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Antifungal susceptibilities of *Candida glabrata* species complex, *Candida krusei*, *Candida parapsilosis* species complex and *Candida tropicalis* causing invasive candidiasis in China: 3 year national surveillance

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Objectives: To define the antifungal susceptibility patterns of the most common non-*albicans Candida* spp. in China.

Methods: We evaluated the susceptibilities to nine antifungal drugs of *Candida parapsilosis* species complex, *Candida tropicalis*, *Candida glabrata* species complex and *Candida krusei* isolates from patients with invasive candidiasis at 11 hospitals over 3 years. Isolates were identified by MALDI-TOF MS supplemented by DNA sequencing. MICs were determined by Sensititre YeastOne™ using current clinical breakpoints/epidemiological cut-off values to assign susceptibility (or WT), and by CLSI M44-A2 disc diffusion for fluconazole and voriconazole.

Results: Of 1072 isolates, 392 (36.6%) were *C. parapsilosis* species complex. *C. tropicalis*, *C. glabrata* species complex and *C. krusei* comprised 35.4%, 24.3% and 3.7% of the isolates, respectively. Over 99.3% of the isolates were of WT phenotype to amphotericin B and 5-flucytosine. Susceptibility/WT rates to azoles among *C. parapsilosis* species complex were ≥97.5%. However, 11.6% and 9.5% of *C. tropicalis* isolates were non-susceptible to fluconazole and voriconazole, respectively (7.1% were resistant to both). Approximately 14.3% of *C. glabrata sensu stricto* isolates ($n=258$) were fluconazole resistant, and 11.6% of *C. glabrata sensu stricto* isolates were cross-resistant to fluconazole and voriconazole. All *C. krusei* isolates were susceptible/WT to voriconazole, posaconazole and itraconazole. Overall, 97.7%–100% of isolates were susceptible to caspofungin, micafungin and anidulafungin, but 2.3% of *C. glabrata* were non-susceptible to anidulafungin. There was no azole/echinocandin co-resistance. Disc diffusion and Sensititre YeastOne™ methods showed >95% categorical agreement for fluconazole and voriconazole.

Conclusions: In summary, reduced azole susceptibility was seen among *C. tropicalis*. Resistance to echinocandins was uncommon.

Keywords: China Hospital Invasive Fungal Surveillance Net (CHIF-NET), antifungal susceptibility, non-*albicans Candida* species/species complexes, *C. parapsilosis* species complex, *C. tropicalis*, *C. glabrata* species complex, *C. krusei*

Introduction

Invasive candidiasis (IC) is a major threat to the health of patients in hospitals as well as in the community and its consequent mortality is costly.[1–4] Although *Candida albicans* remains the most common aetiological agent overall, non-*albicans Candida* species are increasingly encountered,[5–7] and in some countries these species account for more episodes of IC, including candidaemia, than *C. albicans*.[8–12] The relative frequency of *Candida* species other than *C. albicans* also varies with region. For example, *Candida glabrata* is the second most common species after *C. albicans* in North America.[13] In Europe, Australia, Latin America and Asia, however, *Candida parapsilosis* or *Candida tropicalis* is relatively more common.[8,9,14] In India, *C. tropicalis* causes even more cases of candidaemia than *C. albicans*.[15,16] In addition, uncommon species such as *Candida guilliermondii* and *Candida rugosa*, and novel species, e.g. *Candida quercitrusa,* are emerging.[17–19]

Given that many non-*albicans Candida* spp. are resistant or less susceptible to antifungal agents,[17–19] there is a continuing need to determine the antifungal susceptibility patterns of these organisms. Further, the data from one region may not be transferable to another. In China, the epidemiology of IC and associated antifungal susceptibility patterns of *Candida* spp. remains incompletely defined.[9,20] To address these deficiencies, a prospective nationwide study, the China Hospital Invasive Fungal Surveillance Net (CHIF-NET), was initiated in 2009 to monitor trends in the epidemiology of yeast infections over a 3 year period and to provide up-to-date susceptibility data on antifungal drugs.[9] The CHIF-NET survey reported the susceptibilities of yeasts collected during the first year of the study ($n=814$ isolates) to fluconazole and voriconazole using the CLSI M44-A2 disc diffusion method.[9,21] However, there were no data on susceptibilities to the echinocandin drugs.

