ORIGINAL RESEARCH

Immunosuppressed Patients with Clinically Diagnosed Invasive Fungal Infections: The Fungal Species Distribution, Antifungal Sensitivity and Associated Risk Factors in a Tertiary Hospital of Anhui Province

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Objective: Since the nosocomial fungal infections increasingly emerge, we extensively investigated the fungal species stratification and antifungal sensitivity profiles, clinical characteristics and associated risk factors of immunosuppressed patients with clinically diagnosed invasive fungal infections (IFIs) in a tertiary hospital of Anhui province.

Methods: In total, 112 subjects with immunosuppressive state were enrolled from a comprehensive tertiary hospital in Central China between July 2019 and December 2021. Eight-one fungal isolates were clinically recovered by fungus-culturing approaches. The identifications were conducted through a mass spectrometry detecting platform. The susceptibilities to antifungals were tested using the broth micro-dilution method, and the possible antifungal azole-resistance mechanism in specific *Candida* species was availably explored by sequencing. Patient medical profiles were accessed via the digitized retrieval system of hospital, from which clinical outcomes and multiple risk factors for immunosuppressed patients with clinically diagnosed IFIs were explicitly documented for evaluation.

Results: *Candida* species predominated in clinically diagnosed IFIs of immunosuppressed patients (accounting for 88.88%), followed by *Trichosporon* and *Aspergillus* species (6.17% and 4.94%, respectively). The source types of specimen were primarily comprised of urine (41.98%), respiratory samples (33.33%) and peripheral blood (9.88%). Frequently isolated *Candida* and *Trichosporon* species exhibited a high level of in vitro sensitivity for amphotericin B and 5-fluorocytosine, whereas a substantial portion of *Candida* species including *C. glabrata, C. parapsilosis* complex and *C. tropicalis*, and *Trichosporon* species showed lowered sensitivity patterns toward itraconazole, fluconazole and voriconazole at different levels. Specifically, gene mutations of *ERG11* were identified in azole-resistant *C. tropicalis*. Distinct risk factors were analyzed to be highly associated with the clinically diagnosed IFI incidence, mainly including hospitalization duration, surgical procedures, immunosuppressive treatments, underlying diseases and other conditions.

Conclusion: *Candida, Trichosporon* and *Aspergillus* species were the top three pathogenic fungal agents causing clinically diagnosed IFIs in immunosuppressed patients. The attenuated sensitivity to azoles in *Candida* and *Trichosporon* species needs close surveillance, and *ERG11* polymorphism might contribute to azole resistance in specific *Candida* species. Multiple featured risk factors for immunosuppressed patients developing clinically diagnosed IFIs require further consideration during clinical practice.

Keywords: invasive fungal infections, immunosuppressed patients, species distribution, antifungal sensitivity, risk factors, treatment outcome

Introduction

Invasive fungal infections (IFIs) are increasingly recognized as a major threat in immunocompromised and critically-ill patients with a high morbidity and mortality.[1,2] It is estimated that approximately 1.5 million invasive fungal deaths occur worldwide annually,[3,4] more than 80% of which is attributable to infections of *Candida, Aspergillus* or *Cryptococcus*.[5] Though advanced approaches of diagnoses and treatments allow patients to survive life-threatening diseases in clinical settings, many of them are gradually involved in immunosuppressive state and subsequently vulnerable to a variety of infectious disorders including clinically diagnosed IFIs during hospitalization. These vulnerable or susceptible hosts are mainly comprised of cancer patients and recipients with solid organ or blood cell/bone marrow transplantations.[2,6–8] In particular, clinically diagnosed IFIs are more likely associated with invasive diagnoses and treatments, and immuno-modulating interventions during clinical practice.[2,9] It is considered that extensive applications of broad-spectrum antibiotics, more potent immunosuppressant drugs for transplantation, and more aggressive anti-cancer chemo-/radio-therapies readily leading to mucositis and neutropenia, would inevitably increase the risk of clinically diagnosed IFIs.[7,10,11] The incidence and severity of these infections also highly correlate with other risk factors, including host underlying diseases, hospitalization time, age, etc.

With the improvement of effective prophylactic strategies and the refinement of immunosuppressive regimens, the occurrence of infectious complications in immunocompromised patients tends to be reduced; however, the clinically diagnosed IFIs are still able to keep hospitalized patients in danger occasionally.[3,11,12] Furthermore, emerging multidrug-resistant fungi of clinical origin would be a fatal threat for the immunosuppressed inpatients and especially for those with transplantations or chemo-/radio-therapies if novel and strong antifungal agents are unavailable.[13–15] Recent studies on new potent antimicrobial drugs against multidrug-resistant *Candida* and filamentous fungi hold a promising perspective to overcome refractory and invasive fungal infections.[13–18] Given the widespread administration of clinically commonly-used antifungals including azoles to a large number of patients experiencing malignancy and transplantation, it is of significant importance to understand the changing trends of clinically diagnosed IFIs in terms of the fungal species stratifications, antifungal sensitivities and possible underlying azole-resistance mechanisms related to sterol$\Delta^{5,6}$-desaturase (*ERG3*) and 14-α-sterol demethylase (*ERG11*) genes,[19,20] as well as clinical characteristics and distinctive risk factors in combination with relevant clinical profiles.

In the present study, therefore, we conducted these integrated analyses for clinically diagnosed IFI patients with immunosuppressed conditions, especially focusing on cancer and/or transplantation cases in regional China. In addition, the antifungal treatment effects and clinical outcomes of the subjects (particularly with azole resistance) were also documented for assessment.

