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ORIGINAL RESEARCH

Fungal Spectrum and Susceptibility Against Nine Antifungal Agents in 525 Deep Fungal Infected Cases

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Background: Deep fungal infection has become an important cause of infection and death in hospitalized patients, and this has worsened with increasing antifungal drug resistance.

Objective: A 3-year retrospective study was conducted to investigate the clinical characteristics, pathogen spectrum, and drug resistance of deep fungal infection in a regional hospital of Guangzhou, China.

Methods: Non-duplicate fungi isolates recovered from blood and other sterile body fluids of in-patients of the clinical department were identified using biochemical tests of pure culture with the API20C AUX and CHROMagar medium. Antifungal susceptibilities were determined by Sensititre YeastOne® panel trays.

Results: In this study, 525 patients (283 female, 242 male) with deep fungal infection were included, half of them were elderly patients (≥60 years) (54.67%, n=286). A total of 605 non-repetitive fungi were finally isolated from sterile samples, of which urine specimens accounted for 66.12% (n=400). Surgery, ICU, and internal medicine were the top three departments that fungi were frequently detected. The mainly isolated fungal species were *Candida albicans* (43.97%, n=266), *Candida glabrata* (20.00%, n=121), and *Candida tropicalis* (17.02%, n=103), which contributed to over 80% of fungal infection. The susceptibility of the *Candida* spp. to echinocandins, 5-fluorocytosine, and amphotericin B remained above 95%, while *C. glabrata* and *C. tropicalis* to itraconazole were about 95%, and the dose-dependent susceptibility of *C. glabrata* to fluconazole was more than 90%. The echinocandins had no antifungal activity against *Trichosporon asahi* in vitro (MIC_{90}>8 μg/mL), but azole drugs were good, especially voriconazole and itraconazole (MIC_{90} = 0.25 μg/mL).

Conclusion: The main causative agents of fungal infection were still the genus of *Candida*. Echinocandins were the first choice for clinical therapy of *Candida* infection, followed with 5-fluorocytosine and amphotericin B. Azole antifungal agents should be used with caution in *Candida glabrata* and *Candida tropicalis* infections.

Keywords: fungal infection, species distribution, antifungal drugs, drug susceptibility, drug resistance

Introduction

Deep fungal infection has become a serious burden of mortality and mobility in clinic with increasing broad-spectrum antifungal drugs (antibiotics, corticosteroids), immunocompromised individuals, and interventional treatment operations.[1,2] The diagnosis of invasive fungal infection is based on recovery of fungi by culture of a sample obtained by a sterile procedure from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process.[3] The estimated 1.4 million deaths of infectious disease were caused by invasive fungal infection worldwide per year,[4,5] including *Candida*, *Aspergillus*, *Pneumocystis*, and *Cryptococcus*. Among them,

Candida species were the most frequent fungal pathogens in clinic, and were ranked fourth among the most common agents of bloodstream infections,[6] followed by other common bacterial pathogens. *Aspergillus* species are the most common microbial infections in hematopoietic stem cell transplant recipients.[7] The mortality rate ranged from 30~50% in invasive aspergillosis patients and was ~50% in candidemia.[4] Clinical therapy of mycoses lies on the current antifungal interventions, but are often limited due to the emerging resistant clinical isolates.[8,9] Poorantifungal activity and universal side effects of antifungal drugs affect the efficacy on clinical therapy of mycoses, thus concerns are growing regarding the increasing antifungal resistance in clinic.

Polyenes, pyrimidine analogs, echinocandins, and triazoles were mainly used to treat invasive fungal infections.[10] Triazoles were the most used antifungals. Fluconazole is the major triazole in clinical settings due to its high oral availability and tolerability by patients. In this case, the frequent use of azoles in clinical treatment increased the fungal resistance, making the incidence of emerging antifungal resistance in clinical isolates more and more serious. The trend of infection spectrum may also shift accordingly, eg, the agents of candidemia were changed from *Candida albicans* to non-*C. albicans* in clinic.[11] These phenomena also occurred in other invasive aspergillosis patients, as well as superficial dermatophytic infections. Therefore, to track the fungal spectrum and the susceptibility against frontline antifungal agents will be the key to understanding the trends of shifts and guide for clinical antifungal therapy.