Here, we extend the evaluation of antifungal susceptibilities of *C. parapsilosis* species complex, *C. tropicalis*, *C. glabrata* species complex and *C. krusei* collected during the 3 years of the CHIF-NET study to nine antifungal agents. We employed the Sensititre YeastOne™ YO10 (Sensititre; Thermo Scientific, Cleveland, Ohio, USA) system, which is based on CLSI methodology,[22] and used current species-specific clinical breakpoint (CBPs) or epidemiological cut-off values (ECVs) for *Candida* spp. to assign susceptibility or resistance.[23,24] For all isolates, susceptibility to fluconazole and voriconazole was also studied by CLSI M44-A2 disc diffusion testing[9,21] and categorical agreements between the results obtained using the two methods were compared.

Materials and methods

Study design and collection of isolates

The CHIF-NET study was a prospective, laboratory-based, multicentre study of invasive yeast infections beginning in August 2009 as previously described.[9] During the second and third years of the study (ending in July 2012), the number of surveillance sites expanded from 12 to 22 tertiary hospitals. A total of 11 hospitals (eight major cities) participated in the study for the 3 years (see the Acknowledgements section for study sites and hospitals). To ensure the coherence and consistency of epidemiology and antifungal susceptibility data over the 3 years, only isolates from the above 11 hospitals were included in the present study.

The CHIF-NET study inclusion criteria were as previously described.[9] All isolates were forwarded to the Department of Clinical Laboratory, Peking Union Medical College Hospital for species confirmation and antifungal susceptibility testing. The study was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (S-263).

Species identification

A total of 1072 isolates were studied comprising 392 (36.6%) *C. parapsilosis* species complex (325 *C. parapsilosis sensu stricto*, 43 *C. metapsilosis*, 14 *C. orthopsilosis* and 10 *Lodderomyces elongisporus*), 379 (35.4%) *C. tropicalis*, 261 (24.3%) *C. glabrata* species complex (258 *C. glabrata sensu stricto* and 3 *C. nivariensis*) and 40 (3.7%) *C. krusei* strains (Table 1).

Isolates from Year 1 of the study were identified by DNA sequencing of the fungal internal transcribed spacer (ITS) regions as previously reported,[9] while isolates from Years 2 and 3 were identified by MALDI-TOF MS (Vitek MS, bioMérieux, Marcy-l'Étoile, France) supplemented with ITS sequencing according to the algorithm of Zhang *et al.*[25]

Antifungal susceptibility testing

The *in vitro* susceptibility to nine antifungal drugs—fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, micafungin, anidulafungin, amphotericin B and 5-flucytosine—was determined using Sensititre YeastOne™ YO10 methodology (Thermo Scientific) following the manufacturer's instructions. In addition, susceptibility to fluconazole and voriconazole was determined for all 1072 isolates using the CLSI M44-A2 disc diffusion protocol.[9,21] For each run, the quality control strains were *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.

MIC values determined by the Sensititre YeastOne™ YO10 method (Thermo Scientific) were interpreted according to current CLSI species-specific CBPs (Table S1, available as Supplementary data at *JAC* Online).[23] Where there were no CBPs defined, species-specific ECVs were used to define isolates as WT or non-WT.[24] Cross-resistance was defined as resistance to, or of the non-WT phenotype to, ≥2 antifungals of the same drug class.[26]

For fluconazole and voriconazole, categorical agreements between the CLSI M44-A2 disc diffusion and Sensititre YeastOne™ YO10 (Thermo Scientific) results were calculated, with results obtained by the latter as the reference. Major errors were considered to be present when there was a classification of 'resistant' by the disc diffusion test and 'susceptible' by the Sensititre YeastOne™. Very major errors were a classification of susceptible by the disc diffusion method and resistant by Sensititre YeastOne™. Minor errors occurred when the result of one of the tests was susceptible or resistant, and that of the other test was susceptible-dose-dependent (S-DD) or intermediate.[27]

Statistical analysis

All statistical analyses were performed using IBM SPSS software (version 22.0; IBM SPSS Inc., New York, USA). Categorical variables were compared using the χ^2 or Fisher's exact test, and continuous variables by the Mann–Whitney *U* test. A *P* value of 0.05 was considered significant.