Materials and Methods

Study Design

This study was carried out within a 2800-bed comprehensive tertiary teaching hospital of a provincial capital city in Central China, and collected the cases in which all the subjects were under immunosuppressive state (mainly with cancers or transplantations) and were admitted to hospital between July 2019 and December 2021. Specimens from both sterile and non-sterile body sites were carefully screened out for the study. Specifically, samples from the conventionally non-sterile body sites (primarily including urine, sputum and pus materials) were harvested/aspirated aseptically by the paracentesis of bladders or abscesses, or from deep lower respiratory tracts of patients with fungal pneumonia (≥ 50 colonies of fungi) by sterile procedures including ventilating devices or bronchofiberscopes, for direct microscopic examinations and mycological cultures. The criteria of diagnosis for clinically diagnosed IFIs were referenced to the updated Consensus Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group,[2] and/or the local Chinese Medical Association (CMA) Expert Consensus guidelines,[21] based on host factors, clinical features and mycological evidence (Supplementary Figure S1). Sample cultures with same isolates from same subjects were excluded in the present study. Patient medical profiles were accessed through digitized retrieval system, and laboratory testing data were available in patient charts in hospital. Multiple potential risk factors which are highly related to clinically diagnosed IFIs were comprehensively assessed, eg,

immunosuppressive state (with cancers or transplantations), underlying diseases, minimally/aggressively invasive clinical interventions, hospitalization time and other infectious or featured factors. To those repeatedly admitted to hospital, the very first episode of hospitalization was accepted for analysis within the study period.

Organism Identification

As per the study protocol, all the specimens were timely harvested from the inpatients, mainly including urine, peripheral blood, ascitic fluid, pus, drainage fluid, as well as sputum and bronchoalveolar lavage fluid (BALF) selectively from deep lower respiratory tracts. Peripheral blood received rapid culture and expansion processes in a BacT/AlerT 3D automatic microbe detector (bioMérieux, Marcy l'Étoile, France). The rest specimens were aseptically inoculated directly onto Chromogenic *Candida* Agar and/or Sabouraud Dextrose Agar medium plates, and cultured at 35°C for 48-hour or more time period. The isolated fungal colonies were then identified on a VITEK mass spectrometry platform using a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions.

Antifungal Susceptibility

The common antifungal susceptibility assays in clinical laboratories were conducted for the isolated fungal species if applicable using an ATB FUNGUS 3 strip product (bioMérieux, La Balme-Les Grottes, France) following the producer's specifications. Briefly, the mean minimal inhibitory concentrations (MICs) of the antifungal drugs, primarily including amphotericin B (AMB), 5-fluorocytosine (5-FC), and commonly used azoles in clinic, were determined visually and automatically on an Automatic Testing Bacteriology (ATB) Expression Bacteriology Analyzer with expert system (bioMérieux, La Balme-Les Grottes, France). The interpretative criteria of sensitivities and breakpoints for these antifungals complied with the reference method for broth micro-dilution of the CLSI M27-A3.[22] The reference strains of *C. parapsilosis* (ATCC22019) and *C. krusei* (ATCC6258) were set for quality control.

Sequencing of Azole-Resistant Genes

The azole-resistance related genes of *ERG3* and *ERG11* were identified through polymerase chain reaction (PCR) and sequencing with primers of *ERG3*-Forward: 5'-ATGGATATCGTACTAGAAATTTGTG-3', *ERG3*-Reverse: 5'-TCATTGTTCAACATATTCTCTATCC-3', and *ERG11*-Forward: 5'-GTTTTCTACTGGATCCCATG-3', *ERG11*-Reverse: 5'-TACATCTGTGTCTACCACC-3'.[23,24] The PCR programme included: 95°C for 5 min, 35 cycles of 94°C for 30s, 50°C for 90s, and 72°C for 90s, then a final extension of 72°C for 10 min. Genomic DNA was extracted and PCR products were quantified for sequencing (Sangon Biotech Co., Ltd., Shanghai, China). The obtained sequences were then strictly aligned with the corresponding reference sequences of *ERG3* (GenBank accession no. XM002550136.1) and *ERG11* (XM_002550939.1) in *C. tropicalis* (ATCC750).
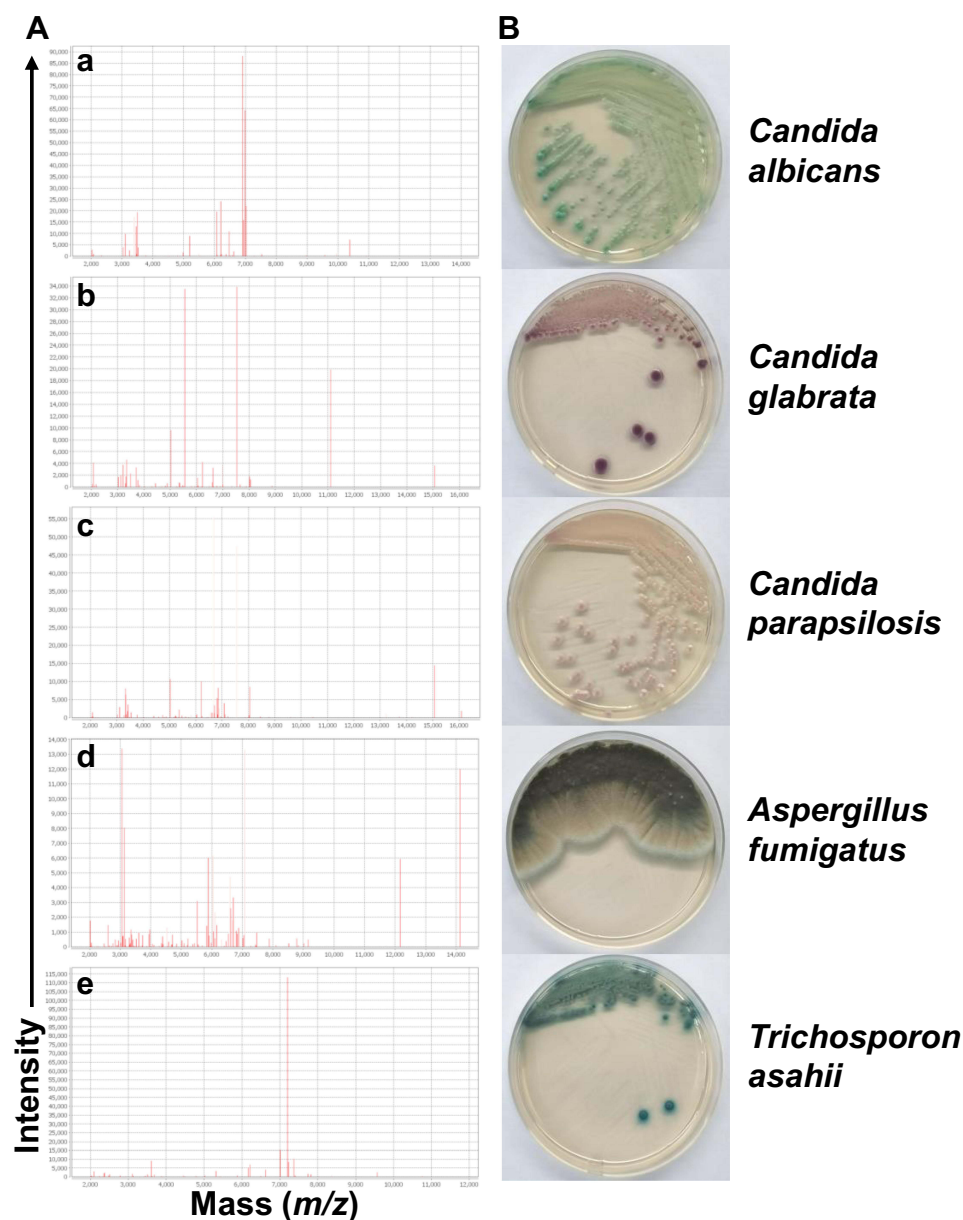
Statistical Analysis

