Infection and Drug Resistance

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ORIGINAL RESEARCH

Clinical and Microbiological Characteristics of Aspergillosis at a Chinese Tertiary Teaching Hospital

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Background: *Aspergillus* spp. infection in immunocompromised patients results in increasing morbidity and mortality. This study investigated clinical and microbiological characteristics of aspergillosis in a Chinese tertiary teaching hospital.

Methods: A total of 114 patients with aspergillosis were included over a 5-year period at Ruijin Hospital. In sum, 114 *Aspergillus* strains were isolated and identified at species level using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, confirmed by ITS gene region and β-tubulin (*BenA*) gene sequencing. Sensititre YeastOne was used in vitro to test susceptibility to antifungal drugs: amphotericin B, itraconazole, voriconazole, posaconazole, isavuconazole, micafungin, anidulafungin, and caspofungin.

Results: The median age of the patients was 61 (19) years, men accounted for 53.5% (n=61) of the sample, about 64% were immunocompromised, and 36% had underlying diseases. Pulmonary diseases accounted for 27.2%. *Aspergillus* isolates were mainly isolated from sputum (n=42, 36.8%). Antifungal therapy was administered to 106 (93.0%) patients and voriconazole (n=76, 66.7%) was the most frequently used as empirical therapy. *Aspergillus fumigatus* (n=69, 60.5%) was the most common species. There was a 73.7% concordance between MALDI-TOF MS and molecular identification. All *Aspergillus* isolates showed good susceptibility to anidulafungin and caspofungin.

Conclusion: Immunocompromised patients are an at-risk population for aspergillosis, and voriconazole was used as empirical therapy in Ruijin Hospital, China. *A. fumigatus* was the predominant *Aspergillus* species causing aspergillosis, and *A. flavus* — as non–*A. fumigatus* species are increasing — the second-leading cause of aspergillosis. Anidulafungin and caspofungin were the most active in vitro against the *Aspergillus* isolates tested. The MALDI-TOF MS method showed good accuracy for identification of common *Aspergillus* spp. In vitro antifungal-susceptibility testing is crucially important for decisions on effective therapy with aspergillosis.

Keywords: aspergillosis, epidemiology, *Aspergillus* spp., MALDI-TOF MS, molecular identification, antifungal susceptibility

Introduction

Aspergillosis is an infection caused by *Aspergillus* spp. that mainly occurs in immunocompromised individuals. Recently, influenza-associated pulmonary aspergillosis and CAPA (COVID-19–associated pulmonary aspergillosis) have been reported in many studies as causing the clinical attention.[1–3] *Aspergillus fumigatus* is the predominant *Aspergillus* species causing aspergillosis. However, the incidence of infections with non–*A. fumigatus* spp., such as *A. flavus*, *A. niger*, and *A. terreus*, to date has been increasing, especially in immunocompromised hosts.[4] Triazoles and polyene amphotericin B have been licensed

Received: 24 September 2022
Accepted: 23 November 2022
Published: 11 December 2022

for primary therapy of aspergillosis. However, triazole resistance to *Aspergillus* spp. have been reported globally and remain a big challenge for the treatment of aspergillosis.[5,6]

With molecular methods, numerous cryptic species and new species have been identified within the genus *Aspergillus*. Many studies have indicated that antifungal susceptibility varies in particular *Aspergillus* spp.[7,8] Thus, *Aspergillus* identification at the species level become crucial for clinicians to make effective treatment of aspergillosis. The partial internal transcribed spacer (ITS1–4) method combined with β-tubulin (*BenA*) gene sequences have been used for the identification of *Aspergillus* spp.[9,10] In addition, matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) has been demonstrated to be a useful method for rapid identification of *Aspergillus* species in a clinical lab.[10]

Although epidemiological surveillance of *Aspergillus* diseases has been reported in many countries,[11] few surveillance data on aspergillosis involving large sets of patients are available in China.[12,13] This 5-year (2017–2021) retrospective study on a large cohort of patients with aspergillosis was conducted to investigate the clinical and microbiological characteristics of aspergillosis in a tertiary teaching hospital in Shanghai, China.