Clinical sentinel hospital-based surveillance was normally employed to monitor regional trends of fungal infection, and could obtain the antifungal susceptibility data as well. In China, the causative agents of both deep fungal infection and superficial fungal infections vary among geographical areas. The archived survey reported the shifts tendency.[12–15] However, in Southern China where a moist and warm environment is sustained for almost 8 months (March to October), fungal infection occurred more frequently than the other regions of China. Indeed, regional mycoses *Talaromyces marneffei*[16] and chromoblastomycosis[17] were rarely encountered in the north part of China. Therefore, there are great needs to understand the fungal spectrum and the susceptibility of invasive fungal strains to antifungal drugs. In this study, we addressed the deep fungal infection and drug susceptibility against nine antifungal agents in 605 mycoses through a retrospective study in a sentinel hospital in Guangzhou, China.

Materials and Methods
Clinical Data of Cases

These retrospective data were obtained from clinical records in the mycology laboratory of Sun Yat-sen Memorial Hospital, a general hospital of Sun Yat-sen University in Guangdong, China, from September 2018 to February 2021. Variety specimens were from in-patients of the clinical department in our hospital. Repetitive strains isolated from the same patient were eliminated. This study was approved by the ethics review board of Sun Yat-sen Memorial Hospital, Sun Yat-sen University (SYSKY-2022-496-01) and was conducted in compliance with the Declaration of Helsinki. Informed consent was obtained from all participants. For patients under 18 years of age, informed consent was provided by a parent or legal guardian.

Sampling, Fungi Isolation, and Identification

All isolates were from sterile clinical samples submitted by inpatients in our hospital, including urine, drainage fluid, blood, catheter, bile, hepatic tissue, cerebrospinal fluid, and articular fluid. Urine, cerebrospinal fluid, dialysate, and drainage samples were centrifuged first at 5,000 rpm for 10 min. The precipitated substances were suspended with 0.5 mL saline and transferred onto Sabouraud Dextrose Agar (chloramphenicol included) (BD, MD, USA) with or without cycloheximide, and CHROMagar medium (CHROMagar Technology, Paris, France). Cultures were incubated at 25°C for 2 weeks and examined twice a week. Blood samples were pre-cultured directly with commercial blood culture medium (Bio-engineering, Sichuan, China) at 37°C for 1 week, and transferred onto the same culture medium and cultured under the same conditions. The same fungi isolated from the same type of samples of the same patient during one hospitalization were excluded.

Characterization of fungal pathogens can be done based on morphological, biochemical, and physiological features for most clinical laboratories.[18–20] The identification of suspected fungi isolates was based on its macroscopic appearance

and the color of the colonies, pigmentation of the medium, and the microscopic morphology, which were mainly used for *Aspergillus* and *Cryptococcus* species. Biochemical tests of pure culture using the API20C AUX (bioMerieux Vitek, Hazelwood, MO) and CHROMagar medium (CHROMagar Technology, Paris, France) were used to identify *Candida* species.

Some of the isolates have also been verified by molecular sequencing using primers targeting the internal transcribed spacer (ITS) regions, ITS4 and ITS5. The result of molecular identification is shown in Supplementary Table 1.

Antifungal Susceptibility Testing

Antifungal susceptibility of nine antifungal drugs was determined by using the Sensititre YeastOne panels trays, which contained serial two-fold dilutions of anidulafungin (0.015–8 μg/mL), micafungin (0.008–8 μg/mL), caspofungin (0.008–8 μg/mL), 5-flucytosine (0.06–64 μg/mL), posaconazole (0.008–8 μg/mL), voriconazole (0.008–8 μg/mL), itraconazole (0.015–16 μg/mL), fluconazole (0.12–256 μg/mL), and amphotericin B (0.12–8 μg/mL) (Thermo Fisher Company, Shanghai, China). A working conidium suspension of approximately 1×10^4 cells/mL was prepared in YeastOne broth. Each well of the dried YeastOne panels was rehydrated with 100 μL of the working conidium suspension delivered by a multichannel pipetting device, and then incubated at 35°C for 5 days in a non-CO_2 incubator. Minimum inhibitory concentration (MIC) values for yeast isolates were determined by the Sensititre YeastOne method and interpreted by species-specific clinical breakpoints (CBPs), as recommended by the CLSI M60[21] method. The colorimetric MIC endpoints were determined by visual reading. Fungal growth was evident as a color change from blue (no growth) to red (growth). Colorimetric MIC results for all the testing drugs were defined as the lowest concentration of antifungal agent that prevented the development of a red color from firstly blue or purple. In the light of CBPs, species can be classified as sensitive (S), intermediate (I), or dose-dependent sensitive (SDD), and drug resistant (R). With regard to species for which no CBPs were available from the CLSI we used epidemiological cutoff values (ECVs) to differentiate wild-type (WT) from non-WT (NWT) isolates according to CLSI M59[22] method. The quality control strains *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were operated in parallel with the test strains.

