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Comparative activity of posaconazole and systemic azole agents against clinical isolates of filamentous fungi from a global surveillance programme

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Objectives: The activity of mould-active azoles was evaluated against 397 filamentous fungi causing invasive mould infections (IMI) worldwide. In addition, a tentative posaconazole epidemiological cut-off value (ECV) against *Aspergillus fumigatus* was investigated.

Methods: Isolates were susceptibility tested by the CLSI reference broth microdilution methods. Species identification was confirmed by MALDI-TOF and/or sequencing analysis.

Results: *Aspergillus* spp. (81.9%) remained the most common organism causing IMI worldwide; approximately two-thirds of *Aspergillus* spp. recovered were *A. fumigatus*. In general, more than 90% of 220 *A. fumigatus* isolates were wild type (WT) to all mould-active azoles, except itraconazole (84.5% WT). The voriconazole non-susceptible (NS) *A. fumigatus* rate was 7.7% overall and was higher in Europe (12.9%) than in the other regions (0%–5.8%). Posaconazole (MIC_{50}/MIC_{90}, 0.25/0.5 mg/L) showed similar or slightly higher activity than voriconazole (MIC_{50}/MIC_{90}, 0.5/0.5 mg/L) and isavuconazole (MIC_{50}/MIC_{90}, 0.5/1 mg/L) against *A. fumigatus*. The mould-active azoles displayed similar activity against non-*fumigatus Aspergillus* (WT rates >93%), but differences were observed among the main species/sections. Posaconazole, voriconazole, and isavuconazole inhibited at their respective ECVs 100%, 97.0%, and 100% of *A.* section *Nigri*; 100%, 100%, and 93.8% of *A.* section *Terrei*; and 97.3%, 100%, and 100% of *A.* section *Flavi* isolates. Posaconazole displayed potency greater than or equal to the other azoles against the Mucorales group and *Scedosporium* spp.

Conclusions: Posaconazole and other mould-active azoles showed good activity against *Aspergillus* spp. causing IMI, but clinicians should be aware of regional rates of voriconazole-NS *A. fumigatus*.

Introduction

The clinical impact of invasive mould infections (IMI) has increased markedly due to the growing number and diversity of immunocompromised hosts.[1] IMI remains difficult to diagnose and treat, threatening recent advances in managing patients who have undergone allogeneic haematopoietic stem cell or solid organ transplant as well as patients who are critically ill or suffer from malignancy, autoimmune, or inflammatory conditions.[2,3] The complexity of detecting and identifying filamentous fungi represents an important barrier to the determination of their epidemiology and the timely treatment of the infections they cause. This problem also contributes to the high mortality rates associated with these infections. However, the creation and widespread use of diagnostic tools and identification methods, such as MALDI-TOF and sequencing-based techniques, have gradually improved the management of IMI and increased clinician knowledge of filamentous fungi epidemiology.[4]

Although *Aspergillus fumigatus* is the leading cause of IMI worldwide, reported non-*Aspergillus* species infections have increased due to the improvement of diagnostic tools and the broader use of antifungal prophylaxis in immunosuppressed individuals. Members of the Mucorales order, which includes *Mucor* spp., *Rhizopus* spp., and *Lichtheimia* spp., are reported to be responsible for ∼5%–15% of IMI cases, while *Fusarium* spp. and *Scedosporium apiospermum* account for a variable proportion of these infections, depending on the geographic area.[4–6] Moreover, emerging fungal pathogens

exhibiting antifungal resistance to multiple classes, such as *Scopulariopsis* and *Microascus* spp., *Lomentospora prolificans*, and cryptic species of *Aspergillus*, are expanding the known list of opportunistic fungi that cause infections.[1]