All analyses were performed using SPSS software (SPSS version 21, Chicago, USA). The comparison of categorical data was determined by the Chi-squared tests. Statistical significance was determined using two-tailed tests. A *P* value of <0.05 was considered significant in all analyses.

Results
Clinical Distribution Characteristics of Clinically Diagnosed IFIs

In total, 112 subjects with immunosuppressed state primarily due to cancers and/or transplantations were screened during the study period, and in these cases a total of 81 patients were with clinically diagnosed IFIs. As shown in Figure 1, the corresponding fungal isolates (n = 81) from the patients were recovered and identified to a species level by a MALDI-TOF MS platform (a VITEK MS system), along with microbial morphological examinations macroscopically and microscopically. For fungal species stratification in the present study, as summarized in Table 1, the most frequently isolated strains belonged to *Candida* spp. (88.89%, n = 72), *Trichosporon* spp. (6.17%, n = 5) and *Aspergillus* spp.

Figure 1 Identification of the fungal isolates cultured in the study. (**A**) Representative identification data of the isolated fungal species by MALDI-TOF MS in this study; a~e: *C albicans, C glabrata, C parapsilosis, A fumigatus* and *T. asahii*. (**B**) Representative macroscopically imaging for the indicated fungal isolates culturing on the Chromogenic *Candida* Agar medium plates.

(4.94%, n = 4). Specifically, *C. albicans* predominated in *Candida* spp. isolated from clinically diagnosed IFI patients, accounting for 32.10%, followed by *C. glabrata* (22.22%), *C. parapsilosis* (9.88%), *C. orthopsilosis* (9.88%), *C. tropicalis* (8.64%) and *C. krusei* (2.47%). These inocula originated from different specimen sources. The major parts of the fungal species (41.98%) were obtained from urine, then sputum (28.40%), peripheral blood (9.88%), BALF (4.94%), ascitic fluid (3.70%), pus (3.70%), drainage fluid (2.47%), and others including bile (1.23%), catheter (1.23%), and secretion (1.23%), respectively.

Antifungal Susceptibility Profiles and Drug-Resistant Host Clinical Features

The clinical commonly administered antifungals were tested for *Candida* and *Trichosporon* species in terms of the in vitro sensitivities, including AMB, 5-FC, fluconazole (FLU), itraconazole (ITR) and voriconazole (VOR). As listed in Table 2, the assay results illustrated that both AMB and 5-FC exerted a potent influence on all the *Candida* spp. at the

Table 1 The Fungal Species Distribution and Characteristics of Specimen Source Types from Clinically Diagnosed IFI Patients

Species	No. (%)	Specimen Source Type, n (%)							
		Urine	Sputum	PB	BALF	AF	Pus	DF	Others[a]
Candida spp.									
C. albicans	26 (32.10)	8 (9.88)	13 (16.05)	/	/	2 (2.47)	1 (1.23)	1 (1.23)	1 (1.23)
C. glabrata	18 (22.22)	8 (9.88)	4 (4.94)	2 (2.47)	2 (2.47)	/	/	/	2 (2.47)
C. parapsilosis	8 (9.88)	6 (7.41)	/	/	2 (2.47)	/	/	/	/
C. orthopsilosis	8 (9.88)	4 (4.94)	/	4 (4.94)	/	/	/	/	/
C. tropicalis	7 (8.64)	2 (2.47)	2 (2.47)	1 (1.23)	/	1 (1.23)	1 (1.23)	/	/
C. krusei	2 (2.47)	1 (1.23)	/	1 (1.23)	/	/	/	/	/
C. guilliermondii	1 (1.23)	1 (1.23)	/	/	/	/	/	/	/
C. pseudotropicalis	1 (1.23)	/	1 (1.23)	/	/	/	/	/	/
C. rugosa	1 (1.23)	/	1 (1.23)	/	/	/	/	/	/
Trichosporon spp.	5 (6.17)	4 (4.94)	/	/	/	/	/	1 (1.23)	/
Aspergillus spp.									
A. fumigatus	2 (2.47)	/	1 (1.23)	/	1 (1.23)	/	/	/	/
A. flavus	2 (2.47)	/	1 (1.23)	/	/	/	1 (1.23)	/	/
Total	81 (100.00)	34 (41.98)	23 (28.40)	8 (9.88)	4 (4.94)	3 (3.70)	3 (3.70)	2 (2.47)	3 (3.70)