Statistical Analysis

All statistical analyses were performed using SPSS software version 24.0 (SPSS Inc., Chicago, IL). The percentage, median of demographical data, distribution of bacteria, and antifungal susceptibility data were calculated, and the counting data were expressed in cases or percentages.

Results

Demographic Information of Cases

From September 2018 to February 2021, a total of 605 sterile clinical samples from 525 in-hospital deep fungal infected cases were collected in Sun Yat-sen Memorial Hospital, including 242 male (46.10%) and 283 female patients (53.90%). Among them, age ranged from 6 to 98 years old, and the median age was 61 years old. In particular, 287 cases were elderly (over 60 years old), accounting for 54.67% of total cases.

Sampling and Fungal Isolation

A total of 3,956 samples of 1,808 deep fungal infected inpatients were sent for examination during the 3 years. There were 605 non-repetitive fungi finally isolated from sterile samples. Of the various types of specimens, over 50% of fungal isolates were recovered from urine (400, 66.12%), followed by drainage fluid (131, 21.65%), blood (40, 6.61%), and catheter (16, 2.64%). In particular, the urine sample is from patients who have not had an indwelling urinary catheter within 7 days before the first positive culture and patients have had at least two positive urine cultures.[23] Only two strains were isolated from cerebrospinal fluid and one strain from articular fluid (Figure 1). Regarding the different clinical departments, 605 specimens of 525 cases came from 10 clinical departments, of which the most frequently isolated fungi was from surgery with a total of 236 strains from 226 cases (39.01%), followed by Intensive Care Unit (ICU) with 203 strains from 155 cases (33.55%) and internal medicine with 103 strains from 97 cases (17.02%) (Figure 2).
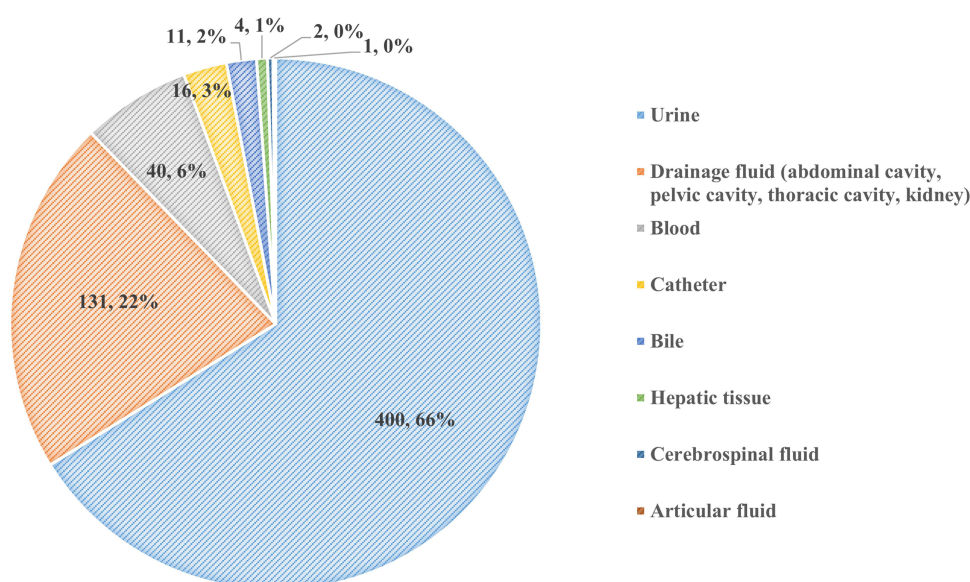
Figure 1 Distribution of isolated strains in sterile samples.
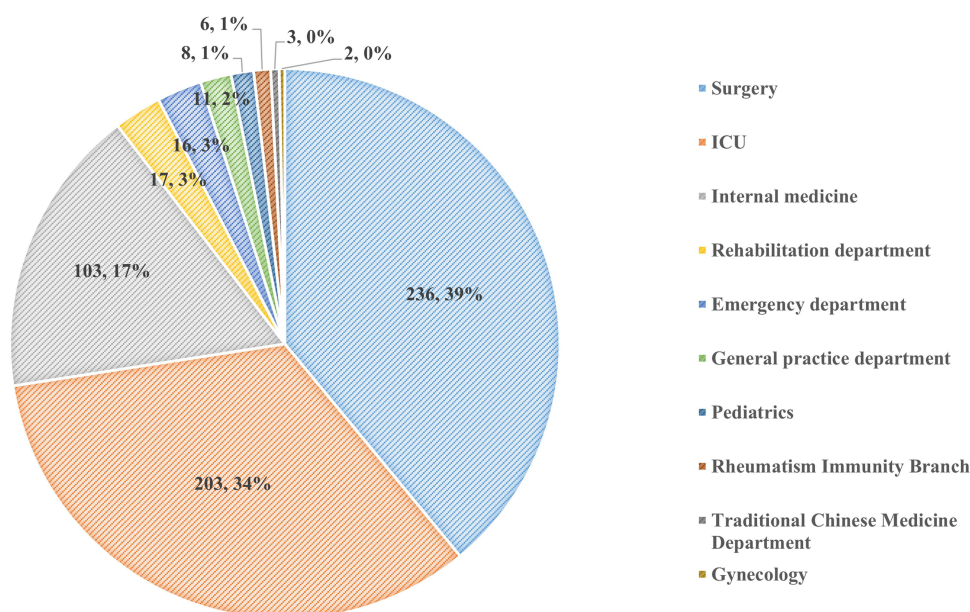
Figure 2 Distribution of isolated strains by clinical department.

