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ORIGINAL ARTICLE

In vitro antifungal activity of amphotericin B and 11 comparators against *Aspergillus terreus* species complex

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Summary

Aspergillus terreus infections are difficult to treat because of the intrinsic resistance to amphotericin B, and higher mortality compared to infections caused by other *Aspergillus* species. The aim of the present study was to determine the in vitro antifungal activity of amphotericin B and 11 comparators against clinical (n = 36) and environmental (n = 45) A. *terreus* isolates. In vitro antifungal susceptibility was performed using the CLSI M38--A2 procedure. Amphotericin B exhibited the highest MICs (MIC range, 0.125--4 μg/mL; MIC90, 2 μg/mL), followed by terbinafine (MIC range, 0.002--1 μg/mL; MIC90, 1 μg/mL). Only one isolate (1/81) showed amphotericin B MIC above the epidemiologic cut--off value (ECV; 4 μg/mL). None of the isolates had a MIC of≥ECV for voriconazole, itraconazole and posaconazole. The reasons for the difference in amphotericin B susceptibility patterns between studies remain unknown. The genetic and species diversity, clinical, environmental and ecological factors in *Terrei* section on various amphotericin B susceptibility profiles in different countries should be considered more as the main reasons associated with these differences.

KEYWORDS
amphotericin B, *Aspergillus terreus* species complex, In vitro antifungal activity, Iran

1 | INTRODUCTION

The emergence of drug resistance in fungal infections is one of the most significant epidemiological changes in current decades.[1] Thus, therapeutic options available for the treatment of these infections have become limited.[2] Amphotericin B is generally considered the mainstay of treatment of severe fungal infections. Invasive fungal infections due to *Aspergillus terreus* are difficult to treat because of the intrinsic resistance to amphotericin B, and higher mortality compared to infections caused by other *Aspergillus* species.[3] The in vitro and in vivo amphotericin B resistance in *A. terreus* isolates is not very well known so far. However, it may be correlated to less absorption and amphotericin B to cause cell death by oxidative damage.[4] In addition, upregulation of ERG5, ERG6 and ERG25 genes (ergosterol biosynthesis genes) could be responsible for decreased ergosterol content in the cell membrane leading to the development of resistance.[4,5] Clinical studies have shown a lack of response to amphotericin B and variable efficacy of azole therapy, particularly for voriconazole, with a high percentage of treatment failure in *A. terreus* infections.[6,7] Moreover, alterations in *cyp51A* such as the M217I mutation are responsible for acquired voriconazole resistance in *A. terreus*.[8] In many countries of the world, there are no epidemiological data about the antifungal susceptibility profiles of *A. terreus* available. The aim of the present study was to determine the in vitro antifungal activity of amphotericin B and 11 comparators against 81 clinical and environmental *A. terreus* isolates from different geographical regions in Iran.

2 | MATERIALS AND METHODS

In all, 81 clinical and environmental of *A. terreus* isolates were obtained from Sari (36.5659°N, 53.0586°E), Tehran (35.6892°N, 51.3890°E), Ahvaz (31.3183°N, 48.6706°E), Mashhad (36.2605°N, 59.6168°E) and Shiraz (29.5918°N, 52.5837°E). The collection consisted of 36 clinical isolates from a variety of specimens comprising, bronchoalveolar lavage fluid (n = 7), biopsy (n = 3), pharynx (n = 3), sinus (n = 1), sputum (n = 8) and nail (n = 14) (Table 1). In addition, 45 environmental isolates collected from air (n = 7) and soil (n = 38) samples in hospital surroundings were included (Table 1). Soil isolates were processed as previously described by Chowdhary et al.[9] and all isolates were deposited at −70°C in the reference culture collection of Invasive Fungi Research Center (IFRC), Mazandaran University of Medical Sciences, Sari, Iran. All isolates were identified to species level by DNA sequencing of partial β--tubulin gene, using primers Bt2a (5`--GGTAACCAAATCGGTGCTGCTTTC--3`) and Bt2b (5`--ACCCTCAG TGTAGTGACCCTTGGC--3`) as previously described by Hong et al[10] In vitro antifungal susceptibility was performed using the CLSI M38--A2 procedure.[11] Antifungal agents were dispensed into microdilution trays at final concentration ranges of 0.016--16 μg/mL for amphotericin B (Bristol--Myers--Squib, Woerden, Netherlands), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer, Central Research, Sandwich, United Kingdom), posaconazole (Merck, Whitehouse Station, NJ) and isavuconazole (Basilea Pharmaceuticals, Basel, Switzerland), 0.063--64 μg/mL for fluconazole (Pfizer, Groton, CT, USA), 0.001--1 μg/mL for lanoconazole and luliconazole (Nihon Nohyaku Co, Osaka, Japan), 0.004--4 μg/mL for terbinafine (Novartis Research Institute, Vienna, Austria), caspofungin (Merck Sharp & Dohme, Haarlem), micafungin (Astellas Pharma, Ibaraki, Japan) and anidulafungin (Pfizer, Central Research, Sandwich, United Kingdom). MICs were compared with previously published epidemiological cut--off values (ECVs) for AMB (4 μg/mL), ITC (1 μg/mL); VRC (1 μg/mL); POS (0.5 μg/mL); ISA (1 μg/mL) and CFG (0.25 μg/mL).[12,13] *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) reference strains were included as quality controls.[11] All tests were performed in duplicate and the Students t--test was used to compare MICs distribution between clinical and environmental *A. terreus* species complex isolates, with the SPSS statistical package (version 7.0). *P* values of <.05 were considered statistically significant.