Methods

Patients and Fungal Isolation

In this study, 114 patients diagnosed with aspergillosis were recruited from 2017 to 2021 in Ruijin Hospital, Shanghai, China, a tertiary care teaching hospital that has 3624 beds and admits around 130,000 patients each year. Diagnosis of aspergillosis was based on guidelines of the Infectious Diseases Society of America (IDSA).[14] Ethics approval (2019204) for the study was obtained from the Ethics Committee of Ruijin Hospital, and all patients involved understood and agreed to the use of these clinical specimens.

Patients' clinical information collected comprised age, sex, specimen type, site of isolation, underlying diseases and antifungal treatment based on medical records. Specimens from patients with aspergillosis — bronchoalveolar lavage fluid (BALF), exudate, and drainage fluid — were sent to the Clinical Microbiology Department at Ruijin Hospital for fungal detection. All patients had *Aspergillus* cultures detected twice in consecutive weeks.

Mycological criteria were positive direct microscopy with hyphae presented in specimen and positive culture of *Aspergillus*, with Sabouraud glucose agar (SGA; Difco, Detroit, MI, USA) containing chloramphenicol (50 mg/L) used for the isolation of *Aspergillus* spp., repeated culturing of the *Aspergillus* spp. for three times and samples, and molecular identification of the *Aspergillus* isolates. Direct microscopic examination was performed using a 20% KOH solution. All isolation plates were incubated at 27°C for up to 4 weeks.

Identification Based on MALDI-TOF MS

All 114 *Aspergillus* isolates were identified using MALDI-TOF MS (Knowledge Base 3.0 system, BioMérieux, Marcy-I'Etoile, France) and the Mould kit and Auto kits to prepare samples, for which the overall rate of correct identification was excellent. Sample preparation strictly followed the manufacturer's instructions. Briefly, cotton swabs were dipped into about 1 cm^2 mold colony, then added to 900 μL ethanol (70%) and spun for 2 min at 14,000 *g*. Next, the ethanol was removed and the residual pellet resuspended in 40 μL 70% formic acid and 40 μL acetonitrile for extraction of proteins. After centrifugation for 2 min at 14,000 *g*, 1 μL supernatant was spotted onto a slide, dried naturally, then covered with 1 μL saturated alphacyano-4-hydroxycinnamic acid matrix solution in 50% acetonitrile + 2.5% trifluoroacetic acid. Instrument calibration (using a reference strain of *Escherichia coli* ATCC 8739) and the quality control (*A. fumigatus* ATCC 204305) was performed every 12 samples.

Sequence-Based Identification

DNA was extracted with cultures grown on SGA plates for 5–7 days at 27°C using a genomic DNA isolation kit (Sangon Biotech, Shanghai, China). All 114 isolates were identified by sequencing a part of *BenA* gene and ITS1–4 DNA primer sequences and PCR reaction conditions described previously.[15,16] The obtained sequences were compared to the NCBI

nucleotide database (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). GenBank accession numbers for the generated ITS1–4 and *BenA* sequences are listed in the CNCB Genome Warehouse under accession number PRJCA01048.

Antifungal-Susceptibility Testing

The in vitro susceptibility of all the *Aspergillus* isolates to the antifungal drugs itraconazole (Itr), voriconazole (Vor), posaconazole (Pos), isavuconazole (Isa), micafungin (Mcf), anidulafungin (Afg), and caspofungin (Cas) was determined with Sensititre YeastOne CMC1JHY methodology (Thermo Fisher Scientific). An amphotericin B (AmB) microbroth dilution kit (Bio-Kont, Wenzhou, China) was used for AmB. A standard *A. fumigatus* ATCC MYA-3626 strain was used as the quality control. The results were interpreted based on the clinical breakpoints or epidemiological cutoff values (ECVs) recommended by the Clinical and Laboratory Standards Institute (Itr ECV ≥1 μg/mL, Vor ≥2 mg/mL, and Isa ≥1 μg/mL for *A. fumigatus*).[17]

Statistical Analysis

Continuous variables are given as medians and interquartile ranges, and categorical variables as frequencies and percentages. Statistical analyses were performed using SPSS 26.0. $P<0.05$ was considered statistically significant.