Fungal Spectrum Tendency in Variety Clinical Samples and Departments

Among 605 clinical isolates, *Candida* was the predominant genus (98.18%, n=594) in a variety of clinical samples, followed by *Trichosporon asahi* (1.65%, n=10), and unique *Cryptococcus neoformans* isolate. Within the genus of *Candida*, *C. albicans*, *C. glabrata*, and *C. tropicalis* accounted for 43.97%, 20.00%, and 17.02%, respectively. These three *Candida* species were ranked on the top three causative agents in 605 deep fungal infection cases (Table 1).

The fungi isolated from urine were mainly *C. albicans* (41.50%, n=166), followed by *C. glabrata* (24.25%, n=97) and *C. tropicalis* (18.25%, n=73). The distribution of fungi species isolated from drainage fluid (including from the abdominal cavity, pelvic cavity, thoracic cavity, or kidney) was *C. albicans* (49.62%, n=65), *C. tropicalis* (17.56%, n=23), and *C. glabrata* (11.45%, n=15). Moreover, *C. albicans* was also the major agent of blood samples (n=18, 45%), followed by *C. parapsilosis* (25%, n=10), *C. tropicalis* (12.5%, n=5), and *C. glabrata* (12.5%, n=5) (Table 2).

Table 1 Fungal Species Distribution of Fungal Isolates

Fungal Species	Number	Percentage (%)
Candida albicans	266	43.97
Candida glabrata	121	20.00
Candida tropicalis	103	17.02
Candida parapsilosis	25	4.13
Candida krusei	3	0.50
Candida lusitaniae	3	0.50
Candida guilliermondii	1	0.17
Candida albicans and *Candida glabrata**	12	1.98
Candida albicans and *Candida tropicalis**	3	0.50
Candida parapsilosis and *Candida tropicalis**	2	0.33
Candida glabrata and *Candida tropicalis**	2	0.33
Candida parapsilosis and *Candida albicans**	1	0.17
Candidaandida growth (unknown)	52	8.60
Trichosporon asahi	10	1.65
Cryptococcus neoformans	1	0.17
Total	605	100.00

Note: *These isolates are from the same sample.