Note: [a]The others included: one bile (1.23%), one catheter (1.23%), and one secretion (1.23%).
Abbreviations: PB, peripheral blood; BALF, bronchoalveolar lavage fluid; AF, ascitic fluid; DF, drainage fluid.

in vitro level. One hundred percent of the *Candida* isolates were fully susceptible to AMB, and nearly each member presented in this study showed 100% of sensitivities to 5-FC except *C. glabrata* (94.40%). VOR was also observed 100% sensitive for most *Candida* isolates, apart from *C. parapsilosis* and *C. orthopsilosis* (87.50% and 75.00%, respectively). However, the *Candida* species primarily exhibited relatively lowered susceptibilities to both FLU (ranging from 96.20% to 62.50%) and ITR (from 96.20% to 42.90%). This study found several rare *Candida* species whose susceptibility rates for the five drugs were all able to reach 100% if intrinsic resistance was not taken into account. In addition, for *Trichosporon* spp., their susceptibilities for FLU were the highest and up to 100%, whereas all these isolates attenuated sensitivities to 5-FC, ITR, FLU and VOR (50.00%, 50.00%, 75.00% and 75.00%, respectively). Incidentally, among all the isolated fungal species confirmed definitely resistant to azoles (Table 3), the *C. tropicalis* was spotted out to harbor altered drug resistant genes of *ERG3* and *ERG11* through sequencing, trying to hold a clue to possible reasons for the in vitro resistance to azole antifungals. Briefly, after aligned with the reference sequences in GenBank, our sequencing analysis proved the presence of one silent mutation of G366A in *ERG3*, two silent mutations of T225C and G264A in *ERG11*, but two homozygous missense mutations of A395T and C461T in *ERG11* which were able to consequently produce changes of Y132F and S154F, respectively, at the amino acid level. In particular, of all the seven azole-resistant patients, five underwent prior treatment of VOR, one of them even received sequential administration of VOR and then caspofungin (CAS) separately. Four patients receiving final antifungal monotherapy of FLU, VOR or CAS turned out to be unsatisfactory (with infection persistence/progression, or moribund); whereas the other two eventually did very well post the switch of VOR or CAS monotherapy into a combined antifungal strategy of VOR plus CAS. Notably, half of the patients with azole-resistant *Candida* spp. presented infections at more than one site, without desirable treatment effects compared to those at a single one (Table 3).

Clinical Characteristics, Outcomes and Risk Factors of Clinically Diagnosed IFIs

The clinical characteristics of the cohort for fungal culture-positive subjects under immunosuppressive state are described in Table 4. Among the clinically diagnosed IFI patients, males accounted for 59.26% (48/81) and females for 40.74% (33/81) who appeared more susceptible compared to the control cohort without IFIs. The most commonly encountered invasive *Candida* spp. in males were *C. albicans* (31.25%, 15/48) and *C. glabrata* (22.92%, 11/48), which in females were *C. albicans* (33.33%, 11/33) and *C. parapsilosis* (24.24%, 8/33) instead. In particular, all the rare *Candida* spp.

Table 2 In vitro Drug Susceptibility Profile for *Candida* and *Trichosporon* Species

Species (No.)	Antifungal	MIC (µg/mL)			Sensitivity (%)
		MIC$_{50}$	MIC$_{90}$	Range	
C. albicans (26)	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	1.00	1.00	1.00~16.00	96.20
	Itraconazole	0.13	0.13	0.12~1.00	96.20
	Voriconazole	0.06	0.06	0.05~1.00	100.00
C. glabrata (18)	Amphotericin B	0.50	0.50	0.25~1.00	100.00
	5-fluorocytosine	4.00	4.00	2.00~16.00	94.40
	Fluconazole	1.00	64.00	1.00~64.00	77.80
	Itraconazole	0.13	1.00	0.12~4.00	66.70
	Voriconazole	0.06	1.00	0.06~1.00	100.00
C. parapsilosis (8)	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	1.00	32.00	1.00~32.00	87.50
	Itraconazole	0.13	0.50	0.12~0.50	87.50
	Voriconazole	0.06	2.00	0.06~2.00	87.50
C. orthopsilosis (8)	Amphotericin B	0.50	1.00	0.50~1.00	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	4.00	64.00	1.00~64.00	62.50
	Itraconazole	0.13	1.00	0.12~1.00	50.00
	Voriconazole	1.00	2.00	0.06~2.00	75.00
C. tropicalis (7)	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	1.00	64.00	1.00~64.00	85.70
	Itraconazole	0.25	1.00	0.12~1.00	42.90
	Voriconazole	0.13	0.50	0.06~0.50	100.00
C. krusei (2)[a]	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	-	-	-	-
	Itraconazole	-	-	-	-
	Voriconazole	0.06	0.13	0.06~0.12	100.00
Others (3)[b]	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	4.00	4.00~4.00	100.00
	Fluconazole	2.00	2.00	2.00~2.00	100.00
	Itraconazole	0.13	0.13	0.12~0.12	100.00
	Voriconazole	0.06	0.06	0.06~0.06	100.00
Trichosporon spp. (5)	Amphotericin B	0.50	0.50	0.50~0.50	100.00
	5-fluorocytosine	4.00	16.00	4.00~16.00	50.00
	Fluconazole	1.00	128.00	1.00~128.00	75.00
	Itraconazole	0.13	2.00	0.12~2.00	50.00
	Voriconazole	0.13	4.00	0.06~4.00	75.00

