1
Meyerozyma caribbica	1 (0.1)		100		1/0
Rhodotorula mucilaginosa	1 (0.1)			100	1/0
Trichosporon asahii	7 (0.9)	85.7	14.3		3/4
Trichosporon dermatis	1 (0.1)		100		0/1
Total	814 (100)	84.5	12.2	3.3	349/465

[a] Initial identification was performed at each surveillance site by phenotypic and biochemical methods; molecular identification was performed by sequencing of the ITS region and/or D1/D2 domain of the 28S rRNA gene.

[b] A minor error was defined as (i) correct identification of an isolate to the genus level but inability to identify it to the species level (e.g., *C. catenulata* identified as *Candida* spp. by phenotypic methods) or (ii) initial correct identification of an isolate to the species complex level but not to the species level (e.g., *C. metapsilosis* or *C. orthopsilosis* identified as *C. parapsilosis* complex and *C. nivariensis* as *C. glabrata* complex). A major error was defined as other disagreements between the initial identification and molecular identification.

non-*Candida* yeast strains were most often isolated from inpatients in ICUs and surgical wards (40% and 41.1% of the isolates, respectively) (Table 2). *C. neoformans* was more likely to be isolated from patients in medical wards (44/58 cases, 75.8%) than were *Candida* spp. (57/680 or 18.3%; $P < 0.001$) and were not recovered from ICU patients.

Yeast species. A total of 27 yeast species were identified by sequencing of the ITS and/or D1/D2 regions of the rRNA gene during this study.

Initial species identification was concordant with the molecular identification of 688/814 (84.5%) isolates (Table 3; see Table S1 in the supplemental material). Major errors occurred in 99 identifications (12.2% of the cases, involving 21 species), and minor errors occurred in 27 (3.3%) isolate identifications (eight species). Initial identification of *C. albicans*, *C. parapsilosis* (*sensu stricto*), *C. tropicalis*, *C. glabrata* (*sensu stricto*), and *C. neoformans* showed good agreement (86.2 to 97.2%) with molecular identification; however, for the remaining yeast species ($n = 113$ isolates, 22 species), the overall agreement was significantly lower (mean, 35.4%); for example, all three *Candida quercitrusa* strains were identified as *Candida lusitaniae* (see Table S1 in the supplemental material). All isolates were identified to the species level by DNA sequencing.

Overall, *C. albicans* was the most common *Candida* species (282/737 isolates, 38.3%), followed by *C. parapsilosis* complex (23.3%) and *C. tropicalis* (16.7%) (Table 3). All 63 strains of *Cryptococcus* were *C. neoformans*; there were no isolates of *Cryptococcus gattii*. *Trichosporon* species were uncommon ($n = 8$ isolates; 1% of

all yeast strains). Of 172 isolates of the *C. parapsilosis* complex, 22 (12.8%) were *C. metapsilosis*, 4 (2.3%) were *C. orthopsilosis*, and 3 (1.8%) were *Lodderomyces elongisporus*. Only 2/92 *C. glabrata* complex isolates were *C. nivariensis*.

Comparison of blood isolates with those recovered from sites other than blood. Among *Candida* spp., *C. albicans* was significantly more likely to be recovered from sites other than blood (50.1% of the *Candida* isolates) than from blood cultures (23%; $P < 0.001$). In contrast, the *C. parapsilosis* complex—*C. parapsilosis sensu stricto* ($n = 90$ isolates), *C. metapsilosis* ($n = 11$), *C. orthopsilosis* ($n = 4$), and *L. elongisporus* ($n = 2$)—the most common cause of candidemia, was disproportionately represented in blood cultures compared with nonblood specimens (107/322 isolates, 33.2%, versus 65/415 isolates, 15.6%; $P < 0.001$). The likelihood of isolating *C. glabrata*, including *C. nivariensis* ($n = 2$) and uncommon *Candida* spp. such as *C. quercitrusa*, from blood was also significantly higher ($P = 0.01$ and $P < 0.001$, respectively, Table 3). Three of eight *Trichosporon* isolates were blood culture isolates (all *T. asahii*), and the remaining five were from ascitic fluid ($n = 2$), BAL fluid, CSF, and a CVC tip ($n = 1$ each). Other rare yeast strains found in blood were *Geotrichum capitum*, *Kodamaea ohmeri*, *Meyerozyma caribbica*, and *Rhodotorula mucilaginosa* ($n = 1$ each).