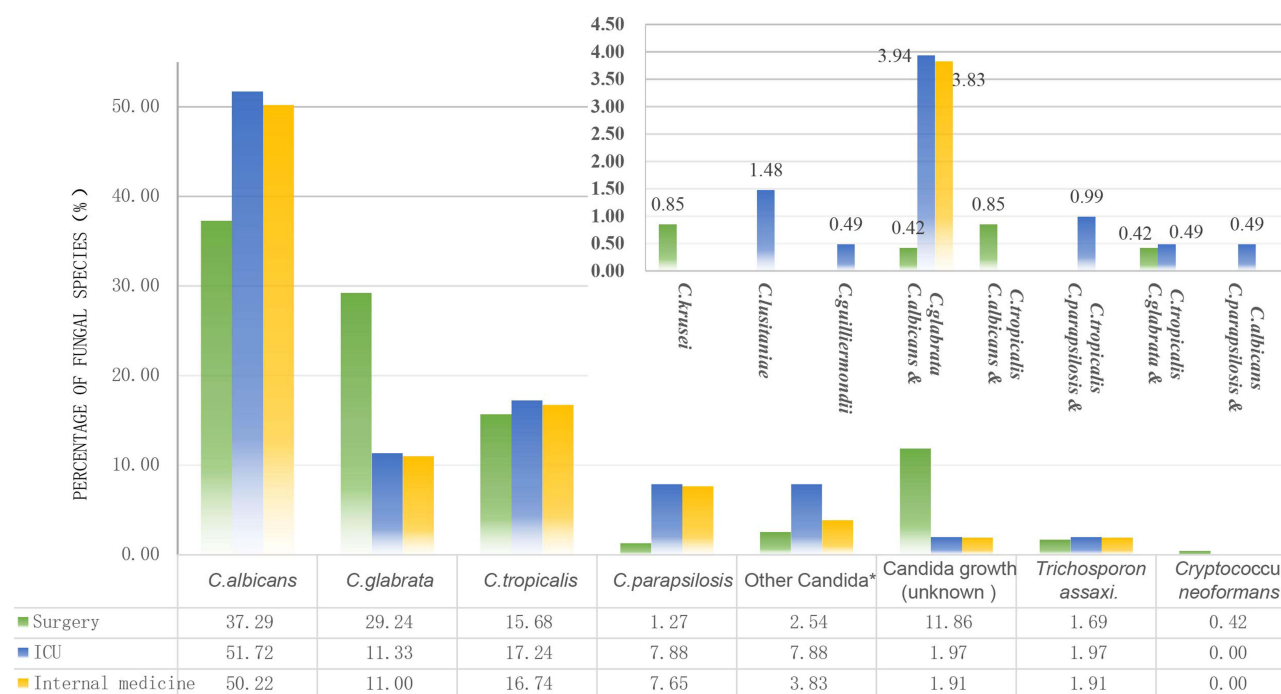**Table 2** Distribution of Fungal Species According to Clinical Samples

Fungal Species	Urine		Drainage Fluid		Blood	
	n	%	n	%	n	%
Candida albicans	166	41.50	65	49.62	18	45.00
Candida glabrata	97	24.25	15	11.45	5	12.50
Candida tropicalis	73	18.25	23	17.56	5	12.50
Candida parapsilosis	1	0.25	10	7.63	10	25.00
Candida krusei	–	–	1	0.76	1	2.50
Candida lusitaniae	1	0.25	2	1.53	–	–
Candida guilliermondii	–	–	–	–	1	2.50
Candida albicans and *Candida glabrata**	4	1.00	6	4.58	–	–
Candida albicans and *Candida tropicalis**	3	0.75	–	–	–	–
Candida parapsilosis and *Candida tropicalis**	1	0.25	–	–	–	–
Candida glabrata and *Candida tropicalis**	–	–	2	1.53	–	–
Candida parapsilosis and *Candida albicans**	–	–	1	0.76	–	–
Candidaandida growth (unknown)	47	11.75	3	2.29	–	–
Trichosporon asahi	7	1.75	3	2.29	–	–
Cryptococcus neoformans	1	0.42	–	–	–	–
Total	400	100.00	131	100.00	40	100.00

Note: *These isolates are from the same sample.

Regarding the clinical department distribution of fungal isolates, *C. albicans* accounted for 51.72% of fungal isolates in the ICU, 43.69% in internal medicine, and 37.29% in surgical wards, respectively. We should note that *C. glabrata* became a more frequent isolated species than *C. tropicalis* in medical departments (20.39%, n=21 vs 15.53%, n=16) and surgical medicine (29.24%, n=69 vs 15.68%, n=37), while it was the converse in ICU Wards, with *C. tropicalis* (17.24%, n=35) vs *C. glabrata* (11.33%, n=23) (Figure 3).