Systemic triazoles are commonly administered to immunocompromised patients to prevent and treat invasive fungal infections. Four mould-active azoles are clinically available: itraconazole, voriconazole, posaconazole, and isavuconazole. Although these agents have activity against filamentous fungi, differences based on organism groups and species are noted as well as whether these agents have been approved for different indications. While posaconazole is approved by the United States Food and Drug Administration (US FDA) for prophylaxis of invasive *Aspergillus* and *Candida* infections and the treatment of oropharyngeal candidiasis, the remaining azoles are approved for treating invasive aspergillosis as well as other indications (package inserts). Nevertheless, the Infectious Diseases Society of America (IDSA) guidelines recommend posaconazole as salvage therapy in patients with refractory or progressive invasive aspergillosis.[2] Recently, a study evaluating the safety and efficacy of posaconazole versus voriconazole for the treatment of invasive aspergillosis has been completed and results are under analysis (NCT01782131). Posaconazole and isavuconazole display a broad-spectrum activity that includes *Aspergillus* spp. and most Mucorales organisms, while voriconazole is inactive against the latter group.[7] In the present investigation, we evaluated the *in vitro* activity of posaconazole and other mould-active azoles against a collection of *Aspergillus* spp. and other rare moulds collected worldwide in 2018. In addition, a tentative posaconazole epidemiological cut-off value (ECV) against *A. fumigatus* was determined and applied for comparison.[2]

Materials and methods

Organism collection

A total of 397 non-duplicate mould isolates causing invasive infections was collected from 41 medical centres located in North America (211 isolates from 21 medical centres in 2 countries), Europe (126 isolates from 14 centres in 11 countries), the Asia-Pacific region (APAC; 52 isolates from 4 medical centres in 3 countries), and Latin America (LATAM; 8 isolates from 2 medical centres in 2 countries). Participating medical centres submitted consecutively collected fungal isolates deemed by local criteria to cause invasive infections to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) as a part of the 2018 SENTRY Antimicrobial Surveillance Program. Fungal isolates were identified by MALDI-TOF (Bruker Daltonics, Bremen, Germany). Isolates not scoring ≥2.0 by spectrometry were submitted to confirmatory identification by sequencing and analysis of 28S ribosomal subunit (all isolates), and one of the following genes: β-tubulin for *Aspergillus* spp., translation elongation factor (TEF) for *Fusarium* spp., or internal transcribed spacer (ITS) for all other species of filamentous fungi.[8] Nucleotide sequences were analysed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared with available sequences through the internet using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). TEF sequences were analysed using the *Fusarium* multilocus sequence typing (MLST) database (http://www.westerdijkinstitute.nl/fusarium/).

Antifungal susceptibility testing

Isolates were tested for susceptibility by broth microdilution following the guidelines in the CLSI M38 document.[9] The following azoles were included in this study: posaconazole, isavuconazole, itraconazole, and voriconazole. Quality control was performed and interpreted as recommended in the CLSI documents M38 and M61 using *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Aspergillus flavus* ATCC 204304, *A. fumigatus* MYA-3626, and *Hamigera insecticola* ATCC MYA-3630.[9,10] The voriconazole clinical breakpoints approved by the CLSI for *A. fumigatus* (susceptible ≤0.5 mg/L; resistant ≥2 mg/L) were applied.[11] ECVs were applied following those criteria published in the CLSI M59 for posaconazole and comparators agents against *Aspergillus* spp., where available.[12]

ECV calculation

Since ECVs for posaconazole are not available for *A. fumigatus* and are not found in the CLSI method, we calculated the ECVs for 220 *A. fumigatus* isolates based on a 97.5% cut-off using following the ECOFFinder method described by the CLSI M57 document and Turnidge *et al.*[13] To verify our results, isavuconazole and voriconazole ECVs also were calculated for the same collection and compared with corresponding ECVs for *A. fumigatus* published by CLSI (Table S1, available as Supplementary data at *JAC-AMR* Online). The calculated *A. fumigatus* ECV for isavuconazole and voriconazole was not applied to azole non-wildtype MICs in this collection. CLSI ECVs were used instead.