***In vitro* susceptibilities.** The majority (>94%; Table 3) of the *C. albicans*, *C. parapsilosis* group, and *C. tropicalis* strains were susceptible to fluconazole. Among the *C. glabrata* complex strains, 12.2% (11/90) of the *C. glabrata sensu stricto* isolates were categorized as resistant to fluconazole and 87.8% were S-DD, as were both *C. nivariensis* isolates. All *Trichosporon* strains were susceptible. Fluconazole-resistant yeast isolates were *Candida krusei* and uncommon *Candida* species, including *Candida pelliculosa* and *C. quercitrusa*. The rate of resistance to fluconazole among uncommon *Candida*/non-*Candida* yeast strains was 31.9%.

Overall, most yeast strains were susceptible to voriconazole. Sixteen (17.4%) of 92 *C. glabrata* isolates were categorized as non-WT according to species-specific ECVs. In addition, voriconazole resistance was present in 7/123 *C. tropicalis* isolates (5.7%), 2 *C. krusei* isolates, *C. catenulata*, *Candida lipolytica*, *C. pelliculosa*, and *R. mucilaginosa* (Table 4). The rate of resistance to voriconazole among uncommon *Candida*/non-*Candida* yeast strains was 13.3%. Of 51 fluconazole-resistant non-*C. glabrata* isolates, nine strains of *C. lipolytica*, seven of *C. tropicalis*, two of *C. catenulata* and *C. krusei*, and one each of *C. albicans*, *C. pelliculosa*, and *R. mucilaginosa* (23 isolates in all) were cross-resistant to voriconazole. No isolate was voriconazole resistant but susceptible to fluconazole.

Geographic variation in species distribution and *in vitro* susceptibilities. *C. albicans* was the most common pathogen in all of the study centers, except site LR (northeastern China) and site HX (central China), where *C. glabrata sensu stricto* (8/24 yeast isolates, 33.3%) and *C. parapsilosis sensu stricto* (21/79 isolates, 26.6%) were predominant, respectively (Fig. 1 and 2). *C. neoformans* was recovered in <10% of the cases, except at sites HX and TJ (21.5% and 16.2% of the yeast isolations, respectively).

Of note, uncommon *Candida* or non-*Candida* yeast species were relatively common at sites H1 and H4, Harbin, northeastern China (Fig. 2). Further, a greater proportion of isolates from these two centers were fluconazole resistant (27% at H1 and 20% at H4), as well as being voriconazole resistant and in the non-WT category (19.1% at H1, 10% at H4). This compares with resistance rates of 2.5 to 15% for fluconazole and 0 to 16.7% for voriconazole at other centers (data not shown). Three fluconazole-resistant yeast species (*C. catenulata*, *C. lipolytica*, and *C. quercitrusa*, 14 isolates in all) were identified only at H1, and one species (*C. pelliculosa*, 4 isolates) was identified only at H4. Six of seven *C. tropicalis* isolates cross-resistant to fluconazole and voriconazole were identified at H1, including five isolates that were identified over 35 days (September to October in 2009) and one that was identified in July 2010.

TABLE 4 *In vitro* susceptibilities to fluconazole and voriconazole of 814 yeast strain isolates in the CHIF-NET study, 2010

Organism	*In vitro* susceptibility[a] to:			
	Fluconazole		Voriconazole	
	% S	% R	% S	% R
C. albicans	99.3	0.4	99.3	0.7
C. parapsilosis complex				
C. parapsilosis sensu stricto	98.6	1.4	100	0
C. metapsilosis	100	0	100	0
C. orthopsilosis	100	0	100	0
L. elongisporus	100	0	100	0
C. tropicalis	94.3	5.7	94.3	5.7
C. glabrata complex				
C. glabrata sensu stricto	NA[b]	12.2	82.2[c]	17.8[c]
C. nivariensis	NA	0	100[c]	0[c]
C. krusei	0	100	83.3	11.1
C. guilliermondii	100	0	100	0
C. pelliculosa	50.0	33.3	66.7	8.3
C. lipolytica	10.0	90.0	10.0	90.0
C. lustitaniae	83.3	0	100	0
C. quercitrusa	0	100	100	0
C. catenulata	0	100	0	100
C. fabianii	100	0	100	0
C. famata	100	0	100	0
C. haemulonii	100	0	100	0
C. kefyr	100	0	100	0
C. norvegensis	100	0	100	0
C. neoformans	90.5	6.3	100	0
Other yeast species				
Geotrichum capitum	100	0	100	0
Kodamaea ohmeri	100	0	100	0
Meyerozyma caribbica	100	0	100	0
Rhodotorula mucilaginosa	0	100	0	100
Trichosporon asahii	85.7	0	100	0
Trichosporon dermatis	100	0	100	0