Results

Species distribution of common non-albicans Candida species

Of 1072 *Candida* isolates, members of the *C. parapsilosis* species complex were the most common (392 isolates; 36.6%) followed by *C. tropicalis* (35.4%) and *C. glabrata* species complex (24.3%) (Table 1). *C. krusei* was uncommon (3.7%). Among the *C. parapsilosis* species complex, 17.1% of strains were those other than *C. parapsilosis sensu stricto*, while 98.9% of *C. glabrata* species complex isolates were *C. glabrata sensu stricto*.

Table 1. Susceptibility results of the four common non-*albicans Candida* species/species complexes studied to nine antifungal agents[a]

Species (no. tested)	FLC	VRC	ITC	POS	CAS	MCF	ANF	AMB	5FC
C. parapsilosis species complex (*n*=392)									
range	≤0.12–128	≤0.008–0.5	≤0.015–0.25	≤0.008–0.25	0.03–2	0.015–2	0.015–2	≤0.12–2	≤0.06–1
GM MIC (mg/L)	0.53	0.006	0.03	0.02	0.23	0.44	0.43	0.47	0.05
S or WT (%)	97.7	99.2	100	100	100	100	100	100	99.2
S-DD or I (%)	0.8	0.8	—	—	0	0	0	0	—
R or non-WT (%)	1.5	0	0	0	0	0	0	0	0.8
C. parapsilosis sensu stricto (*n*=325)									
range	≤0.12–16	≤0.008–0.5	≤0.015–0.25	≤0.008–0.25	0.06–2	0.12–2	0.12–2	≤0.12–2	≤0.06–1
GM MIC (mg/L)	0.54	0.006	0.03	0.02	0.29	0.56	0.56	0.48	0.06
S or WT (%)	97.5	99.4	100	100	100	100	100	100	97.7
S-DD or I (%)	1.0	0.6	—	—	0	0	0	0	—
R or non-WT (%)	1.5	0	0	0	0	0	0	0	2.3
C. metapsilosis (*n*=43)									
range	≤0.12–128	≤0.008–0.5	≤0.015–0.25	≤0.008–0.12	0.03–0.5	0.06–0.5	0.03–1	0.25–1	≤0.06–1
GM MIC (mg/L)	0.89	0.008	0.02	0.01	0.08	0.18	0.13	0.49	0.04
S or WT (%)	97.7	97.7	100	100	100	100	100	100	100
S-DD or I (%)	0	2.3	—	—	0	0	0	0	—
R or non-WT (%)	2.3	0	0	0	0	0	0	0	0
C. orthopsilosis (*n*=14)									
range	≤0.12–1	≤0.008–0.008	0.015–0.06	0.008–0.03	0.03–0.5	0.12–0.5	0.12–1	0.25–0.5	≤0.06–0.06
GM MIC (mg/L)	0.27	0.005	0.03	0.02	0.17	0.26	0.30	0.43	0.04
S or WT (%)	100	100	100	100	100	100	100	100	100
S-DD or I (%)	0	0	—	—	0	0	0	0	—
R or non-WT (%)	0	0	0	0	0	0	0	0	0
L. elongisporus (*n*=10)									
range	≤0.12–1	≤0.008–0.008	≤0.015–0.12	0.008–0.12	0.03–0.06	0.015	0.015	0.12–0.25	≤0.06–0.5
GM MIC (mg/L)	0.13	0.004	0.02	0.02	0.03	0.02	0.02	0.22	0.08
S or WT (%)	100	100	100	100	100	100	100	100	100
S-DD or I (%)	0	0	—	—	0	0	0	0	—
R or non-WT (%)	0	0	0	0	0	0	0	0	0
C. tropicalis (*n*=379)									
range	0.25–>256	≤0.008–>8	0.015–>16	0.008–>8	0.015–0.25	≤0.008–0.06	≤0.015–0.5	0.25–1	≤0.06–>64
GM MIC (mg/L)	1.90	0.08	0.18	0.13	0.04	0.03	0.05	0.68	0.04
S or WT (%)	88.4	90.5	98.7	68.3	100	100	99.7	100	98.9
S-DD or I (%)	3.4	4.2	—	—	0	0	0.3	0	—
R or non-WT (%)	8.2	5.3	1.3	31.7	0	0	0	0	1.1