Characterization of mutations in the sterol 14α-demethylase-encoding gene

A. fumigatus isolates displaying posaconazole MIC values above the calculated ECV and randomly selected isolates non-wildtype for any other azole were submitted to molecular detection of *CYP51A* and *CYP51B* mutations as previously described.[14] Sequences were compared with GenBank sequences available under the accession numbers *AAK73659.1* for *CYP51A* and *AAK73660.1* for *CYP51B*.

Results

The most frequent filamentous fungi isolated during the 2018 SENTRY Antimicrobial Surveillance Program were *A. fumigatus* (220 isolates; 55.4%), followed by *A.* section *Flavi* (37 isolates; 9.3%) and *A.* section *Nigri* (33 isolates; 8.3%). *A.* section *Terrei* and other species of *Aspergillus* combined contributed to 4.0% and 4.8% of all mould isolates, respectively. *Scedosporium* spp. (4.8%), *Fusarium* spp. (4.3%), and the Mucorales group (3.8%) were the most frequent organism groups found after *Aspergillus* (Table 1).

A. fumigatus contributed 57.3%, 55.6%, and 46.2%, of all isolates collected from North America, Europe, and APAC, respectively. Only eight filamentous fungi isolates were collected from LATAM,: five were *A. fumigatus*, two were *Aspergillus* section *Flavi*, and one was *Aspergillus* section *Nigri*. Among the non-*fumigatus Aspergillus* species, *A.* section *Nigri* was the most frequent organism group in North America (6.6%), followed by the equally distributed *A.* section *Flavi*, *Fusarium* spp., and *Scedosporium* spp. (4.7%). Conversely, *A.* section *Flavi* isolates were more frequently observed in the other regions (Europe, 13.5%; APAC, 15.4%), closely followed by *A.* section *Nigri* in Europe (10.3%). The APAC region showed a similar frequency of *A.* section *Nigri* and *Scedosporium* spp. (9.6% each) isolates.

In general, more than 90% of all *A. fumigatus* isolates were wild-type (WT) to all mould-active azoles, except itraconazole (84.5% WT). Based on MIC_{50} values, posaconazole (MIC_{50}/MIC_{90}, 0.25/0.5 mg/L) exhibited 2- to 4-fold greater activity than that observed for isavuconazole (MIC_{50}/MIC_{90}, 0.5/1 mg/L), voriconazole (MIC_{50}/MIC_{90}, 0.5/0.5 mg/L), and itraconazole (MIC_{50}/MIC_{90},

Table 1. MIC distribution for posaconazole when tested against a worldwide collection of moulds (2018)

Organism/organism group (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (mg/L) of:										MIC_{50}	MIC_{90}
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8		
Aspergillus fumigatus[a] (220)		0	10	114	90	5	0	1			0.25	0.5
		0.0	4.5	56.4	97.3	99.5	99.5	100.0				
North America (121)		0	2	61	57	1					0.25	0.5
		0.0	1.7	52.1	99.2	100.0						
Europe (70)		0	6	34	25	4	0	1			0.25	0.5
		0.0	8.6	57.1	92.9	98.6	98.6	100.0				
APAC (24)		0	1	18	5						0.25	0.5
		0.0	4.2	79.2	100.0							
LATAM (5)		0	1	1	3						0.5	–
		0.0	20.0	40.0	100.0							
Voriconazole-NS *A. fumigatus* (≥1 mg/L) (17)			0	4	7	5	0	1			0.5	1
			0.0	23.5	64.7	94.1	94.1	100.0				
Aspergillus section *Flavi* (37)		0	2	13	21	1					0.5	0.5
		0.0	5.4	40.5	97.3	100.0						
Aspergillus section *Nigri* (33)		0	2	1	21	9					0.5	1
		0.0	6.1	9.1	72.7	100.0						
Aspergillus section *Terrei* (16)		0	2	13	1						0.25	0.25
		0.0	12.5	93.8	100.0							
Other *Aspergillus* spp.[b] (19)		0	2	3	6	4	0	2	0	2	0.5	>8
		0.0	10.5	26.3	57.9	78.9	78.9	89.5	89.5	100.0		
Fusarium spp.[c] (17)					0	1	2	2	0	12	>8	>8
					0.0	5.9	17.6	29.4	29.4	100.0		
Mucorales group[d] (15)			0	1	7	5	1	0	1		0.5	2
			0.0	6.7	53.3	86.7	93.3	93.3	100.0			
Scedosporium spp.[e] (19)			0	1	1	3	13	0	0	1	2	2
			0.0	5.3	10.5	26.3	94.7	94.7	94.7	100.0		
Scedosporium apiospermum species complex (17)			0	1	1	3	11	0	0	1	2	2
			0.0	5.9	11.8	29.4	94.1	94.1	94.1	100.0		