[a] Susceptibility data were interpreted (i) by species-specific interpretive criteria for *C. albicans*, *C. tropicalis*, *C. parapsilosis* complex, the *C. glabrata* complex, and *C. krusei* as described by Pfaller et al. (27, 28) and (ii) in accordance with CLSI document M44-S3 (8) for other yeast species.

[b] The latest species-specific interpretive criteria for *C. glabrata* complex susceptibility to fluconazole were defined as ≤14 mm for R and ≥15 mm for S-DD (28); thus, category S is not applicable (NA) to the *C. glabrata* complex.

[c] As CBPs for *C. glabrata* complex susceptibility to voriconazole are not available, the ECV (a zone diameter of ≥16 mm) was used to differentiate wild-type (listed in category S) from non-wild-type (listed in category R) strains of this complex, as described by Pfaller et al. (27).

DISCUSSION

The epidemiology of invasive yeast infections and the associated antifungal susceptibility patterns are poorly defined in China. De-

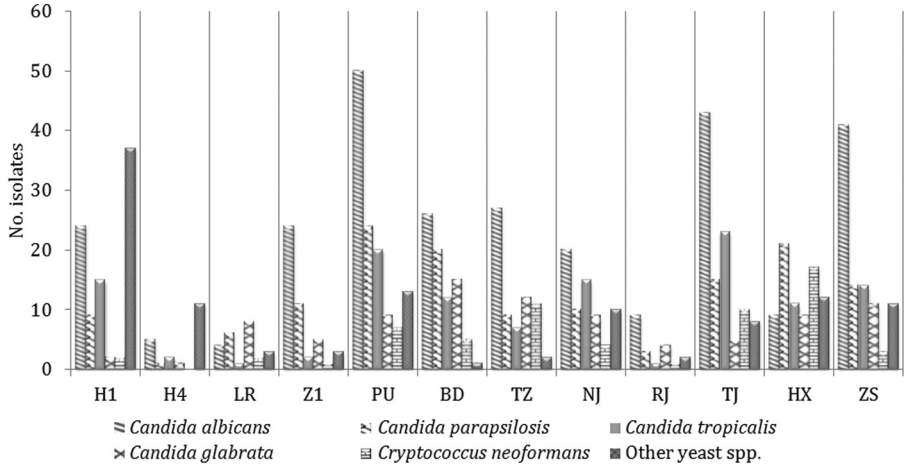

FIG 2 Geographic variations of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *Cryptococcus neoformans*, and other yeast species at 12 surveillance sites.

spite some data on susceptibility to antifungal agents reported through the ARTEMIS study (31, 32), these were not clearly separate from those of other Asia-Pacific regions, more recent data are lacking, and there is no ongoing surveillance. The results of the first year of this national surveillance program have provided clinically relevant data regarding causative pathogens and *in vitro* susceptibility to fluconazole and voriconazole; important regional differences were identified.

Accurate identification of yeast strains by using robust molecular methods (14, 15, 19) was essential to the aims of the study. Though the overall agreement between phenotypic and molecular identification was 84.5%, the rate of major errors was high (14.3 to 100%) for yeast strains, including *C. krusei* and rare *Candid*a species (Table 3; see Table S1 in the supplemental material). This is not surprising, since yeast isolates may be "misidentified" on chromogenic media because of atypical color and because of database limitations of phenotypic identification systems, particularly for uncommon yeast strains. As with other studies, molecular but not phenotypic methods distinguished species within the *C. parapsilosis* complex (21, 22, 24) and the *C. glabrata* complex (4, 10). The results are also noteworthy for having identified three cases of bloodstream infection due to *C. quercitrusa*, highlighting the utility of molecular methods in identifying novel pathogens. Although molecular tools were used in the present study, their use in the routine identification of yeast strains is not feasible for most clinical microbiology laboratories in China because of high costs and a lack of standardized methods and quality control procedures. The contribution of active surveillance networks such as CHIF-NET is thus pivotal to providing these data.