Notes: [a]*C. krusei* is inherently resistant to fluconazole and itraconazole. [b]The others included one *C. guilliermondii*, one *C. pseudotropicalis*, and one *C. rugose*.

including *C. guilliermondii, C. pseudotropicalis* and *C. rugosa*, as well as all the *Trichosporon* spp., were exclusively derived from male cases. And of *Aspergillus* spp., *A. fumigatus* was isolated from males and *A. flavus* from the opposite sex. Moreover, it's believed that patients' age may play a role in the infections. Of the clinically diagnosed IFI patients, those over 65 years of age were the most vulnerable to the infections, accounting for 40.74% (33/81), in which *C. albicans* infections were prominent with 51.52% (17/33, *P* = 0.002 compared to the other age groups), followed by the infections of *C. glabrata* (15.15%, 5/33), *C. parapsilosis* (9.09%, 3/33) and *C. tropicalis* (9.09%, 3/33). While those

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Table 3 Characteristics of All the Immunosuppressed Patients with Azole-Resistant Fungal Species in This Study

Item	Individual Patient with Azole-Resistant IFI[a]						
	P₁	**P₂**	**P₃**	**P₄**	**P₅**	**P₆**	**P₇**
Age (year)/Sex	32/M	53/M	85/M	59/M	51/M	23/M	56/M
Infectious pathogen	*Trichosporon*	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. glabrata*	*C. orthopsilosis*	*C. orthopsilosis*
Infection site	1	2	2	2	1	1	1
Gene point mutation	/	/	G366A[b], T225C/G264A/A395T/C461T[c]	/	/	/	/
Prior azole use	VOR	VOR	NA	NA	VOR, CAS	VOR	VOR
Final treatment	VOR	CAS	FLU	FLU	VOR plus CAS	VOR plus CAS	/
Clinical outcome	Moribund	Moribund	Not desirable	Not desirable	Cured	Cured	Discharged

Notes: [a]The P₁~P₇ for each individual azole-resistant patient found in this study, and P₇ was voluntarily discharged without completion of antifungal treatment with unknown reasons. Not desirable, infection persistence or progression. [b]A mutation in *ERG3* gene. [c]Mutations in *ERG11* gene.
Abbreviations: M, male; NA, not applicable; VOR, voriconazole; FLU, fluconazole; CAS, caspofungin.

Table 4 Clinical Distribution, Characteristics and Outcomes of Clinically Diagnosed IFI Patients

Species (No.)	Sex, n (%)		Age/Year, n (%)				Hospitalization Time/Day, n (%)			Outcome[a] n (%)
	Male	**Female**	**<15**	**15~49**	**50~65**	**>65**	**<7**	**7~14**	**>14**	
Candida **spp. (72)**										
C. albicans (26)	15 (57.69)	11 (42.31)	1 (3.85)	1 (3.85)	7 (26.92)	17 (65.38)[b]	2 (7.69)	9 (34.62)	15 (57.69)	4 (15.38)
C. glabrata (18)	11 (61.11)	7 (38.89)	1 (5.56)	4 (22.22)	8 (44.44)	5 (27.78)	1 (5.56)	6 (33.33)	11 (61.11)	4 (22.22)
C. parapsilosis (8)	/	8 (100.00)	/	1 (12.50)	4 (50.00)	3 (37.50)	/	/	8 (100.00)	3 (37.50)
C. orthopsilosis (8)	8 (100.00)	/	/	4 (50.00)	2 (25.00)	2 (25.00)	/	1 (12.50)	7 (87.50)	3 (37.50)
C. tropicalis (7)	4 (57.14)	3 (42.86)	/	2 (28.57)	2 (28.57)	3 (42.86)	/	/	7 (100.00)	2 (28.57)
C. krusei (2)	/	2 (100.00)	/	1 (50.00)	1 (50.00)	/	/	/	2 (100.00)	1 (50.00)
C. guilliermondii (1)	1 (100.00)	/	/	1 (100.00)	/	/	/	/	1 (100.00)	1 (100.00)
C. pseudotropicalis (1)	1 (100.00)	/	/	/	1 (100.00)	/	/	/	1 (100.00)	/
C. rugosa (1)	1 (100.00)	/	/	1 (100.00)	/	/	/	/	1 (100.00)	/
Trichosporon **spp. (5)**	5 (100.00)	/	/	2 (40.00)	2 (40.00)	1 (20.00)	/	1 (20.00)	4 (80.00)	2 (40.00)
Aspergillus **spp. (4)**										
A. fumigatus (2)	2 (100.00)	/	/	/	/	2 (100.00)	/	1 (50.00)	1 (50.00)	1 (50.00)
A. flavus (2)	/	2 (100.00)	/	1 (50.00)	1 (50.00)	/	/	/	2 (100.00)	/
Patients w/ IFIs (81)	48 (59.26)	33 (40.74)	2 (2.47)	18 (22.22)	28 (34.57)	33 (40.74)	3 (3.70)	18 (22.22)	60 (74.07)	21 (25.93)
Patients w/o IFIs (31)	25 (80.65)	6 (19.35)	0 (0.00)	8 (25.80)	12 (38.71)	11 (35.48)	7 (22.58)	8 (25.80)	16 (51.61)	1 (3.23)
P value[c]	0.014		1.000	0.855	0.682	0.610	0.006	0.878	0.023	<0.001

Notes: [a]It indicates the number (or %) of patients with infection deterioration, moribund or discharged with voluntary withdrawal of treatment with unknown reasons. [b]$P=0.002$ when compared with the other age groups all infected with *C. albicans*. [c]Comparison between patients with clinically diagnosed IFIs and those without.