Abbreviations: APAC, Asia-Pacific; LATAM, Latin America; NS, non-susceptible.
[a]*Aspergillus fumigatus* was not grouped into *Aspergillus* section *Fumigati* (222 isolates; including 220 *A. fumigatus* and 2 *A. lentulus*) due to the large number of isolates within this species and the tentative ECV evaluation for this species. Posaconazole MIC values against *A. lentulus* isolates are shown in the Supplementary data (Table S2).
[b]Organisms include: *Aspergillus* lentulus (2), *A. nidulans* species complex (5), *A. ochraceus* species complex (1), *A. sclerotiorum* (1), *A. sydowii* (2), *A. unguis* (1), *A. ustus* (4), *A. versicolor* (2), and *Aspergillus* spp. (1).
[c]Organisms include: *Fusarium* incarnatum-*equiseti* species complex (1), *F. oxysporum* species complex (3), *F. solani* species complex (8), and the *Gibberella fujikuroi* species complex (5).
[d]Organisms include *Lichtheimia* corymbifera (1), *L. ramosa* (1), *Mucor circinelloides/M. ramosissimus* (3), *Rhizomucor pusillus* (1), *Rhizopus microsporus* group (5), *R. oryzae* (3), and *Mucor* spp. (1).
[e]Organisms include: *Scedosporium apiospermum* species complex (17) and *S. aurantiacum* (2).

1/2 mg/L), regardless of the geographic region (Table 2). Applying the recently published clinical breakpoint, 7.7% of *A. fumigatus* isolates recovered in this study were non-susceptible (NS) to voriconazole (Table 2). Voriconazole-NS *A. fumigatus* isolates were most frequently recovered from Europe (12.9%), followed by North America (5.8%) and then the APAC region (1/17 isolates; 4.2%). Voriconazole-NS isolates were not observed in LATAM. Posaconazole (MIC_{50}/MIC_{90}, 0.5/1 mg/L) and isavuconazole (MIC_{50}/MIC_{90}, 1/4 mg/L) inhibited 64.7% and 58.8% of voriconazole-NS *A. fumigatus* isolates at their respective ECV (posaconazole, MIC ≤0.5 mg/L; isavuconazole, MIC ≤1 mg/L). Interestingly, 58.8% of voriconazole non-susceptible isolates were still wild-type for this compound.

The mould-active azoles showed a wide MIC distribution against *A. fumigatus*. Posaconazole MICs ranged from 0.12 to 4 mg/L, while isavuconazole and voriconazole ranged from 0.12 mg/L to >8 mg/L. The mode MIC values for posaconazole, voriconazole, isavuconazole, and itraconazole against *A. fumigatus* isolates were 0.25 mg/L, 0.5 mg/L, 0.5 mg/L, and 1 mg/L, respectively. The posaconazole ECV generated against *A. fumigatus* isolates from this collection at 97.5% was 0.5 mg/L (Table S1). The ECV values calculated at 95%, 97.5%, and 99% for posaconazole,

Table 2. Activity of azoles against *Aspergillus* spp. stratified by species complex and geographic region