2.1 | Patient details

The patients were primary included based on clinically suspected *Aspergillus* infection and confirmed by histopathology and mycology examination. Data on demographics, clinical characteristics, underly-ing condition, radiological features and mycological findings were col-lected with positive *A. terreus* cultures. The patients were categorised in four groups comprising, invasive aspergillosis (IA), aspergilloma, chronic pulmonary aspergillosis (CPA) and onychomycosis according to the diagnostic criteria.[14-16] The patient without proven or prob-able disease was classified into the colonised group. This study was approved by the Ethics Commission of Mazandaran University of Medical Sciences, Sari, Iran (nr. 2206).

3 | RESULTS

Molecular identification showed that 66 and 15 isolates were *A. ter-reus sensu stricto* and *A. citrinoterreus*, respectively. 33.3% (5 of 15) and 26.7% (4 of 15) of *A. citrinoterreus* were isolated from sputum and soil, respectively. 69.7% of *A. terreus sensu stricto* were also isolated from soil (n = 33) and nail (n = 13) samples. However, other cryptic species of *Aspergillus* section *Terrei* were not detected. Nucleotide sequences of all isolates have been deposited in GenBank under the accession numbers MF185011 to MF185091. Table 2 summa-rises the MIC range, mode, geometric mean (GM) MIC, MIC50 and MIC90 for 12 antifungal drugs against 81 *A. terreus* isolates. Among the antifungal drugs, amphotericin B exhibited the highest MICs (MIC range, 0.125--4 μg/mL; MIC90, 2 μg/mL), followed by terbinafine (MIC range, 0.002--1 μg/mL; MIC90, 1 μg/mL), voriconazole (MIC range, 0.125--1 μg/mL; MIC90, 0.5 μg/mL), posaconazole (MIC range, 0.031--0.5 μg/mL; MIC90, 0.5 μg/mL) and itraconazole (MIC range, 0.016--2 μg/mL; MIC90, 0.25 μg/mL). However, the novel imidazoles, that is, lanoconazole (MIC range, 0.001--0.031; MIC90, 0.031 μg/mL) and luliconazole (MIC range, 0.001--0.031; MIC90, 0.008 μg/mL) dem-onstrated potent activity against all *A. terreus* isolates, in comparison