with <15 years old presented barely 2.47% (2/81) in the incidence of clinically diagnosed IFIs. All the *Trichosporon* and *Aspergillus* strains were found in the subjects with >14 years of age. Our analyses unveiled that with the stay length of hospitalization prolonged, the percentage of clinically diagnosed IFI events subsequently occurring in these inpatients grew significantly from 3.70% (3/81, with <7 days, $P = 0.006$) to 74.07% (60/81, with >14 days, $P = 0.023$). As expected, the prominent infectious agent was invariably *C. albicans* for each hospitalization duration group. Interestingly, all of the *Trichosporon* and *Aspergillus* isolates were gained from the patients with >7 days of hospitalization. Next, when compared with the counterparts without IFIs, the clinical outcomes of clinically diagnosed IFI patients were significantly unsatisfactory with infection deteriorations or being moribund ($P < 0.001$), especially to those with azole resistance as described above.

The demographic characteristics of each invasive fungal species along with additional distinctive risk factors associated with clinically diagnosed IFIs were comprehensively investigated. As summarized in Table 5, and a vast majority of the patients developed one or more comorbidities, and the common ones prior to the IFIs as documented were

Table 5 Comparison of Clinically Associated Risk Factors Among Patients with or Without the IFIs

Clinical Risk Factors	All Subjects, n = 112		P
	w/ IFIs, n = 81 (%)	w/o IFIs, n = 31 (%)	
Underlying conditions			
Cancer	57 (70.37)	/	/
Renal failure or dysfunction[a]	23 (28.40)	/	/
Diabetes	15 (18.52)	1 (3.23)	0.039
Cirrhosis	4 (4.94)	1 (3.23)	1.000
Invasive procedures			
Surgery	34 (41.98)	6 (19.35)	0.025
Urinary catheters	28 (34.57)	4 (12.90)	0.023
Arteriovenous cannulation	28 (34.57)	4 (12.90)	0.023
Ventilator support	27 (33.33)	5 (16.13)	0.071
Tracheal intubation	24 (29.63)	5 (16.13)	0.144
Endoscope	13 (16.05)	3 (9.68)	0.575
Dialysis[b]	10 (12.35)	1 (3.23)	0.273
Tracheotomy	7 (8.64)	0 (0.00)	0.210
Immune interventions			
Immuno-modulation[c]	34 (41.98)	5 (16.13)	0.010
Chemo- or radio-therapy	19 (23.46)	8 (25.81)	0.795

Notes: [a]Most patients received renal transplantations later. [b]Hemodialysis or peritoneal dialysis. [c]It mainly included the immunosuppressant tacrolimus (FK506) or cyclosporine A treatment.

cancers (70.37%, especially accompanied with *C. albicans*), severe renal failure or dysfunction (28.40%, many received renal transplantations later, chiefly with *C. orthopsilosis*), and diabetes (18.52% and $P = 0.039$, usually with *C. albicans* or *C. glabrata*) (Table 6). Invasive interventions or surgical procedures as well as host immunosuppressive state were generally considered as highly relevant risk factors for invasive fungal infections. In the present study, the frequently encountered invasive procedures prior to the IFIs were as follows: surgery (41.98% and $P = 0.025$, mainly with *C. albicans*), urinary catheters (34.57% and $P = 0.023$, mainly with *C. parapsilosis* complex), arteriovenous cannulation (34.57%, $P = 0.023$, mainly with *C. parapsilosis*), ventilator support (33.33%), tracheal intubation (29.63%), endoscope (16.05%), and hemodialysis/peritoneal dialysis (12.35%); and those directly affecting host immune systems were involved with immuno-modulations (41.98% and $P = 0.01$, primarily with *C. albicans, C. glabrata* or *C. orthopsilosis*) including immunosuppressant tacrolimus/FK506 or cyclosporine A treatment, and chemotherapy and/ or radiotherapy (23.46%) (Table 6). Other potentially associated risk factors were also explicitly analyzed when

Table 6 Demographic Characteristics Between Fungal Species Distribution and Significant Risk Factors for Clinically Diagnosed IFI Patients

Significant Factors	Candida spp., n (%)							Aspergillus spp. n (%)	Trichosporon spp. n (%)
	Cal	Cgl	Cpa	Cor	Ctr	Ckr	Others[a]		
Cancer	23 (28.40)	12 (14.81)	5 (6.17)	2 (2.47)	7 (8.64)	/	2 (2.47)	3 (3.70)	3 (3.70)
Renal failure/dysfunction	4 (4.94)	4 (4.94)	4 (4.94)	6 (7.41)	/	1 (1.23)	1 (1.23)	1 (1.23)	2 (2.47)
Diabetes	4 (4.94)	4 (4.94)	2 (2.47)	1 (1.23)	1 (1.23)	/	1 (1.23)	1 (1.23)	1 (1.23)
Surgery	11 (13.58)	6 (7.41)	6 (7.41)	4 (4.94)	4 (4.94)	/	1 (1.23)	1 (1.23)	1 (1.23)
Urinary catheters	5 (6.17)	5 (6.17)	6 (7.41)	6 (7.41)	3 (3.70)	1 (1.23)	/	/	2 (2.47)
Arteriovenous cannulation	4 (4.94)	5 (6.17)	6 (7.41)	5 (6.17)	4 (4.94)	/	1 (1.23)	/	2 (2.47)
Immuno-modulation	6 (7.41)	6 (7.41)	4 (4.94)	6 (7.41)	3 (3.70)	1 (1.23)	2 (2.47)	3 (3.70)	3 (3.70)