	MIC_{50}/MIC_{90} (%S[a]/%WT[c])			
Species (no. tested)	Posaconazole[d]	Isavuconazole	Voriconazole	Itraconazole
Aspergillus fumigatus[a] (220)	0.25/0.5 (–/97.3)	0.5/1 (–/95.9)	0.5/0.5 (92.3/96.8)	1/2 (–/84.5)
Voriconazole-NS *A. fumigatus*[b] (17)	0.5/1 (–/64.7)	1/4 (–/58.8)	1/2 (0.0/58.8)	2/8 (–/47.1)
North America (121)	0.25/0.5 (–/99.2)	0.5/1 (–/97.5)	0.5/0.5 (94.2/99.2)	1/2 (–/80.2)
Europe (70)	0.25/0.5 (–/92.9)	0.5/1 (–/91.4)	0.5/1 (87.1/92.8)	1/2 (–/87.1)
APAC (24)	0.25/0.5 (–/100.0)	0.5/1 (–/100.0)	0.5/0.5 (95.8/95.8)	1/1 (–/100.0)
LATAM (5)	0.5/– (–/100.0)	1/– (–/100.0)	0.5/– (100.0/100.0)	1/– (–/100.0)
Aspergillus section *Flavi* (37)	0.5/0.5 (–/97.3)	0.5/1 (–/100.0)	0.5/1 (–/100.0)	1/1 (–/100.0)
Aspergillus section *Nigri* (33)	0.5/1 (–/100.0)	1/4 (–/100.0)	1/2 (–/97.0)	2/4 (–/100.0)
Aspergillus section *Terrei* (16)	0.25/0.25 (–/100.0)	0.5/1 (–/93.8)	0.5/0.5 (–/100.0)	0.5/1 (–/100.0)

Abbreviations: NS, non-susceptible; LATAM, Latin America; APAC, Asia Pacific region.
[a]*Aspergillus fumigatus* was not grouped into *Aspergillus* section *Fumigati* (222 isolates; including 220 *A. fumigatus* and 2 *A. lentulus*) due to the large number of isolates of this single species and the tentative ECV evaluation for this species. The azoles MIC values against *A. lentulus* isolates are shown in Table S2.
[b]Using voriconazole clinical breakpoint per CLSI criteria.
[c]Per CLSI criteria.
[d]Using posaconazole tentative ECV criteria (0.5 mg/L) against *A. fumigatus* calculated in this study and where previously published.[30,31]

Table 3. Summary of CYP alterations detected among azole non-wild-type *Aspergillus fumigatus* isolates

		MIC according to CLSI method (mg/L):				Amino acid substitutions:	
Organism	Country	Posaconazole	Isavuconazole	Itraconazole	Voriconazole	CYP51A	CYP51B
Aspergillus fumigatus	USA	1	1	2	1	I242V	WT
Aspergillus fumigatus	Belgium	1	2	4	2	TR_{34}/L98H	WT
Aspergillus fumigatus	Italy	1	4	8	2	TR_{34}/L98H	WT
Aspergillus fumigatus	Italy	1	4	4	2	TR_{34}/L98H	WT
Aspergillus fumigatus	Italy	1	4	4	2	TR_{34}/L98H	WT
Aspergillus fumigatus	Italy	4	>8	>8	>8	TR_{34}/L98H	WT
Aspergillus fumigatus	USA	0.5	1	2	0.5	I242V	WT
Aspergillus fumigatus	Italy	0.5	2	4	1	TR_{34}/L98H	WT
Aspergillus fumigatus	USA	0.5	4	2	2	WT	Q42L
Aspergillus fumigatus	Canada	0.5	1	2	0.5	WT	WT
Aspergillus fumigatus	Australia	0.25	0.5	1	2	WT	Q42L
Aspergillus fumigatus	Czech Republic	0.5	1	2	1	F46Y, M172V, N248T, D255E, E427K	WT
Aspergillus fumigatus	USA	0.5	1	2	0.5	A9T	WT