TABLE 1 Isolation data of *Aspergillus terreus* species complex in Iran

Species nr (%)	IFRC nr	Beta Tubulin accession nr	Source	Specimen	Age/Sex	Underlying condition	First-line therapy	Origin
A. terreus sensu stricto, 66 (81.5%)	IFRC 1131	MF185016	Environmental	Soil	--	--	--	Tehran
	IFRC 1132	MF185017	Clinical	Pharynx	15/Female	Cystic fibrosis	ITC	Tehran
	IFRC 1133	MF185018	Clinical	BAL	31/Female	Bilateral pulmonary echinococcosis	ITC	Sari
	IFRC 1134	MF185019	Clinical	Nail	29/Male	Diabetes	ND	Shiraz
	IFRC 1135	MF185020	Clinical	Pharynx	21/Male	Cystic fibrosis	ITC	Tehran
	IFRC 1136	MF185021	Clinical	Nail	62/Female	None	ND	Shiraz
	IFRC 1137	MF185022	Clinical	Pharynx	18/Male	Cystic fibrosis	ITC	Tehran
	IFRC 1165	MF185023	Environmental	Soil	--	--	--	Sari
	IFRC 1166	MF185024	Environmental	Soil	--	--	--	Sari
	IFRC 1168	MF185025	Clinical	Biopsy (Lung)	52/Male	Tuberculosis pneumonia	ND	Tehran
	IFRC 1169	MF185026	Clinical	BAL	48/Male	Kidney Transplantation	AMB/VRC	Tehran
	IFRC 1171	MF185027	Clinical	Nail	25/Male	None	ND	Ahvaz
	IFRC 1172	MF185028	Clinical	Nail	25/Male	None	ND	Ahvaz
	IFRC 1173	MF185029	Clinical	Nail	46/Male	Diabetes	ND	Ahvaz
	IFRC 1174	MF185030	Clinical	Nail	46/male	ND	ND	Ahvaz
	IFRC 1175	MF185031	Clinical	Nail	27/Male	None	ND	Ahvaz
	IFRC 1177	MF185032	Clinical	Nail	27/Male	None	ND	Ahvaz
	IFRC 1178	MF185033	Clinical	Nail	53/Female	None	ND	Ahvaz
	IFRC 1179	MF185034	Clinical	Nail	53/Female	Diabetes	ND	Sari
	IFRC 1180	MF185035	Clinical	Nail	52/Male	Diabetes	ND	Sari
	IFRC 1181	MF185036	Environmental	Air	--	--	--	Tehran
	IFRC 1182	MF185037	Environmental	Air	--	--	--	Tehran
	IFRC 1282	MF185038	Clinical	Biopsy (Lung)	45/Male	Kidney transplant/ Diabetes	ITC/VRC	Sari
	IFRC 1283	MF185039	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1284	MF185040	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1286	MF185041	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1287	MF185042	Environmental	Air	--	--	--	Ahvaz
	IFRC 1288	MF185043	Environmental	Soil	--	--	--	Tehran
	IFRC 1290	MF185045	Clinical	BAL	45/Male	Asthma	ND	Tehran
	IFRC 1291	MF185046	Environmental	Air	--	--	--	Ahvaz
	IFRC 1292	MF185047	Environmental	Air	--	--	--	Ahvaz
	IFRC 1296	MF185048	Clinical	Sputum	35/Female	COPD	ND	Tehran
	IFRC 1516	MF185049	Clinical	Nail	63/Male	Diabetes	ND	Sari
	IFRC 1517	MF185050	Clinical	Nail	46/Female	None	ND	Sari
	IFRC 1519	MF185052	Environmental	Soil	--	--	--	Shiraz
	IFRC 1520	MF185053	Environmental	Soil	--	--	--	Shiraz
	IFRC 1521	MF185054	Environmental	Soil	--	--	--	Shiraz
	IFRC 1522	MF185055	Environmental	Soil	--	--	--	Shiraz
	IFRC 1524	MF185057	Clinical	Sputum	52/Male	Bronchitis	ND	Tehran
	IFRC 1531	MF185059	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1532	MF185060	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1533	MF185061	Environmental	Soil	--	--	--	Ahvaz

(Continues)

TABLE 1 (Continued)