IC is the most common invasive yeast infection, and the predominance of *Candida* isolates (90.5%) was expected. Yet although it was the most common *Candida* species overall, *C. albicans* made up only 38.3% of the *Candida* yeast strains (Table 3), a percentage substantially lower than those reported in other large surveys: 62.6% in the multicenter ARTEMIS study (1997 to 2007) (31, 32) and 46.1% in the SENTRY (2009) program (29). Instead, we noted a higher proportion of *Candida* species other than *C. albicans*, including species of the *C. parapsilosis* complex (23.3% of the *Candida* isolates, compared with 5.8% and 1.3% in the ARTEMIS and SENTRY surveys, respectively). While these large surveys analyzed species distribution according to the continent of origin, they did not consider potential differences within regions (30, 32). Additionally, in the present study, but not in the ARTEMIS study, sputum, urine, and genital tract samples were excluded from the analysis; *C. albicans* is the most common *Candida* species isolated from these clinical specimens (33).

A key finding of this study is the confirmation that trends in causative species of *Candida* bloodstream infections are not transferable between regions. While *C. albicans* has remained the most common etiological agent (37 to 70%) worldwide (2, 5, 9, 38, 39), we found the *C. parapsilosis* complex to be the most prevalent species in blood cultures (33.2% versus 23% for *C. albicans*). The high prevalence of *C. parapsilosis* candidemia in China is not readily explained. *C. parapsilosis* is associated with catheter-related fungemia (32, 40) and is known to colonize skin; evaluation of CVC care and infection control practices may be helpful in identifying risk factors for *C. parapsilosis* candidemia. *C. glabrata* candidemia (15.8% of the cases) was less prevalent than that reported in the United States (24 to 25%) (15, 17) but more so than in certain areas of Latin America and Europe (3 to 8% of the cases) (9, 37).

Few large surveys have described patterns of causative *Candida* species in nonbloodstream infections. The present study provides clinically relevant data, at least for Chinese physicians, in that 50.1% of the *Candida* isolates belonged to *C. albicans*, with *C. tropicalis* the second most common species (18.1%); *C. parapsilosis* complex and *C. glabrata* complex isolates were recovered in only 15.7% and 9.9% of the cases, respectively. It could be argued, although this should be supported by additional data, that in this context, selection of initial antifungal therapy need not necessarily encompass species with reduced susceptibility to azoles, e.g., *C. glabrata*.

Recently, a set of new interpretive criteria for disk diffusion testing with regard to fluconazole and voriconazole susceptibility has been recommended (27, 28). Using species-specific CBPs for *C. albicans*, *C. parapsilosis* complex, *C. tropicalis*, and *C. krusei* and ECVs for the voriconazole susceptibility of *C. glabrata*, overall, most of the yeast strains were susceptible to the two azoles, although susceptibility varied with the species. Azole resistance was uncommon in *C. albicans*, *C. parapsilosis*, and *C. tropicalis* (<10%), consistent with previous reports (29, 32). However,

seven *C. tropicalis* strains were resistant to fluconazole, as well as voriconazole, and six of these seven were recovered from patients at a single location (site H1 in Harbin) within 35 days, all in the ICU. Clusters of infections due to *C. tropicalis* have been reported (6, 18). Other *Candida* species, e.g., *C. krusei*, have also been reported to cause clusters of infections (16). We are in the process of investigating the genetic relatedness of the C. *tropicalis* strains as the cause of a possible cluster with its infection control implications. Further, a larger proportion of resistant yeast species were from sites H1 and H4. This is due partly to the larger proportion of infections caused by uncommon *Candida* spp. at these sites (e.g., *C. quercitrusa* was isolated only at site H1, C. *lipolytica* was isolated from patients at site H1, and *C. pelliculosa* was isolated at site H4).

Interpretive criteria for azole susceptibility using disk diffusion methods have not yet been published for non-*Candida* yeast strains. We used the criteria in the CLSI M44-S3 document to assess susceptibility to enable result comparison (8, 31). Resistance to fluconazole was seen in only four *C. neoformans* isolates, and all were voriconazole susceptible. Historically, fluconazole resistance is uncommon in *C. neoformans*, although resistance rates may vary with the geographic region and be increasing (31, 36). In the ARTEMIS study, 7.3% of the strains were resistant to fluconazole from 1997 to 2000 and 11.7% were resistant to fluconazole from 2005 to 2007 (31).