Results

Patient Characteristics and Prevalence of *Aspergillus* Isolates

A total of 114 patients were diagnosed with aspergillosis based on the guidelines of the IDSA[14] from 2017 to 2021 in Ruijin Hospital. Median age was 61 (19) years in both the immunocompromised and underlying disease groups, 53.5% (61/114) were male, 64% (73/114) had immunocompromised conditions, and 36% (41/114) underlying diseases. The most frequent diseases were chronic renal failure (27/114), followed by autoimmune diseases (15/114), solid tumors (15/114), and septic shock (15/114) in the immunocompromised group, and pulmonary disease (31/114) was the most common in patients with underlying disease.

Sputum (42/114, 36.8%) was the most common specimen from which *Aspergillus* isolates were recovered, followed by BALF (38/114, 33.3%), wound (18/114, 15.8%), exudate (9/114, 7.9%), and drainage fluid (7/114, 6.1%). The intensive care unit (ICU) (32/114, 28.1%) and Respiratory Department (31/114, 27.2%) were the wards where *Aspergillus* was recovered frequently, followed by the Burns (9/114, 7.9%), Dermatology, and Cardiology Departments (7, 6.14%). A few isolates (≤5) were recovered from the other hospital wards. In sum, 22 of 69 *A. fumigatus* isolates were recovered from the Respiratory Department and 22 of 69 *A. fumigatus* isolates from ICUs. Eleven of 32 isolates of *A. flavus* were recovered from ICUs (Table S1). Almost all (93%, 106/114) patients received antifungal therapy, while no antifungal treatment was administered for 7% (8/114). Vor (76/114, 66.7%) was the most frequently used empirical therapy, with similar outcomes between the immunocompromised and underlying diseases group. The clinical characteristics of 114 patients diagnosed with aspergillosis are shown in Table 1.

Based on ITS/*BenA* gene sequencing combined with MALDI-TOF MS, *A. fumigatus* (n=69, 60.5%) was the most common *Aspergillus* species causing aspergillosis, followed by *A. flavus* (n=30, 26.3%), *A. niger* (n=6, 5.3%), *A. terreus* (n=4, 3.5%), *A. tubingensis* (n=3, 2.6%), *A. lacticoffeatus* (n=1), and *A. nidulans* (n=1) (Table 2). A 73.7% concordance between MALDI-TOF MS and molecular identification was found, and MALDI-TOF MS allowed the identification of the four common species — *A. fumigatus*, (accuracy 20/30, 66.7%), *A. flavus* (accuracy 3/6, 50%), *A. niger* and *A. terrus* (accuracy 4/4, 100%) — but failed to identify *A. tubingensis*, *A. nidulans*, and *A. lacticoffeatus*.

Antifungal Susceptibility

Minimum inhibitory concentration (MIC)/minimum effective concentration (MEC) ranges, geometric mean, distribution, modal MIC/MEC, and MIC/MEC for 90% patients (MEC_{90}) of the eight antifungal agents against 114 *Aspergillus* isolates are presented in Table 3. The lowest modal MIC/MEC (<0.008 μg/mL) was Mcf, followed by Afg, and Pos (both 0.016 μg/mL), while 90% of isolates were inhibited at 0.5 μg/mL of MIC for Vor and Itr. Cas, Mcf, and AmB showed great activity against all *Aspergillus* isolates tested. *A. fumigatus* was susceptible to echinocandins (Cas, Mcf, Afg, MIC_{90}