Species nr (%)	IFRC nr	Beta Tubulin accession nr	Source	Specimen	Age/Sex	Underlying condition	First-line therapy	Origin
	IFRC 1535	MF185062	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1537	MF185063	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1539	MF185065	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1541	MF185066	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1542	MF185067	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1543	MF185068	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1544	MF185069	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1546	MF185070	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1547	MF185071	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1548	MF185072	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1549	MF185073	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1603	MF185074	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1604	MF185075	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1605	MF185076	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1606	MF185077	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1607	MF185078	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1608	MF185079	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1609	MF185080	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1613	MF185083	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1614	MF185084	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1665	MF185088	Clinical	Sputum	32/Male	Asthma	ND	Tehran
	IFRC 1685	MF185089	Clinical	BAL	51/Female	Lymphoma	ND	Mashhad
	IFRC 1686	MF185090	Clinical	BAL	43/Female	ND	ND	Mashhad
	IFRC 1687	MF185091	Environmental	Air	--	--	--	Mashhad
A. citrinoterreus, 15(18.5%)	IFRC 493	MF185011	Environmental	Air	--	--	--	Sari
	IFRC 1127	MF185012	Clinical	Biopsy	27/Male	Liver transplantation	AMB	Shiraz
	IFRC 1128	MF185013	Clinical	BAL	ND	COPD	AMB	Tehran
	IFRC 1129	MF185014	Clinical	Nail	21/Female	ND	ND	Sari
	IFRC 1130	MF185015	Clinical	Sinus	31/Female	None	ND	Tehran
	IFRC 1289	MF185044	Environmental	Soil	--	--	--	Tehran
	IFRC 1518	MF185051	Clinical	BAL	32/Female	Asthma	ND	Tehran
	IFRC 1523	MF185056	Clinical	Sputum	21/Female	Asthma	ND	Tehran
	IFRC 1525	MF185058	Clinical	Sputum	35/Female	Pneumonia	ND	Tehran
	IFRC 1538	MF185064	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1610	MF185081	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1612	MF185082	Environmental	Soil	--	--	--	Ahvaz
	IFRC 1662	MF185085	Clinical	Sputum	31/Female	Asthma	ND	Tehran
	IFRC 1663	MF185086	Clinical	Sputum	42/Male	Asthma	ND	Tehran
	IFRC 1664	MF185087	Clinical	Sputum	57/Male	Asthma	ND	Tehran

AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; BAL, Bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; IFRC, Invasive Fungi Research Center; ND, not determined.

to triazoles. Basically, the GM MIC value of luliconazole against all *A. terreus* isolates was >2 log2 dilutions lower than that of lanocona-zole. 1.2% (1/81) of the isolates showed amphotericin B MICs of≥4 μg/mL. None of the isolates had MICs of >2 μg/mL for voriconazole and itraconazole or >0.5 μg/mL for posaconazole. All *A. terreus* iso-lates had low MECs of caspofungin (MEC range, 0.004--0.016 μg/mL;

TABLE 2 In vitro activity of amphotericin B and 11 comparators antifungal agents against clinical and environmental *Aspergillus terreus* species complex

Strains and	MICs or MECs (μg/mL)															
antifungal drugs	Range	MIC_{50}/MIC_{90}	G mean	≤0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	≥16	≥64
Clinical (n = 36)																
AmB	0.25--2	1/2	0.8642						1	7	**24**	4				
FLU	64-->64	64/64	64													**36**
ITC	0.016--2	0.125/0.5	0.1321		3	5	3	**10**	9	3	2	1				
VRC	0.25--1	0.25/1	0.3762						**18**	13	5					
POS	0.125--0.5	0.125/0.5	0.1815					**25**	7	4						
ISA	0.0016--0.125	0.031/0.063	0.0339		**15**	4	14	3								
LCZ	0.001--0.031	0.016/0.016	0.0078	**17**	15	4										
LLCZ	0.001--0.031	0.001/0.008	0.0019	**35**		1										
TER	0.002--1	1/1	0.2646	5	1	2	2			9	**19**					
CFG	0.004--0.016	0.008/0.008	0.008	**35**	1											
AFG	0.008	0.008/0.008	0.008	**36**												
MFG	0.008--0.016	0.008/0.016	0.0093	**27**	9											
Environmental (n = 45)																
AmB	0.125--4	2/2	1.234					1	2	6	13	**22**	1			
FLU	64-->64	64/64	64													**45**
ITC	0.016--0.5	0.125/0.25	0.1427		1	5	3	16	**17**	3						
VRC	0.125--1	0.5/0.5	0.3407					5	16	**23**	1					
POS	0.031--0.5	0.125/0.5	0.1753			4		**25**	5	11						
ISA	0.001--0.063	0.031/0.063	0.0218	5	**15**	11	14									
LCZ	0.001--0.031	0.001/0.031	0.0026	**35**	4	6										
LLCZ	0.001--0.016	0.001/0.004	0.0014	**44**	1											
TER	0.002--1	0.031/1	0.0423	**13**	5	9	1		1	9	7					
CFG	0.004--0.008	0.008/0.008	0.0077	**45**												
AFG	0.008	0.008/0.008	0.008	**45**												
MFG	0.008--0.016	0.008/0.016	0.0086	**42**	3											
All isolates (n = 81)																
AmB	0.125--4	1/2	1.050					1	3	13	**37**	26	1			
FLU	64-->64	64/64	64													**81**
ITC	0.016--2	0.125/0.25	0.1379		4	10	6	26	**27**	5	2	1				
VRC	0.125--1	0.5/0.5	0.3564					5	34	**36**	6					