C. glabrata species complex (*n*=261)									
range	0.5–>256	≤0.008–4	0.06–>16	0.03–>8	≤0.008–0.25	≤0.008–0.06	≤0.015–0.25	≤0.12–1	≤0.06–0.5
GM MIC (mg/L)	13.86	0.22	0.73	1.03	0.07	0.01	0.03	0.58	0.03
S or WT (%)	—	88.1	90.8	90	99.2	100	97.7	100	100
S-DD or I (%)	85.8	—	—	—	0.8	0	1.9	0	—
R or non-WT (%)	14.2	11.9	9.2	10	0	0	0.4	0	0
C. glabrata sensu stricto (*n*=258)									
range	0.5–>256	≤0.008–4	0.06–>16	0.03–>8	≤0.008–0.25	≤0.008–0.06	≤0.015–0.25	≤0.12–1	≤0.06–0.5
GM MIC (mg/L)	14.18	0.22	0.74	1.05	0.07	0.01	0.03	0.58	0.03
S or WT (%)	—	88	90.7	89.9	99.2	100	97.7	100	100
S-DD or I (%)	85.7	—	—	—	0.8	0	1.9	0	—
R or non-WT (%)	14.3	12	9.3	10.1	0	0	0.4	0	0
C. nivariensis (*n*=3)									
range	2	0.03	0.25	0.25	0.06	0.015	0.015	0.5–1	0.12
GM MIC (mg/L)	2	0.03	0.25	0.25	0.06	0.02	0.02	0.79	0.12
S or WT (%)	—	100	100	100	100	100	100	100	100
S-DD or I (%)	100	—	—	—	0	0	0	0	—
R or non-WT (%)	0	0	0	0	0	0	0	0	0
C. krusei (*n*=40)									
range	16–128	0.12–0.5	0.06–0.5	0.03–0.5	0.12–0.5	0.06–0.12	0.015–0.12	0.5–2	16
GM MIC (mg/L)	36.76	0.18	0.19	0.20	0.23	0.11	0.04	0.76	16
S or WT (%)	—	100	100	100	95	100	100	100	100
S-DD or I (%)	—	0	—	—	5	0	0	0	0
R or non-WT (%)	100	0	0	0	0	0	0	0	0

FLC, fluconazole; VRC, voriconazole; ITC, itraconazole; POS, posaconazole; CAS, caspofungin; MCF, micafungin; ANF, anidulafungin; AMB, amphotericin B; 5FC, 5-flucytosine; S, susceptible; I, intermediate; S-DD, susceptible-dose dependent; R, resistant.

[a]Susceptibility categories were determined by species-specific CBPs as per CLSI M27-S4 (S, S-DD or I and R) or ECVs (categories WT and non-WT) when CBPs were not available.

Table 2. Isolates that were cross-resistant to more than two azole drugs and their resistance/non-WT profiles

Resistance/non-WT profile	No. of isolates (% of the species)
C. tropicalis	
FLC, VRC	1 (0.3)
FLC, POS	7 (1.8)
FLC, VRC, POS	14 (3.7)
FLC, VRC, ITC, POS	5 (1.3)
C. glabrata sensu stricto	
FLC, VRC	4 (1.5)
FLC, VRC, POS	2 (0.8)
FLC, VRC, ITC, POS	24 (9.3)

FLC, fluconazole; VRC, voriconazole; POS, posaconazole; ITC, itraconazole.

In vitro susceptibility to azoles

In general, *C. parapsilosis* species complex was susceptible to all four azoles tested. Overall, susceptibility to fluconazole and voriconazole was observed for 97.7% (383/392) and 99.2% (389/392) for the species complex, respectively. All isolates were of the WT phenotype to itraconazole and posaconazole (Table 1). The rates of susceptibility to fluconazole and voriconazole were 97.5% (317/325 isolates) and 99.4% (323/325), respectively, for *C. parapsilosis sensu stricto*, while 97.7% (42/43) of the strains of *C. metapsilosis* and all of the *C. orthopsilosis* (14/14) and *L. elongisporus* (10/10) isolates were susceptible to these azoles.

C. tropicalis isolates were less susceptible to the azoles [11.6% of isolates (44/379) and 9.5% (36/379) tested non-susceptible to fluconazole and voriconazole, respectively] (Table 1). In addition, 31.7% (120/379) of the *C. tropicalis* isolates were non-WT to posaconazole, and 1.3% (5/379) of the isolates were non-WT to itraconazole. Geometric mean (GM) MIC values for the azoles were 2- to 4-fold higher against *C. tropicalis* than against *C. parapsilosis* species complex ($P<0.01$) (Table 1). Twenty-seven (7.1%) *C. tropicalis* isolates were cross-resistant to azoles, including five (1.3%) that were resistant to, or were of the non-WT phenotype to, all four azoles (Table 2).