WT, wild type.

isavuconazole, itraconazole, and voriconazole are displayed in Table S1. At 97.5%, the voriconazole and isavuconazole ECVs assessed by this study were equivalent to the corresponding ECV values published in the CLSI M59 document; however, itraconazole yielded an ECV of 2 mg/L, which is one dilution higher than the published ECV.[12] Among the 220 *A. fumigatus* isolates tested, 97.3%, 95.9%, 96.8%, and 84.5% were wild-type for posaconazole, isavuconazole, voriconazole, and itraconazole (using the CLSI ECV), respectively. Among the six *A. fumigatus* isolates displaying a non-wildtype (NWT) profile to posaconazole, one isolate was from the US, one was from Belgium, and four were from a single medical centre in Italy. All the European isolates were also NWT to all other tested azoles and displayed L98H mutations in combination with a 34 base pair tandem repeat in the promoter region (TR_{34}/L98H) of the *CYP51A* gene (Table 3). Furthermore, among the seven randomly selected isolates displaying NWT to any other azole, the TR_{34}/L98H genotype was observed in one *A. fumigatus* from Italy that displayed an itraconazole and isavuconazole NWT phenotype. One *A. fumigatus* isolate from the Czech Republic harboured multiple mutations in *CYP51A* (F46Y, M172V, N248T, D255E, E427K) but was only NWT to itraconazole.[14] A Cyp51B Q42L mutation was detected in two isolates (from the US and Australia).Both isolates

were voriconazole NWT and one of them (USA isolate) was NWT to itraconazole and isavuconazole as well. In addition to *A. fumigatus*, only two *Aspergillus lentulus* isolates belonging to the *Aspergillus* section *Fumigati* were recovered in this study. Both *Aspergillus lentulus* isolates displayed a posaconazole MIC of 0.5 mg/L, while the other azoles showed MIC values of 2 mg/L (Table S2).

The mould-active azoles displayed similar activity (MIC_{50}/MIC_{90} ranges, 0.5–1/1 mg/L) against *Aspergillus* section *Flavi* isolates (Table 2). All isolates showed a WT phenotype to azoles, except one *A.* section *Flavi* isolate from Thailand, which displayed a posaconazole NWT phenotype (MIC, 1 mg/L). All *Aspergillus* section *Nigri* isolates were WT to posaconazole, isavuconazole, and itraconazole, and 97% of these isolates were WT to voriconazole. Based on MIC_{90} values, voriconazole (2 mg/L) and posaconazole (1 mg/L) were more potent than isavuconazole (4 mg/L) and itraconazole (4 mg/L) against *Aspergillus* section *Nigri*. All isolates of *Aspergillus* section *Terrei* were WT to posaconazole, voriconazole, and itraconazole. Only one *Aspergillus* section *Terrei* isolate from the US displayed an isavuconazole MIC value (2 mg/L) above the ECV criteria. *Aspergillus* section *Nidulantes* were generally WT to these agents, with MIC values of 0.12–1 mg/L for posaconazole, 0.015–0.25 mg/L for isavuconazole, 0.03–0.25 mg/L for voriconazole, and 0.12–2 mg/L for itraconazole (Table S2). Likewise, MIC values for *Aspergillus* section *Versicolores* ranged from 0.12 to 2 mg/L. *Aspergillus* section *Usti* isolates showed high MIC values for all azole agents (MIC ranged from 2 mg/L to >8 mg/L).