In conclusion, this study has provided clinically useful data on the epidemiology of invasive yeast infections in China. Molecular methods are essential for the identification of uncommon yeast strains. *C. parapsilosis* was the most common pathogen in bloodstream infections due to *Candida* spp. but with geographical variation. Both fluconazole and voriconazole demonstrated good activity against *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. neoformans* but not against *C. glabrata*; cross-resistance to both azoles was noted in *C. glabrata* and uncommon yeast strains. Continued surveillance is warranted.

ACKNOWLEDGMENT

The CHIF-NET surveillance program is supported by an Investigator-Initiated Research grant from Pfizer, China.

We have no conflict of interest to declare.

REFERENCES

1. **Arendrup MC, et al.** 2008. Semi-national surveillance of fungaemia in Denmark 2004-2006: increasing incidence of fungaemia and numbers of isolates with reduced azole susceptibility. Clin. Microbiol. Infect. **14**:487–494.
2. **Arendrup MC, et al.** 2011. Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. J. Clin. Microbiol. **49**:3300–3308.
3. **Arnold HM, et al.** 2010. Hospital resource utilization and costs of inappropriate treatment of candidemia. Pharmacotherapy **30**:361–368.
4. **Borman AM, et al.** 2008. *Candida nivariensis*, an emerging pathogenic fungus with multidrug resistance to antifungal agents. J. Clin. Microbiol. **46**:933–938.
5. **Chen S, et al.** 2006. Active surveillance for candidemia, Australia. Emerg. Infect. Dis. **12**:1508–1516.
6. **Chou HH, Lo HJ, Chen KW, Liao MH, Li SY.** 2007. Multilocus sequence typing of *Candida tropicalis* shows clonal cluster enriched in isolates with resistance or trailing growth of fluconazole. Diagn. Microbiol. Infect. Dis. **58**:427–433.
7. **Clinical and Laboratory Standards Institute.** 2009. Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline, 2nd ed, M44-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Clinical and Laboratory Standards Institute.** 2009. Zone diameter interpretive standards, corresponding minimal inhibitory concentration (MIC) interpretive breakpoints, and quality control limits for antifungal disk diffusion susceptibility testing of yeasts; informational supplement, 3rd ed, M44-S3. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Colombo AL, et al.** 2006. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J. Clin. Microbiol. **44**:2816–2823.
10. **Cuenca-Estrella M, et al.** 2011. Prevalence of *Candida bracarensis* and *Candida nivariensis* in a Spanish collection of yeasts: comparison of results from a reference centre and from a population-based surveillance study of candidemia. Med. Mycol. **49**:525–529.
11. **De Pauw B, et al.** 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. **46**:1813–1821.
12. **Falagas ME, Apostolou KE, Pappas VD.** 2006. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur. J. Clin. Microbiol. Infect. Dis. **25**:419–425.
13. **Garey KW, et al.** 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. **43**:25–31.
14. **Guo LN, et al.** 2011. Three-locus identification, genotyping, and antifungal susceptibilities of medically important *Trichosporon* species from China. J. Clin. Microbiol. **49**:3805–3811.
15. **Hajjeh RA, et al.** 2004. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. **42**:1519–1527.
16. **Hautala T, et al.** 2007. A cluster of *Candida krusei* infections in a haematological unit. BMC Infect. Dis. **7**:97. doi:10.1186/1471-2334-7-97.
17. **Horn DL, et al.** 2009. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin. Infect. Dis. **48**:1695–1703.
18. **Jang SJ, et al.** 2005. PFGE-based epidemiological study of an outbreak of *Candida tropicalis* candiduria: the importance of medical waste as a reservoir of nosocomial infection. Jpn. J. Infect. Dis. **58**:263–267.
19. **Leaw SN, et al.** 2006. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J. Clin. Microbiol. **44**:693–699.
20. **Li C-R, Peng S-H, Li D, Li Y.** 2002. A hospital acquired infection of deep fungus: a clinical investigation and drug resistance. Chin. J. Nosocomiol. **12**:485–487.
21. **Lockhart SR, Messer SA, Pfaller MA, Diekema DJ.** 2008. Geographic distribution and antifungal susceptibility of the newly described species *Candida orthopsilosis* and *Candida metapsilosis* in comparison to the closely related species *Candida parapsilosis*. J. Clin. Microbiol. **46**:2659–2664.
22. **Lockhart SR, Messer SA, Pfaller MA, Diekema DJ.** 2008. *Lodderomyces elongisporus* masquerading as *Candida parapsilosis* as a cause of bloodstream infections. J. Clin. Microbiol. **46**:374–376.
23. **Miceli MH, Diaz JA, Lee SA.** 2011. Emerging opportunistic yeast infections. Lancet Infect. Dis. **11**:142–151.
24. **Mirhendi H, et al.** 2011. Differentiation of *Candida glabrata*, *C. nivariensis* and *C. bracarensis* based on fragment length polymorphism of ITS1 and ITS2 and restriction fragment length polymorphism of ITS and D1/D2 regions in rDNA. Eur. J. Clin. Microbiol. Infect. Dis. **30**:1409–1416.
25. **Pappas PG, et al.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **48**:503–535.
26. **Parkins MD, Sabuda DM, Elsayed S, Laupland KB.** 2007. Adequacy of empirical antifungal therapy and effect on outcome among patients with invasive *Candida* species infections. J. Antimicrob. Chemother. **60**:613–618.
27. **Pfaller MA, et al.** 2011. Clinical breakpoints for voriconazole and *Candida* spp. revisited: review of microbiologic, molecular, pharmacodynamic, and clinical data as they pertain to the development of species-specific interpretive criteria. Diagn. Microbiol. Infect. Dis. **70**:330–343.
28. **Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D.** 2010. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist. Updat. **13**:180–195.
29. **Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN.** 2011. Echinocandin and triazole antifungal susceptibility profiles for *Candida*