Approximately 85.7% (221/258) *C. glabrata sensu stricto* isolates were S-DD (MICs $\leq$ 32 mg/L) to fluconazole and 14.3% (37/258) were resistant (MICs $\geq$64 mg/L). The non-WT rates of *C. glabrata sensu stricto* for voriconazole, itraconazole and posaconazole ranged between 9.3% and 12.0%. All three *C. nivariensis* isolates were S-DD to fluconazole and of the WT phenotype to voriconazole, itraconazole or posaconazole. The GM MIC values of voriconazole, itraconazole and posaconazole against *C. glabrata* species complex isolates were 1- to 3-fold higher than those against *C. tropicalis* ($P<0.01$) and 4- to 6-fold higher than those against *C. parapsilosis* species complex ($P<0.01$) (Table 1). Thirty (11.6%) *C. glabrata sensu stricto* isolates were cross-resistant to fluconazole and voriconazole, and 9.3% were resistant/non-WT to all four azoles (Table 2).

Although resistant to fluconazole, all *C. krusei* were susceptible to voriconazole and were of the WT phenotype to itraconazole and posaconazole (Table 1).

In vitro susceptibilities to the echinocandins

The susceptibility rates to all three echinocandins tested were similar for all the non-*albicans Candida* species studied. Caspofungin, micafungin and anidulafungin exhibited >99% susceptibility rates against all isolates with the exception of caspofungin against *C. krusei* [95% (38/40) of isolates were susceptible] (Table 1) and anidulafungin against *C. glabrata sensu stricto* [97.7% (252/258) susceptible]. Echinocandin GM MIC values against *C. parapsilosis* species complex were 2- to 6-fold higher than those against the other three non-*albicans Candida* species ($P<0.01$) except for caspofungin against *C. krusei* ($P=0.13$) (Table 1). The GM MIC values for *L. elongisporus* were 3- to 5-fold lower than those for other species within the *C. parapsilosis* species complex ($P<0.01$) (Table 1).

In vitro susceptibilities to amphotericin B and 5-flucytosine

All isolates studied showed a WT phenotype to amphotericin B. The MIC_{50} and MIC_{90} values of amphotericin B for all four species/species complexes were either 0.5 or 1 mg/L (Table 1) and all isolates were inhibited at drug concentrations of $\leq$1 mg/L except for one isolate each of *C. parapsilosis sensu stricto* and *C. krusei* (MIC 2 mg/L).

Only 0.8% (3/392) of *C. parapsilosis* species complex and 1.1% (4/379) of *C. tropicalis* isolates were non-WT to 5-flucytosine. The GM MIC values of 5-flucytosine against *C. parapsilosis* species complex, *C. tropicalis* and *C. glabrata* species complex ranged from 0.04 to 0.12 mg/L. The MICs of all the *C. krusei* isolates were 16 mg/L (Table 1).

Three year trends of antifungal susceptibilities

The susceptibility rates of *C. parapsilosis* species complex isolates remained stable (97.4%–100%) for all the agents tested (Figure 1). For *C. tropicalis*, the susceptibility rate to fluconazole decreased by over 4% to 85.1% in the third year of the study but this was not significant ($P=0.29$); similarly, the proportion of posaconazole WT strains dropped from 73.8% to 64.2% ($P=0.11$) (Figure 1). There were no significant trends in susceptibility to voriconazole, itraconazole and posaconazole among *C. glabrata* species complex strains over the 3 years (Figure 1).

Specimen source and antifungal susceptibilities

Data on the distribution of isolates by specimen source, and the proportions of isolate that exhibited fluconazole resistance, are summarized in Table 3. Isolates recovered from blood cultures comprised 25%–58.2% of the strains (depending on the species) and those from ascitic fluid accounted for 15.1%–26.4% (Table 3). All six fluconazole-resistant *C. parapsilosis* species complex isolates came from blood culture. In comparison, *C. tropicalis* isolates from tissue, central venous catheter (CVC), bronchoalveolar lavage (BAL) fluid and pus had rates of resistance to fluconazole that were higher (22.2%, 16.7%, 14.3% and 11.8%, respectively) than the mean rate (8.2%); *C. glabrata* species complex isolates from CVC and BAL fluid had higher fluconazole