(Continues)

TABLE 2 (Continued)

Strains and antifungal drugs	MICs or MECs (μg/mL)															
	Range	MIC_{50}/MIC_{90}	G mean	≤0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	≥16	≥64
POS	0.031--0.5	0.125/0.5	0.1781			4		**50**	12	15						
ISA	0.001--0.125	0.031/0.063	0.0266	5	**30**	14	29	3								
LCZ	0.001--0.031	0.008/0.031	0.0042	**52**	19	10										
LLCZ	0.001--0.031	0.001/0.008	0.0016	**79**	1	1										
TER	0.002--1	0.5/1	0.0972	18	6	11	3		1	18	**24**					
CFG	0.004--0.016	0.008/0.008	0.0078	**80**	1											
AFG	0.008	0.008/0.008	0.008	**81**												
MFG	0.008--0.016	0.008/0.016	0.0089	**69**	12											
A. *terreus sensu stricto* (n = 66)																
AmB	0.125--4	1/2	0.9792					1	3	13	**30**	18	1			
FLU	64	64/64	64													**66**
ITC	0.016--1	0.125/0.5	0.1332		3	8	6	**21**	**21**	5	2					
VRC	0.125--1	0.5/0.5	0.3462					5	28	**30**	3					
POS	0.031--0.5	0.125/0.5	0.1576			4		**40**	8	14						
ISA	0.004--0.125	0.031/0.063	0.0287	5	**25**	10	24	2								
LCZ	0.001--0.031	0.008/0.031	0.0048	**42**	16	8										
LLCZ	0.001--0.031	0.001/0.008	0.0018	**64**	1	1										
TER	0.001--1	0.5/1	0.1192	12	5	10	1		1	15	**22**					
CFG	0.004--0.008	0.008/0.008	0.0079	**66**												
AFG	0.008	0.008/0.008	0.008	**66**												
MFG	0.008--0.016	0.008/0.016	0.0086	**59**	7											
A. citrinoterreus (n = 15)																
AmB	1--2	2/2	1.4472								7	**8**				
FLU	64	64/64	64													**15**
ITC	0.016--2	0.125/0.5	0.1504		1	2		**5**	**5**	1		1				
VRC	0.25--1	0.5/1	0.4352						**6**	**6**	3					
POS	0.125--0.5	0.125/0.25	0.1649					**10**	**4**	1						
ISA	0.016--0.125	0.031/0.063	0.0329		**5**	**5**	**4**	1								
LCZ	0.001--0.031	0.008/0.016	0.0045	**10**	3	2										
LLCZ	0.001--0.031	0.001/0.008	0.0019	**14**		1										
TER	0.001--1	0.063/1	0.0691	**5**	1	1	2			3	4					

(Continues)

TABLE 2 (Continued)

Strains and antifungal drugs	Range MIC	MICs or MECs (g/mL)μ																	
		50/MIC 90	G mean	≤0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	≥16	≥64			
CFG	0.004--0.016	0.008/0.008	**14**0.008	1															
AFG	0.008	0.008/0.008	**15**0.008																
MFG	0.008--0.016	0.008/0.016	**10**0.01007		5														

AMB, amphotericin B; ITC, itraconazole; FLU, fuconazole; VRC,ladfungin;MFG,micafungin;Numbersinboldaremodalvalue. ISA, isavuconazole; LCZ, voriconazole; POS, posaconazole; lanoconazole; LLC Z, e; luliconazol e; TE R, e; terbinafin e; CFG , caspofungin; AFG, anid u-

MEC90, 0.008 μg/mL), micafungin (MEC range, 0.008--0.016 μg/mL; MEC90,0.016 μg/mL) and anidulafungin (MEC range, 0.008 μg/mL; MEC90, 0.008 μg/mL). There were significant differences in MICs of terbinafine ($P < .0001$) and amphotericin B ($P < .002$) between clinical and environmental isolates. However, no significant differences were found in the activities of terbinafine, amphotericin B, lanoconazole, luliconazole and micafungin between *A. terreus* and *A. citrinoterreus (P* > .05).