The activity of the azoles against non-*Aspergillus* moulds varied by organism and antifungal agent. In general, the non-*Aspergillus* moulds had higher MIC values with the tested agents when compared with *Aspergillus* spp. isolates (Tables 1, 4 and Table S2). Posaconazole, isavuconazole, and itraconazole exhibited activity against the Mucorales group, although differences were observed among the genera (Table 4). *Mucor* spp. isolates displayed higher MIC values (MIC range, 1–8 mg/L) for these azoles than *Rhizopus* spp., *Lichtheimia* spp., and *Rhizomucor* spp. isolates (MIC range, 0.25–2 mg/L). Voriconazole showed poor activity against Mucorales isolates (MIC range, 4 to >8 mg/L). Posaconazole ($MIC_{50/90}$, 2/2 mg/L) and voriconazole ($MIC_{50/90}$, 0.5/4 mg/L) exhibited greater activity against *Scedosporium* spp. isolates (mostly belonging to the *S. apiospermum* species complex) than isavuconazole ($MIC_{50/90}$, 8/8 mg/L) and itraconazole ($MIC_{50/90}$, 8/>8 mg/L). All triazoles showed poor activity against *Fusarium* spp., as these MIC values usually were greater than 8 mg/L, regardless of the species complex. Only one *Fusarium incarnatum-equiseti* species complex and two *Gibberella fujikuroi* species complex isolates displayed posaconazole MIC values ≤2 mg/L.

Discussion

To treat and manage IMI infections, clinicians should consider the fungal species, the immune status of the patient, the patient's previous exposure to antifungal agents, and the local or regional rates of azole resistance. IMI mortality rates are extremely high, approaching 50% in general and reaching 80%–100% among high-risk patients infected with azole-resistant *A. fumigatus* strains.[15,16] Despite wide and effective use of prophylaxis in high-risk patients, the overall incidence of invasive fungal infections continues to increase over time, mainly due to the increased immunocompromised population and improved diagnosis.[17,18] Identification of mould pathogens based on morphological features is challenging and requires well-trained personnel. Gold-standard sequencing identification is only accessible in reference or large medical institutions and thus is not routinely performed. MALDI-TOF has filled the gap as a cost-effective method with a

Table 4. Antimicrobial activity of posaconazole and comparator agents tested against mould isolates other than *Aspergillus* spp

Organism/organism group (no. of isolates)	Posaconazole		Isavuconazole		Itraconazole		Voriconazole	
	MIC range	MIC_{50}/MIC_{90}	MIC range	MIC_{50}/MIC_{90}	MIC range	MIC_{50}/MIC_{90}	MIC range	MIC_{50}/MIC_{90}
Fusarium spp. (*n* = 17)	1–>8	>8/>8	2–>8	>8/>8	>8	>8/>8	2–>8	8/>8
F. solani species complex (8)	>8	>8/–	8–>8	>8/–	>8	>8/–	8–>8	8/–
Gibberella fujikuroi species complex (5)	1–>8	4/–	2–>8	>8/–	>8	>8/–	2–>8	8/–
F. oxysporum species complex (3)	4–>8	>8/–	4–>8	>8/–	>8	>8/–	4–8	8/–
Fusarium incarnatum-equiseti species complex (1)	2	–	8	–	>8	–	2	–
Mucorales (*n* = 15)	0.25–8	0.5/2	0.5–8	1/2	1–8	2/4	4–>8	8/>8
Rhizopus spp. (8)	0.25–1	0.5/–	0.5–2	1/–	1–2	1/–	4–8	4/–
Mucor spp. (4)	1–8	1/–	2–8	4/–	2–8	4/–	>8	>8/–
Lichtheimia spp. (2)	0.5–1	0.5/–	2	2/–	1–2	1/–	>8	>8/–
Rhizomucor spp. (1)	0.5	–	1	–	1	–	8	–
Scedosporium spp. (*n* = 19)	0.25–>8	2/2	0.5–8	8/8	0.5–>8	8/>8	0.12–8	0.5/4
Scedosporium apiospermum species complex (17)	0.25–>8	2/2	0.5–8	8/8	0.5–>8	4/>8	0.12–8	1/4
S. aurantiacum (2)	2	2/–	8	8/–	8	8/–	0.5	0.5/–

MIC_{90} values were calculate for organism groups containing ≥10 isolates.

rapid turn-around time and has become an accurate identification method for the most frequent species causing IMI, adding great value to clinical practice and epidemiological studies.