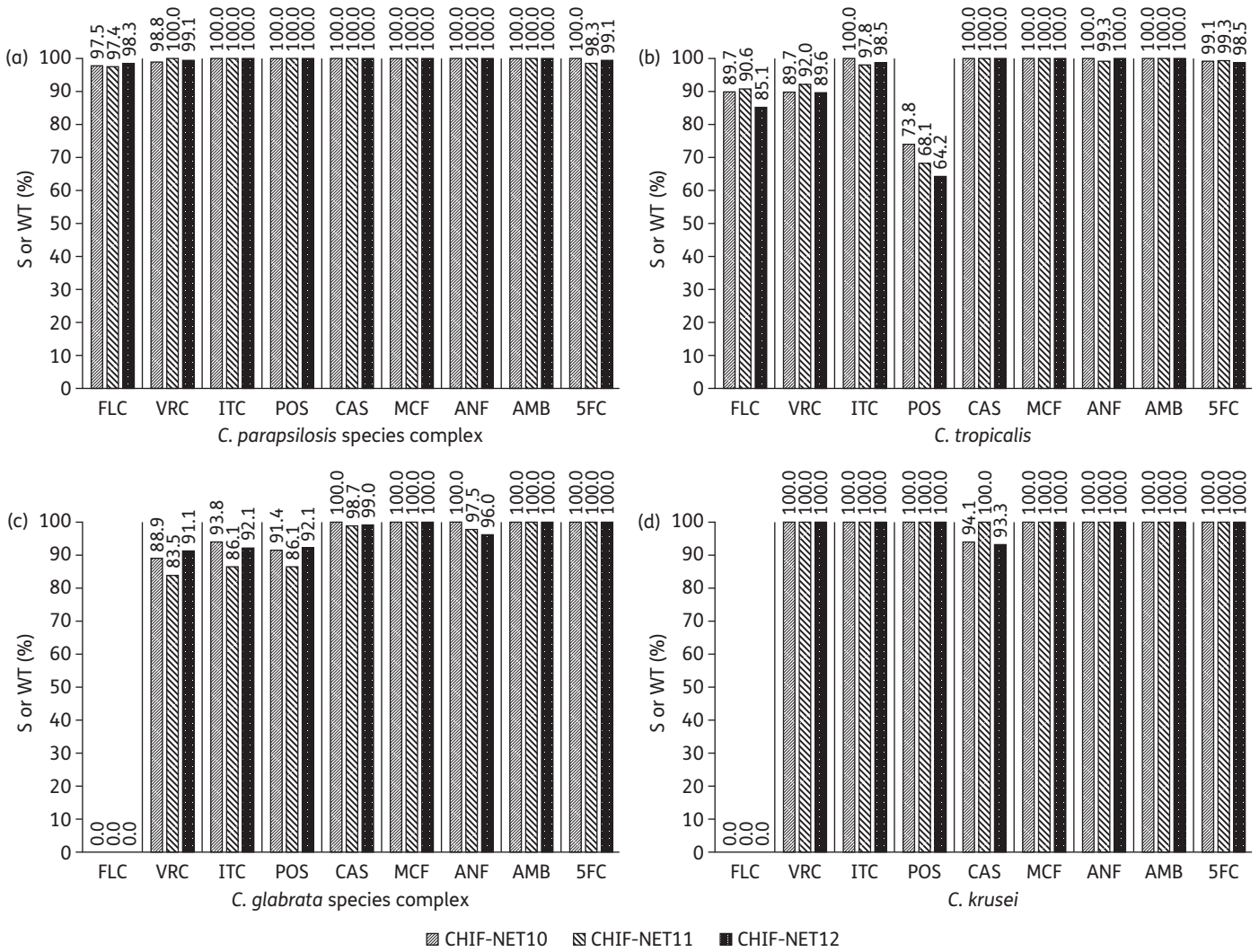

Figure 1. Trends of susceptible (S) or WT rates of *C. parapsilosis* species complex, *C. tropicalis*, *C. glabrata* species complex and *C. krusei* through the CHIF-NET study (2010–12).

resistant rates (both 20%) than average (14.2%) (Table 3; *P* = not significant).