Notes: [a]The others: *C. guilliermondii, C. pseudotropicalis*, and *C. rugose*.
Abbreviations: *Cal, C. albicans; Cgl, C. glabrata; Cpa, C. parapsilosis; Cor, C. orthopsilosis; Ctr, C. tropicalis; Ckr, C. krusei.*

Table 7 Potentially Featured Risk Factors for Clinically Diagnosed IFI Patients Infected with the Major Fungal Species

Potential Factors	Total No. (%)	*Candida*, n (%)	*Aspergillus*, n (%)	*Trichosporon*, n (%)
Aberrant tumor biomarkers[a]	42 (51.85)	36 (44.44)	2 (2.47)	4 (4.94)
Virus mediated infections[b]	17 (20.99)	12 (14.81)	2 (2.47)	3 (3.70)
Abnormal cytokines release[c]	10 (12.35)	8 (9.88)	/	2 (2.47)
Mycoplasma pneumoniae infection	3 (3.70)	2 (2.47)	1 (1.23)	/
Treponema pallidum infection	1 (1.23)	1 (1.23)	/	/

Notes: [a]Highly associated tumor biomarker expressions were assessed if applicable. [b]Hepatitis caused by hepatitis B virus, and infections by Epstein-Barr virus, BK virus or cytomegalovirus if any. [c]It mainly included interleukin-1β (IL-1β), IL-2R, IL-6, IL-8, IL-10, and tumor necrosis factor-α.

available, and a large portion of these factors were immune-related and relevant to *C. albicans*-mediated clinically diagnosed IFIs in this study: (1) aberrant tumor biomarker expressions (51.85%), such as alpha-fetoprotein, carcinoembryonic antigen, carbohydrate antigen 19–9 (CA19-9), CA12-5, cytokeratin 19 fragment (CYFRA 21–1), neuron-specific enolase, tumor abnormal protein (TAP), ferritin, etc.; (2) virus-mediated infections (20.99%), primarily involving hepatitis caused by hepatitis B virus (HBV) rather than HAV or HCV, and infections by Epstein-Barr virus, BK virus or human cytomegalovirus (but without the coronavirus), and interestingly one patient was found to be infected with influenza B virus who received kidney transplantation and suffered candidemia with *C. orthopsilosis*; (3) abnormal cytokines release (12.35%), basically including interleukin-1β (IL-1β), IL-2R, IL-6, IL-8, IL-10, and tumor necrosis factor-α; (4) *Mycoplasma pneumoniae* infection (3.70%); and (5) *Treponema pallidum* infection (1.23%) (Table 7).

Discussion

To date, an increasing number of clinical practices engage directly or indirectly with host immune systems, which inevitably poses a potential or substantial impact on the immune defense functions within patients, especially for those cancer patients receiving chemo/radio-therapies, as well as patients receiving transplantations and continuous immunosuppressant drugs. Though constant progress has been achieved from invasive medical interventions over the past decades, the incidence of clinically diagnosed invasive fungal infections has risen concomitantly.[6,25–28] The increase in such infections gives rise to relatively high morbidity and mortality among the inpatients with immunosuppressive state. However, current clinical studies of the IFIs in immunosuppressed subjects are rarely involved in central part of China, indicating relatively limited knowledge is available for local areas in the country on the epidemiology, antifungal susceptibility patterns, and associated risk factors for clinically diagnosed IFI patients under immunosuppressive status. Therefore, this study provided potentially valuable information to bedside on the management of these patients with the infections in local regions.

With the ongoing outbreak of coronavirus disease 2019, the sample size of this study was relatively affected, however it may otherwise truly reflect the distinctive features of clinically diagnosed IFIs in immunosuppressed cases in the region during the special time period. We believe it would be highly informative to investigate whether the coronavirus would potentially be an important risk factor for the clinically diagnosed IFIs locally, though the present study did not access to any coronavirus-positive cases (with PCR assays).[29,30] Additionally, with recent implementation of the policy of hierarchical medical system in China,[31] severe patients have a priority to visit our tertiary teaching hospital with a higher medical hierarchy, relatively leading to a high local incidence of clinically diagnosed IFIs. Previous studies reported that the occurrence of invasive fungal infections was largely attributable to *Candida* spp., then *Cryptococcus* spp. and *Aspergillus* spp.[32–35] The present study observed a similar phenomenon that *Candida* spp. remained a causative agent for clinically diagnosed IFIs in immunosuppressed patients; and *Trichosporon* and *Aspergillus* species were the two primary non-*Candida* spp. causing the clinically diagnosed invasive mycoses. The specimen sources in this research were of diverse origins. The top three sources of culture-positive specimens were urine, sputum, and peripheral blood. In general, the incidence and severity of clinically diagnosed IFIs were influenced by a variety of factors, for instance, patient age, hospitalization duration, underlying diseases, clinical invasive interventions, immune status and others.[25,36] It turned out that patients' age played a significant role in raising the clinically diagnosed IFI incidence. Those developing the infections in this study were almost elderly patients who also underwent significantly prolonged hospitalization

duration, ie, over 70% of them were more than 50 years of age and experienced hospitalization for over two weeks. The highly relevant underlying diseases were mainly comprised of cancers (particularly with aberrant tumor biomarker expressions), organ transplantations and diabetes. These conditions, together with invasive procedures (eg, surgery, catheters and arteriovenous cannulation), immunosuppressive treatment and other infectious or featured factors (eg, virus- or mycoplasma-mediated infections, abnormal cytokines release, etc.), would likely offer massive opportunities for the IFIs or transmission of the causative fungal species among those inpatients. Interestingly, we observed that one subject was infected with influenza B virus who underwent kidney transplantation and developed *C. orthopsilosis*-mediated candidemia, holding a clue that influenza might be a potential risk factor for clinically diagnosed IFIs here.[37] Other researchers stated that the risk factors for *Trichosporon* infections dealt with neutropenia (an abnormally low amount of neutrophils), transplantations, end-stage renal diseases, HIV infections, and use of immuno-regulatory agents and invasive medical devices.[38] Our analyses echoed that statement, and it is worth noting that the clinically diagnosed IFIs caused by *Trichosporon* in immunocompromised patients are likely to be one of life-threatening complications.