Antifungal Susceptibility

The results of susceptibility testing in vitro of the main *Candida* isolates against nine frontline antifungal agents are summarized in Table 3. *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* all showed high susceptibility to

Figure 3 Distribution of fungal species according to different wards. The asterisk (*) indicates the species and distribution of other Candida on the top right panel.

echinocandin (anidulafungin, micafungin, and caspofungin) and 5-flucytosine (S>95%), while *T. asahi* was completely resistant to echinocandin drugs. *Candida* showed variable resistance to azole drugs (voriconazole, itraconazole, fluconazole, posaconazole). *C. albicans* showed slight resistance to voriconazole and fluconazole (R<5%), followed by posaconazole (R=9.02%), and higher resistance to itraconazole (R=17.67%). On the contrary, *C. glabrata* and *C. tropicalis* were more sensitive to itraconazole (S=99.17%/94.17%), among which *C. glabrata* was highly sensitive to fluconazole in a dose-dependent manner (SDD=90.91%). It is worth noting that the resistance of *C. tropicalis* to posaconazole was close to 50%. *C. parapsilosis* was completely sensitive to itraconazole, voriconazole, and posaconazole, but resistant to fluconazole (R=16%). As there is no recommended CBPs of *T. asahi* for reference presently, the sensitivity and drug resistance cannot be clearly determined. The results showed that echinocandins had no antifungal activity against *T. asahi* in vitro (MIC$_{90}$>8 μg/mL). Unlike echinocandins, the antifungal activity of azole drugs was good: MIC$_{90}$ of voriconazole, itraconazole, fluconazole, and posaconazole were 0.25 μg/mL, 0.25 μg/mL, 8 μg/mL, and 0.25 μg/mL, respectively. MIC$_{90}$ of amphotericin B and 5-fluorocytosine is 1 μg/mL and 16 μg/mL, respectively (Supplementary Table 2).

Discussion

Isolation of pathogenic fungi from sterile sites is the key for the diagnosis of invasive fungal infections.[24] In this study, we determined the fungal spectrum and characteristic resistance to antifungal drugs used in the clinic. Causative agents of fungi were isolated from sterile samples of hospitalized deep fungal infection patients in a general hospital in Guangzhou, China, where a moist and warm environment suits fungal survival and transmission. The results showed that patients mainly came from surgical, ICU, and internal medicine wards, and more than half of them were over 60 years old (54.48%). The high isolation rate of fungi was in surgical and ICU patients, which may link to invasive surgery, long-term hospitalization, and the use of broad-spectrum antibiotics for infection.[25] In addition, the large amount of elderly infected patients may be due to the aging of organ function and the decline of immune function, which make them more vulnerable to fungal infection.[26,27]

Despite the global trend toward decreased frequency of *C. albicans* and increased frequency of non-*C. albicans* species,[28] the epidemiological survey of fungi in China shows that the genus *Candida* were still the dominant fungal

Table 3 In vitro Susceptibilities of Fungal Isolates to Eight Antifungal Agents

Antifungal Agents	AND		MCF		CAS		5-FC		VRC		ITR		FLC		AMB		POS	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Candida albicans (n=266)																		
S/WT	266	100.00	266	100.00	265	99.62	257	96.62	237	89.10	219	82.33	238	89.47	266	100.00	242	90.98
I/SDD	0	0.00	0	0.00	0	0.00	1	0.38	24	9.02	-	-	16	6.02	-	-	-	-
R/NWT	0	0.00	0	0.00	1	0.38	8	3.00	5	1.88	47	17.67	12	4.51	0	0.00	24	9.02
Candida glabrata (n=121)																		
S/WT	117	100.00	116	95.87	116	95.87	120	99.17	93	76.86	120	99.17	0	0	121	100.00	101	83.47
I/SDD	2	1.65	2	1.65	4	3.31	0	0.00	-	-	-	-	110	90.91	-	-	-	-
R/NWT	2	1.65	3	2.48	1	0.82	1	0.83	28	23.14	1	0.83	11	9.09	0	0.00	20	16.53
Candida tropicalis (n=103)																		
S/WT	102	99.03	103	100.00	102	99.03	101	98.06	67	65.05	97	94.17	71	68.93	103	100.00	57	55.34
I/SDD	1	0.97	0	0.00	1	0.97	0	0.00	17	16.50	-	-	8	7.77	-	-	-	-
R/NWT	0	0.00	0	0.00	0	0.00	2	1.94	19	18.45	6	5.83	24	23.30	0	0.00	46	44.66
Candida parapsilosis (n=25)																		
S/WT	25	100.00	24	96.00	25	100.00	25	100.00	25	100.00	25	100.00	21	84.00	25	100.00	25	100.00
I/SDD	0	0.00	1	4.00	0	0.00	0	0.00	0	0.00	-	-	0	0.00	-	-	-	-
R/NWT	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	4	16.00	0	0.00	0	0.00