spp., *Cryptococcus neoformans*, and *Aspergillus fumigatus*: application of new CLSI clinical breakpoints and epidemiologic cutoff values to characterize resistance in the SENTRY Antimicrobial Surveillance Program (2009). Diagn. Microbiol. Infect. Dis. **69**:45–50.
30. **Pfaller MA, Diekema DJ.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20**:133–163.
31. **Pfaller MA, et al.** 2009. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J. Clin. Microbiol. **47**:117–123.
32. **Pfaller MA, et al.** 2010. 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. **48**:1366–1377.
33. **Pfaller MA, et al.** 2007. Results from the ARTEMIS DISK Global Antifungal Surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of *Candida* species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J. Clin. Microbiol. **45**:1735–1745.
34. **Pfaller MA, et al.** 2006. Correlation of MIC with outcome for *Candida* species tested against voriconazole: analysis and proposal for interpretive breakpoints. J. Clin. Microbiol. **44**:819–826.
35. **Pfaller MA, Diekema DJ, Sheehan DJ.** 2006. Interpretive breakpoints for fluconazole and *Candida* revisited: a blueprint for the future of antifungal susceptibility testing. Clin. Microbiol. Rev. **19**:435–447.
36. **Pfaller MA, et al.** 2005. Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). J. Clin. Microbiol. **43**:2163–2167.
37. **Sabino R, et al.** 2010. Epidemiology of candidemia in oncology patients: a 6-year survey in a Portuguese central hospital. Med. Mycol. **48**:346–354.
38. **Sandven P, et al.** 2006. Candidemia in Norway (1991 to 2003): results from a nationwide study. J. Clin. Microbiol. **44**:1977–1981.
39. **Sofair AN, et al.** 2006. Epidemiology of community-onset candidemia in Connecticut and Maryland. Clin. Infect. Dis. **43**:32–39.
40. **Trofa D, Gacser A, Nosanchuk JD.** 2008. *Candida parapsilosis*, an emerging fungal pathogen. Clin. Microbiol. Rev. **21**:606–625.
41. **Xu X-L, Yang P-H, Sun Y-Q, Fan X.** 2007. Deep fungi infection: flora distribution and drug resistance. Chin. J. Nosocomiol. **17**:349–351.
42. **Zilberberg MD, Shorr AF, Kollef MH.** 2008. Secular trends in candidemia-related hospitalization in the United States, 2000-2005. Infect. Control Hosp. Epidemiol. **29**:978–980.