3.1 | Patient isolates

In all, 36 *A. terreus* isolates were collected in six tertiary care centres from five different cities. The mean age was 59.2 ± 14.2 years and 21 patients (58.3%) were male. In total, 11 (30.5%) of 36 patients were classified into the colonised group and 25 (69.6%) of 36 into the aspergillosis group. Among the patients with aspergil-losis, IA was diagnosed in 6 (16.7%), aspergilloma in 2 (5.6%), CPA in 3 (8.3%) and onychomycosis in 14 (38.9%) patients. According to diagnostic criteria, two cases out of six IA patients were classi-fied as proven IA and the other four cases identified as probable IA. The risk factors for IA patients were associated with COPD (n = 2), solid organ transplants (n = 2), lymphoma (n = 1) and his-tory of tuberculosis (n = 1). Due to the lack of available treatment data for all patients, we were unable to analyse outcome. Among three IA patients with treatment information, two received ampho-tericin B alone and one was treated with amphotericin B followed by voriconazole. Five of 6 patients with IA had a fatal outcome within 3--5 weeks of diagnosis. One of five patients died of other causes not related to IA. All the three cases with CPA received itra-conazole for 2--18 weeks and two patients died during treatment. Two patients with aspergilloma were treated with a combination of surgery and antifungal therapy (the first one received itraconazole alone and the second itraconazole for 1 week followed by voricon-azole for 3 months).

4 | DISCUSSION

Aspergillus citrinoterreus as a new species was found in patients with (n = 2) and without IA. The majority of *A. citrinoterreus* had been isolated from sputum and was regarded as coloniser (33.3%). It is difficult to make firm conclusions of this new species because of the lack of a comprehensive patient history and laboratory data.

In the present study, 98.8% (80/81) of all isolates showed amphotericin B MICs of<ECV (4 μg/mL). Although several studies have shown that *A. terreus* species complex has intrinsic resistance to amphotericin B, some reports described *A. terreus* isolates with low MICs.[4,17-20] The reasons for the difference in amphotericin B susceptibility patterns between studies remain unknown, but it has been speculated that genetic and species diversity in *Terrei* sec-tion may play an important role. However, Tortorano et al[21] and Neal et al[22] were unable to show a relationship between low amphotericin B MICs and particular genotypes. Interestingly, in our

study, a large number of *A. terreus sensu stricto* isolates were found with low amphotericin B MICs. The data in the present study are in concordance with a study by Risslegger et al,[23] who reported low amphotericin MICs (MIC range, 0.25--0.5 mg/L) in 6.3% *A. terreus sensu stricto.* Investigation of the impact of clinical, environmental and even ecological factors on various amphotericin B susceptibil-ity profiles in different countries can be considered. In the present report, terbinafine has shown low in vitro activity against these isolates, consistent with data published by Garcia--Effron et al[24] with MIC range (0.003--4 μg/mL) and MIC90 (1 μg/mL). However, Fernandez et al. reported lower values (MIC range, 0.003--0.25 μg/ mL; MIC90, 0.25 μg/mL).[17] The variable methodologies employed in previously published studies, the failure of molecular identifica-tion and the emergence of cryptic species may be the main rea-sons associated with these differences. A prospective international

A. terreus analysis with 370 cases from 21 countries showed low susceptibility to amphotericin B against the majority of cryptic species, the amphotericin B profiles of *A. terreus* on a worldwide scale is still poorly understood and more research is needed.[23] Overall, in vitro susceptibility testing can help to select an appropriate therapy and to improve the management of patients infected with *A. ter-reus*. Susceptibility trends of *A. terreus* in surveillance studies can aid this objective.

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CONFLICT OF INTEREST

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