Table 1 Clinical characteristics of 114 patients with aspergillosis

	Immunocompromised	Underlying diseases
n	58	56
Median age, years (range)	59 (24–93)	59 (26–92)
Male/female	37/21	24/32
Chronic pulmonary diseases	0	31
Diabetes	0	5
Burn	0	5
Chronic renal failure	27	0
Autoimmune disease	15	0
Solid tumors	15	0
Septic shock	15	0
Hematologic malignancy	1	0
Antifungal therapy		
Vor	43	33
Vor + AmB	10	/
Flu + Vor	5	2
Flu + AmB	12	/
Ketoconazole	1	/
No treatment	3	5
Outcomes		
Survived	37	36
Died	21	20

Abbreviations: Vor, voriconazole; AmB, amphotericin B; Flu, fluconazol.

Table 2 Species identification of 114 *Aspergillus* isolates based on ITS/BenA gene sequencing and MALDI-TOF MS

Isolate	Molecular-based method	MALDI-TOF MS kit method
57	*A. fumigatus*	*A. fumigatus*
12	*A. fumigatus*	No results
20	*A. flavus*	*A. flavus/oryzae*
10	*A. flavus*	No results
1	*A. lacticoffeatus*	No results
1	*A. nidulans*	No results
3	*A. niger*	*A. niger*
3	*A. niger*	No results
3	*A. tubingensis*	No results
4	*A. terreus*	*A. terreus*

0.016 μg/mL, 0.008 μg/mL, and 0.016 μg/mL, respectively) and AmB (MIC_{90} 1 μg/mL). Two isolates of *A. fumigatus* exhibited MICs values ≥2 mg/mL for three azoles.

All isolates of *A. flavus* showed similar susceptibility to echinocandins (Cas, Mcf, and Afg, MIC_{90} 0.06 μg/mL, 0.03 μg/mL, and 0.016 μg/mL, respectively) and AmB (MIC_{90} 0.006 μg/mL) as those of *A. fumigatus*. One isolate of *A. flavus* had an MIC value ≥4 μg/mL to Isa, while others were susceptible to Itr, Vor, and Pos. For the one isolate each of *A. lacticoffeatus* and *A. terrus* in this study, AmB was the most active drug against *A. lacticoffeatus* in vitro with the lowest MIC (0.03 μg/mL), followed by Pos (0.12 μg/mL), Itr, Vor, and Isa (0.25 μg/mL), Mcf (0.5 μg/mL), Afg (1 μg/mL), and Cas (2 μg/mL). For *A. terrus*, AmB was the most active drug with the lowest MIC (0.008 μg/mL), followed by Pos, Isa, and Afg (0.03 μg/mL), Itr (0.06 μg/mL), and Vor (0.12 μg/mL).

Table 3 MIC/MEC ranges, geometric mean, distribution, and modal MIC/MEC, MIC/MEC$_{90}$ of eight antifungal agents against 114 *Aspergillus* isolates

Species	Drugs	Range	GM	MEC/MIC$_{90}$	Number of isolates with MEC/MIC (μg/mL)												
					0.008	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
A. fumigatus (69)	AmB	0.25–1	0.558	1						4	**50**	15					
	Itr	0.12–16	0.274	0.25					3	**61**	3					2	
	Vor	0.015–8	0.274	0.25		1		1	1	**60**	2	2		1	1		
	Pos	0.015–1	0.14	0.12		1		8	**51**	7		2					
	Isa	0.008–8	0.376	0.25	1				2	28	**37**			1	1		
	Mcf	0.008–0.03	0.01	0.008	**66**	2	1										
	Afg	0.015	0.016	0.016		**69**											
	Cas	0.06	0.063	0.016				**69**									
A. flavus (30)	AmB	0.25–1	0.707	1							13	**17**					
	Itr	0.03–0.5	0.263	0.25					2	**25**	3						
	Vor	0.03–2	0.277	0.5					1	**20**	7	1	1				
	Pos	0.015–0.5	0.141	0.5				3	6	**20**	1						
	Isa	0.015–4	0.377	0.5					1	9	**18**	1		1			
	Mcf	0.008–0.03	0.01	0.03	7	**13**	10										
	Afg	0.015–0.03	0.016	0.016		**24**	6										
	Cas	0.06–0.12	0.063	0.06				**25**	5								
A. niger (6)	AmB	0.12–0.5	0.397	0.5					1	1	**4**						
	Itr	0.5–1	0.5	1							2	**4**					
	Vor	0.06–1	0.278	0.5				1			**5**	1					
	Pos	0.03–0.5	0.143	0.25				1	1	**4**	1						
	Isa	0.06–2	0.386	2				1				**3**	2				
	Mcf	0.008	0.01	0.008	**6**												
	Afg	0.015	0.016	0.015		**6**											
	Cas	0.06	0.063	0.06				**6**									