Categorical agreement between Sensititre YeastOne™ YO10 and disc diffusion methods

Overall, the Sensititre and disc diffusion methods exhibited good concordance in susceptibility testing results for fluconazole and voriconazole (categorical agreement >95%). Very major error and major errors occurred for 1.1% and 0.2% of isolates, respectively, in testing fluconazole susceptibilities, and 0.9% and 0.3% of isolates, respectively, in testing voriconazole susceptibilities. In addition, 3.3% and 3.2% minor errors occurred in testing fluconazole and voriconazole, respectively (Table 4). The occurrence of very major errors was higher in *C. tropicalis* against fluconazole and *C. glabrata* species complex against voriconazole, although it did not exceed 2.7%. However, a major error rate of 7.5% (3/40 isolates) was found in testing voriconazole activity against *C. krusei* (Table 4).

Discussion

Reliable, contemporary epidemiological data on species distribution and susceptibility are essential for informing early, targeted antifungal therapy in patients with IC and candidaemia, particularly for clinicians working in hospitals where antifungal susceptibility tests are not routinely carried out. Given that such epidemiological data for invasive yeast infections in China have been largely restricted either to studies in single centres or to a limited number of yeast species,[28] this large multicentre study provides clinically useful data on the susceptibilities to nine antifungal agents to treat common non-*albicans Candida* species across China.

A noteworthy finding of the present study is that the most common non-*albicans Candida* species encountered in Chinese hospitals was *C. parapsilosis* species complex (36.6%), followed closely by *C. tropicalis* (35.4%). Conversely, *C. glabrata* accounted for only 24.3% of isolates and *C. krusei* was uncommon. The results were in contrast to findings in the USA, where *C. glabrata*

Table 3. Comparison of fluconazole susceptibility results for *C. parapsilosis* species complex, *C. tropicalis*, *C. glabrata* species complex and *C. krusei* by different specimen sources

	C. parapsilosis species complex		*C. tropicalis*		*C. glabrata* species complex		*C. krusei*	
Characteristic	*n* (%)	FLC R%	*n* (%)	FLC R%	*n* (%)	FLC R%	*n* (%)	FLC R%
Blood	228 (58.2)	2.6	148 (39.0)	8.1	139 (53.3)	15.8	10 (25.0)	100.0
Ascitic fluid	59 (15.1)	0.0	100 (26.4)	5.0	61 (23.4)	14.8	7 (17.5)	100.0
CVC	42 (10.7)	0.0	12 (3.2)	16.7	10 (3.8)	20.0	1 (2.5)	100.0
Pus	26 (6.6)	0.0	17 (4.5)	11.8	13 (5.0)	7.7	3 (7.5)	100.0
BAL	9 (2.3)	0.0	28 (7.4)	14.3	10 (3.8)	20.0	7 (17.5)	100.0
Bile	8 (2.0)	0.0	28 (7.4)	7.1	8 (3.1)	12.5	3 (7.5)	100.0
Pleural fluid	1 (0.2)	0.0	23 (6.0)	8.7	11 (4.2)	0.0	7 (17.5)	100.0
CSF	5 (1.3)	0.0	13 (3.4)	0.0	3 (1.1)	0.0	0 (0.0)	—
Tissue	3 (0.8)	0.0	9 (2.4)	22.2	5 (1.9)	0.0	2 (5.0)	100.0
Peritoneal dialysate fluid	10 (2.6)	0.0	1 (0.3)	0.0	0 (0.0)	—	0 (0.0)	—
Bone marrow	0 (0.0)	—	0 (0.0)	—	1 (0.4)	0.0	0 (0.0)	—
Hydrarthrosis	1 (0.2)	0.0	0 (0.0)	—	0 (0.0)	—	0 (0.0)	—
Total	392 (100.0)	1.5	379 (100.0)	8.2	261 (100.0)	14.2	40 (100.0)	100.0

FLC, fluconazole; R, resistant.

Table 4. Fluconazole and voriconazole susceptibility category agreements between broth microdilution (as standard) and disc diffusion methods

			Errors (%)		
Species/species complex	Total no. of isolates	Agree (%)	very major	major	minor
Fluconazole	1072	95.4	1.1	0.2	3.3
C. parapsilosis species complex	392	97.9	0.8	0.5	0.8
C. tropicalis	379	93.4	2.4	0.0	4.2
C. glabrata species complex	261	93.9	0.0	0.0	6.1
C. krusei	40	100.0	0.0	0.0	0.0
Voriconazole	1072	95.6	0.9	0.3	3.2
C. parapsilosis species complex	392	99.0	0.0	0.0	1.0
C. tropicalis	379	95.2	0.8	0.0	4.0
C. glabrata species complex	261	92.3	2.7	0.0	5.0
C. krusei	40	87.5	0.0	7.5	5.0