Abbreviations: AND, Anidulafungin; MCF, Micafungin; CAS, Caspofungin; 5-FC, 5-Flucytosine; VRC, Voriconazole; ITR, Itraconazole; FLC, Fluconazole; AMB, Amphotericin B; POS, Posaconazole; S, sensitive; I, intermediate; R, drug resistant; SDD, dose-dependent sensitive; WT, wild-type; NWT, non-wild-type.

pathogens, in which the proportion of non-*C. albicans* and some uncommon *Candida* were increasing and *Aspergillus* and *Cryptococcus neoformans* were also on the rise.[29] In our study, *C. albicans* was the main pathogen of fungal infection, accounting for 43.97% of all *Candida* species, which is close to the archived data reported by CHIF-NET (44.9%),[30] but lower than that in Europe (52.5%).[11] In reports in Russia (42.86%),[31] France (55.8%),[32] Belgium (50.4%),[33] and Spain (45.6%),[34] *C. albicans* is also the main pathogen of fungal infections. *C. tropicalis* and *C. glabrata* were ranked as the top two non-*C. albicans* species, of which the tropical region accounts for 17.34% of the total number of *Candida*, higher than that in the Asia Pacific region (11.7%).[35] That is in line with the concept of regional differences in the distribution of *C. tropicalis* proposed by research of ARTEMI. These results also concur with the prevalence of *Candida* species causing candidemia in Asia, which recorded *C. albicans* as the leading (41.3%) isolate causing candidemia.[36] However, the prevalence rate of non-*C. albicans* varies considerably given that they found *C. tropicalis* (25.4%) and *C. glabrata* (13.9%) as the main species.[37]

Echinocandin showed good antifungal activity against *Candida*, and was recommended as the first choice for the treatment of candidemia in the clinic.[38] In our study, all four candida species showed high susceptibility to echinocinoid reagents (anidulafungin, micafungin, and caspofungin). *C. albicans* and *C. parapsilosis* were relatively sensitive to azole drugs, which is consistent with the statistical results of domestic CHIF-NET[30] and global SENTRY monitoring.[39] At present, CLSI has no standard document specifically for the in vitro drug sensitivity test of *Trichosporon*. The existing operations refer to *Candida*, and there is no reference standard for the sensitivity judgment break point, so it is unable to calculate the sensitivity rate and other indicators. From the distribution of MIC values in this study, echinocandin has no antifungal activity against *T. asahi* in vitro, which is consistent with domestic and foreign reports.[40,41] The azole drugs showed good antifungal activity in vitro, especially voriconazole and itraconazole. Azole drug plays an important role in the treatment of *Trichosporon* infection, which was confirmed by Suzuki et al.[40]

Our research has some limitations. The clinical incidence rate of invasive aspergillosis is increasing yearly.[42] We did not observe invasive aspergillosis, thus antifungal drug sensitivity tests of *Aspergillus* were not determined. As a retrospective study, our data may be affected by the integrity of demographic and laboratory data. In addition, the clinical diagnosis of deep fungal infection was referred to the clinical symptoms and laboratory data (microscopy detection, API20C AUX, and CHROMagar medium), while molecular detection was not carried out.[43–45]

In conclusion, we addressed the fungal spectrum and characteristics of the resistance to nine antifungal drugs through 525 hospitalized deep fungal infection patients in Guangzhou, China, a hyperepidemic region of fungal infection. The genus *Candida* was ranked the top deep fungal infection, while *Aspergillus* and *Penicillium* were rarely encountered. Instead, we detected appreciable *T. asahi* infected cases. Echinocandins were the first choice for clinical therapy of *Candida* infection, followed with 5-fluorocytosine and amphotericin B. Besides, azole antifungal agents should be used with caution in *Candida glabrata* and *Candida tropicalis* infections. The data presented in this study may help in the clinical diagnosis and therapy in similar endemic regions of fungal infection in China, as well as neighboring southeast Asia countries, eg, Thailand and Vietnam, which share a similar climate to Guangdong, China.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interest in this work.

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