(*Continued*)

https://doi.org/10.2147/IDR.S391069

Table 3 (Continued).

Species	Drugs	Range	GM	MEC/MIC$_{90}$	Number of isolates with MEC/MIC (μg/mL)												
					0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
A. tubingensis (3)	AmB	0.25	ND	0.25						**3**							
	Itr	0.06–0.25	ND	0.12				1	**1**	1							
	Vor	0.06–0.25	ND	0.12				1	**1**	1							
	Pos	0.03–0.12	0.083	0.06			1	**1**	1								
	Isa	0.03–0.5	0.5	0.03			**3**										
	Mcf	0.06–0.12	ND	0.25					**2**	1							
	Afg	0.015	ND	0.015		**3**											
	Cas	0.06–0.12	0.5	0.12				**2**	1								
A. terreus (4)	AmB	0.25	ND	0.25				1	**2**	1							
	Itr	0.06–0.25	0.264	0/25				1	**2**	1							
	Vor	0.06–0.25	0.27	0.25				1	**2**	1							
	Pos	0.06–0.25	0.134	0.12				**2**	1	1							
	Isa	0.06–0.25	0.358	0.12				1	**2**	1							
	Mcf	0.008–0.015	0.01	0.015	1	**3**											
	Afg	0.015	0.016	0.015		**4**											
	Cas	0.06–0.12	0.062	0.06				**3**	1								

Notes: Modal MIC/MEC: most frequent MICs (value in bold). MICs are shown for AmB, Itr, Vor, Pos, Isa; MECs are shown for Mcf, Afg and Cas.
Abbreviation: ND, not determined.

Discussion

Here, we presented a 5-year retrospective study on the clinical and microbiological characteristics of aspergillosis at a Chinese tertiary teaching hospital. Of infected people among all age-groups, more than 50% of cases occurred in adulthood, with about 50% between the ages of 35 and 70 years, in agreement with a previous study.[18,19] Previous studies had found that sputum and BALF were the most common specimens from which *Aspergillus* isolates were recovered[20,21] and *A. fumigatus* and *A. flavus* were mainly found in respiratory tract isolates.[22] Half were immunocompromised patients, in line with that reported in previous studies.[23,24]

Our study highlights that *A. fumigatus* (60.5%, 69/114) was the predominant species causing invasive aspergillosis in Shanghai, China, similar to that reported from previous studies in China[25] and outside China, such as Italy[26] and the UK.[27] However, it is different from results reported in Cameroon,[28] Turkey,[29] India,[30] and Iran,[19] where *A. niger*, *A. terreus*, and *A. flavus* were the most common *Aspergillus* spp., respectively. *A. flavus* (26.3%, 30/114) were also the second-leading cause of aspergillosis at Ruijin Hospital, in agreement with Pasqualotto, where *A. flavus* was the second-leading cause of invasive and noninvasive aspergillosis.[31] *Aspergillus niger* and *A. tubingensis* have previously been reported as an emerging causal agents of aspergillosis.[32] In our study, *A. niger* (n=6, 5.3%), *A. tubingensis* (n=3, 2.6%), and *A. terreus* (n=4, 3.5%) were frequently isolated from aspergillosis, which is in line with previous reports.[33]