is the most common non-*albicans Candida* species (>20% in candidaemia),[13,29] as well as in India, where *C. tropicalis* is most common (~40% of all cases of candidaemia).[15,16] In addition, the prevalence of species other than *C. parapsilosis sensu stricto* among the *C. parapsilosis* species complex (e.g. *C. metapsilosis*, *C. orthopsilosis* and *L. elongisporus*) in this study (17.1%) was higher than that observed in the global ARTEMIS study and in other European and Latin American studies (prevalence <10%).[5,30–33] These findings emphasize the need to perform locally relevant epidemiological studies.

In vitro susceptibility results showed that members of the *C. parapsilosis* species complex were predominantly (≥97.5%) susceptible (or were WT) to all nine antifungals tested, with only a small proportion (≤1.5%) of *C. parapsilosis sensu stricto* isolates being resistant to fluconazole and voriconazole. The results are in general similar to those obtained from a global surveillance of the antifungal susceptibility of *Candida* species.[7,29] Of note, although fluconazole-resistant *C. parapsilosis* species complex isolates were rare, all six were recovered from blood culture samples. Despite relatively higher echinocandin MICs, *C. parapsilosis* infections may be treated with the echinocandins with good outcomes. Our data indicate that these observations can be extended to IC caused by *L. elongisporus* as GM MICs of echinocandins for *L. elongisporus* were 3- to 5-fold lower than for other members of this species complex ($P<0.01$).

For *C. glabrata* species complex, we observed that 9.3% of *C. glabrata sensu stricto* isolates were resistant/non-WT to all four azoles tested. We also found that *C. glabrata* species complex isolates were largely (99.2%, 100% and 97.7%, respectively) susceptible to caspofungin, micafungin and anidulafungin. These results are comparable to those from the ARTEMIS and SENTRY studies.[7,29] Importantly, we did not observe co-resistance to azoles and the echinocandins among *C. glabrata.* This contrasts with recent US studies where >11% of fluconazole-resistant *C. glabrata* isolates were co-resistant to one or more echinocandins.[34,35] Since we encountered only three *C. nivariensis* isolates, we were unable to determine varying drug susceptibilities between individual members of the *C. glabrata* species complex. *C. nivariensis* has previously been reported to be more resistant to azoles and flucytosine than *C. glabrata sensu stricto*.[36]

Interestingly, 7.1% of *C. tropicalis* isolates were resistant to two or more azole agents. By applying the new CLSI CBPs,[23] the overall fluconazole and voriconazole susceptibilities against *C. tropicalis* isolates dropped by 4.7% and 5.3%, respectively (Table S2). Although the azole non-susceptible isolates came from all 11

hospitals, clustered cases were noted in Hospital H1 in North-East China (see the Acknowledgements section; data not shown). The increased resistance of *C. tropicalis* to fluconazole and outbreaks of infection caused by fluconazole-resistant *C. tropicalis* have previously been recognized in Taiwan.[37,38] Molecular typing is in progress to determine the genetic relatedness of the isolates in the present study.

Importantly, all the species studied here were susceptible to amphotericin B and 5-flucytosine, and although these two agents are not first-line antifungals in the treatment of IC and candidaemia in most clinical contexts,[39] they may have a role where the azoles or echinocandins cannot be used, or in salvage therapy.

As previously noted, the results obtained by Sensititre YeastOne™ and CLSI M44 disc diffusion were comparable.[40–43] The overall category agreements between the two methods was >95% for both fluconazole and voriconazole, while very major and major errors only occurred in <1.5% of cases. We noted that some previous studies had set up ECVs against common non-*albicans Candida* species based on Sensititre YeastOne™ methods, and the ECVs were within one dilution of those determined by the CLSI broth microdilution methods.[44,45] Because ECVs based on Sensititre YeastOne™ methods had not been widely validated, we applied CLSI CBPs where applicable.[46]

In conclusion, the study has provided useful data on the antifungal susceptibility of common non-*albicans Candida* species isolates from patients with invasive infections in China. *C. parapsilosis* species complex, the most common non-*albicans Candida* species, was susceptible to the azoles and echinocandins. In contrast, reduced susceptibility was observed among, e.g. *C. tropicalis* for the azoles. Studies to correlate *in vitro* resistance with clinical outcomes should be a priority.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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