The emergence of drug-resistant fungal strains is becoming a major health concern globally. We next made an in-depth analysis of the antifungal susceptibility features of clinically diagnosed IFI patients. Antifungal agents are generally categorized into four major classes including polyenes (to target cell membrane), fluoropyrimidine analogs (to block nucleic acid synthesis), echinocandins (to inhibit cell wall synthesis), and azoles (to inhibit ergosterol biosynthesis).[5] Especially, the decreased sensitivity of azoles among *Candida* and *Aspergillus* species mounts a rigorous challenge in current clinical settings, and echinocandin- or multidrug-resistant (MDR) isolates of certain *Candida* spp. also become a compelling concern.[33] Recent emergence of new drugs or disinfectant agents against MDR *Candida* spp. during invasive and ocular *Candida* infections holds a promising perspective particularly for immunosuppressed patients.[15] For instance, ophthalmic solutions containing hexamidine diisethionate 0.05% (Keratosept) or povidone-iodine 0.6% (IODIM®) have been evaluated to control ocular fungal infections with potent antimicrobial activities against *Candida* spp. as recently reported by Pinna et al.[13–15] Moreover, many plant essential oils have increasingly been assessed to present promising broad-spectrum antifungal activities to overcome MDR *Candida* spp. and even filamentous fungi, including the essential oils from *Ruta graveolens, Myrtus communis* and *Austroeupatorium inulaefolium*.[16–18] In China, the antifungal susceptibility rate differs significantly from region to region.[39] It is likely due to the extensive and/or irrational utilization of antifungals within clinical and veterinary settings.[40] In the present study, we found that AMB was fully sensitive to all of the invasive *Candida* and *Trichosporon* species. And ITR sensitivity rate was the lowest among all *Candida* and *Trichosporon* species, followed by FLU, VOR and 5-FC. Since ITR currently has only oral formulations available and is regularly used for mucosal mycoses, it is not highly recommended for IFI patients that might prefer an intravenous formulation if applicable in future.[41] Hence, the antifungal susceptibility assay for ITR here merely reflected an in vitro activity for *Candida* and *Trichosporon* species, but it still may provide instrumental information for possible cross resistance between azoles and combined fungal infections. To be specific, a substantial number of *C. orthopsilosis* and *Trichosporon* spp. were identified with remarkably lowered sensitivities to FLU, ITR and VOR, even prone to cross resistance. Owing to relatively limited sample size, the underlying mechanisms for azole resistance found here were yet fully discussed. However, incidentally through sequencing azole-resistance associated genes of *ERG3* and *ERG11* in the *C. tropicalis* resistant against azoles in this study, it might get a clue to a possible resistance mechanism that the existence of homozygous point mutations of A395T and C461T (causing Y132C and S154C amino acid residue changes) in *ERG11* might contribute to such resistance at any rate in *C. tropicalis* here, which is expected to jeopardize the ability of ERG11 proteins to binding azoles.[20] Other researchers also did gene sequencing studies on azole-resistant *C. glabrata* and seldom found significant antifungal missense mutations in local regions.[42] Our ongoing project is proceeding to investigate genetic polymorphisms pertaining to the mechanisms of azole resistance in invasive fungal infections. And consistent with the reported synergistic effects of azole plus caspofungin on multidrug-resistant *Candida* infections,[43–45] the limited data here also appear to imply that a combinatorial antifungal strategy of azole plus echinocandins might be of benefit to the immunosuppressed patients with clinically diagnosed IFIs, albeit more conclusive evidence needed.

Conclusion

Taken together, the current research concretely emphasized the fungal species distribution and antifungal sensitivity profiles, as well as clinical characteristics and the complexity of risk factors associated with immunocompromised patients that were infected with clinically diagnosed invasive mycoses during hospitalization in a comprehensive teaching hospital of a provincial capital city in Central China. It concluded that *Candida* species was the primary pathogen resulting in clinically diagnosed IFIs in patients under immunosuppressive state, followed by *Trichosporon* and *Aspergillus* species. The findings further imply that patient age, duration of hospitalization, multiple underlying diseases and diverse invasive interventions, as well as certain featured conditions would likely be regarded as the highly associated risk factors for clinically diagnosed IFIs. The critical gene mutations of *ERG11* might contribute to attenuated sensitivity to azoles in *C. tropicalis* here. To maintain effective antifungal susceptibilities for better clinical outcomes of clinically diagnosed IFI patients, more efforts ought to be put to standardize the rational application of common antifungals at bedside.

Abbreviations

IFI, invasive fungal infection; ERG3, sterol $\Delta^{5,6}$-desaturase; ERG11, 14-α-sterol demethylase; BALF, bronchoalveolar lavage fluid; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; MIC, minimum inhibitory concentration; MIC_{50} or MIC_{90}, MIC at which 50% or 90% of the isolates tested were inhibited; AMB, amphotericin B; 5-FC, 5-fluorocytosine; FLU, fluconazole; ITR, itraconazole; VOR, voriconazole; CAS, caspofungin; CLSI, Clinical and Laboratory Standards Institute; PCR, polymerase chain reaction; PB, peripheral blood; AF, ascitic fluid; DF, drainage fluid; HBV, hepatitis B virus; IL, interleukin.

Ethical Considerations

The study protocol was reviewed, approved by and carried out following the recommendations of the Ethics Committee of the First Affiliated Hospital of Anhui Medical University. Written informed consents were obtained from patients as per the Declaration of Helsinki.

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

All the authors declared no any competing interests.

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