A multicenter study from the US reported that Vitek MS 3.0 provided a 98% accuracy rate on identification of filamentous fungi when considering all isolates tested in the database.[34] However, a study in France reported that MALD-ITOF MS had lower accuracy in identification of filamentous fungi of 51% for the MS system database 3.0.[35] Recently, a South Korean study evaluated the performance of the Vitek MS 3.0, with accuracy of 79.6% for filamentous fungi.[36] In our study, only three cryptic species were misidentified, due to those species not being included in the Vitek MS 3.0 system. Our results indicated that the MALDI-TOF MS method could be a useful tool for identification of filamentous fungi in a routine clinical laboratory.

In vitro susceptibility tests revealed that most *A. fumigatus* isolates were susceptible to all the antifungals tested in this study, similar to a recent report by a Chinese research group.[37] A retrospective study from Portugual reported that the activity of Afg and Cas against *A. fumigatus* isolates was 100%, while ITC, Vor, and Pos were effective against 95.8%, 97.4%, and 84.7% of *A. fumigatus* isolates, respectively, which is in good agreement with the results in our study.[38] The susceptibility results also showed that AmB was more effective than triazoles against *A. fumigatus* at multiple hospitals in Shanghai, China,[39] due to the fact that AmB is a polyene fungicidal agent with excellent activity.[40] For fungicidal actions, AmB, Afg, and Cas were superior to triazoles. On the other hand, the nephrotoxicity of AmB hinders its clinical use. Afg, Mcf, and Cas could be alternative agents to AmB for treatment of *Aspergillus* infection.

A 20-year antifungal-susceptibility study of *Aspergillus* spp. at a Chinese tertiary hospital[21] revealed that Vor was more active than Itr and AmB against *A. flavus*, in agreement with our findings. However, our results were limited by the retrospective design and data collection being dependent on information in medical records perhaps not representing characteristics of aspergillosis in China overall. In our hospital, the physicians administered prophylactic antifungal and empirical therapy according to the patient's clinical manifestations and risk factors. Vor is recommended by the IDSA guidelines,[14] and was the most used empirical antifungal therapy during the study period. There was a significant difference between outcomes of patients who received Vor antifungal therapy and those who did not.

Conclusion

Aspergillosis commonly occurs in immunocompromised patients and is mainly treated with empirical therapy in China. *A. fumigatus* was found to be the predominant *Aspergillus* species causing aspergillosis. Of note, *A. flavus*, a non–*A. fumigatus* species, is increasing, and is the second-leading cause of aspergillosis. In vitro antifungal-susceptibility testing is crucially important for decisions on effective therapy with aspergillosis. The Vitek MS method has demonstrated fast and effective identification of common *Aspergillus* spp. in clinical laboratories.

Data Sharing

The data presented in this study are openly available at the CNCB Genome Warehouse under the accession number PRJCA010148.

Ethics Approval and Informed Consent

The study was approved by the Ethics Committee of Shanghai JiaoTong University School of Medicine (protocol RJ2019204, August 2, 2019), conducted according to the guidelines of the Declaration of Helsinki, and required written informed consent to be taken from each participant (or a parent/legal guardian for patients under the age of 18 years) before enrollment in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, interpretation, or all these areas, took part in drafting, revising, or critically reviewing the article, gave final approval to 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.

Funding

This work was supported by grants from the Key Discipline of Public Health in Shanghai (GWV-10.1-XK04) and Excellent Technology Leader in Shanghai (20XD1434500), partly supported by a grant from Suzhou Bureau of Science and technology (SKY2022037) and from the People's Hospital of SND (SGY2019D02).

Disclosure

The authors declare no conflicts of interest in this work.

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