Mould antifungal susceptibility tests also are not routinely performed in most clinical laboratories, although this is recommended for all patients suspected of having an invasive infection caused by an azole-resistant isolate or who are unresponsive to antifungal agents.[2,19] Antifungal management of IMI mainly relies on local epidemiology and regional or global antifungal surveillance data. Contemporary surveillance programmes that apply accurate methods for fungal identification and standard susceptibility testing for new and old antifungal drugs are important to monitor the management of these challenging infections. The results from 2018 SENTRY surveillance programme showed that, overall, the newer azoles (posaconazole, isavuconazole, and voriconazole) displayed greater activity against the 397 isolates of filamentous fungi than itraconazole. Specifically, the activity of posaconazole was equivalent to or greater than that displayed by other azoles against the most common filamentous fungi that cause invasive infections worldwide, including *A. fumigatus*, *A.* section *Flavi*, *A.* section *Nigri*, and *A.* section *Terrei*. These findings are concordant with earlier studies, which stated that posaconazole, voriconazole, and isavuconazole were more active than itraconazole against all *Aspergillus* species tested.[20,21] Notably, the posaconazole MIC_{50} and MIC_{90} values against *Aspergillus* groups remained stable when compared with the results described in a previous report against the same organism groups recovered in 2000.[21] Our findings continue to support the use of mould-active azoles against invasive aspergillosis, since *A. fumigatus* and non-*fumigatus Aspergillus* spp. displayed WT rates >95% to posaconazole, voriconazole, and isavuconazole. Although this data reaffirms the current clinical practices for managing of invasive aspergillosis, the increase in infections caused by azole non-susceptible *A. fumigatus* isolates in the past two decades has increased concern about how to better treat and prevent these infections.[2,19] Overall, the voriconazole susceptibility rate among *A. fumigatus* isolates causing invasive infections decreased from 98% in 2000[21] to 92.3% in the present study, and was only 87.1% in Europe. Notably, as reported previously, posaconazole NWT without itraconazole NWT was not observed among *A. fumigatus* isolates.[19] In a recent study including four European medical centres in the Netherlands and Belgium, the majority of voriconazole-resistant *A. fumigatus* isolates recovered displayed TR34/L98H or TR_{46}/Y121F/T289A mutations in the *CYP51A* gene.[22] Similarly, we found that TR34/L98H was the most frequent alteration among posaconazole NWT *A. fumigatus* isolates from Europe. This genotype confers resistance to itraconazole and variable susceptibility phenotypes to voriconazole, posaconazole, and isavuconazole.[23] Other mutations in Cyp51A or its homologue, Cyp51B, have been previously reported and associated with elevated azole MIC results in *A. fumigatus*.[14]

Despite the improvement of IMI outcomes due to the introduction of new triazoles, the management of these infections remain suboptimal for immunocompromised patients.[24,25] As *Aspergillus* spp. remains the most common group of filamentous fungi causing invasive infection worldwide, and *A. fumigatus* accounts for approximately two-thirds of the isolates grouped in this collection, the emergence of non-*Aspergillus* mould infections is worrisome since they are associated with poor outcomes. Overall, non-*Aspergillus* mould isolates were responsible for 72 infection events (18.1%). Although triazole therapy is the primary option to treat infections caused by non-*Aspergillus* moulds, antifungal activity disparities among triazole agents are observed and need to be taken into consideration.[26] Our data is aligned with this observation and showed that posaconazole displayed activity against the *Mucorales* group while voriconazole activity was limited against the same group of organisms. Conversely, voriconazole and posaconazole were active against the majority of *Scedosporium* spp. isolates, while isavuconazole and itraconazole displayed limited activity against these organisms. All triazoles exhibited limited activity against *Fusarium* spp. isolates, regardless of the species complex, and only a few isolates showed triazole MIC values $\leq$2 mg/L. Notably, MIC values may have a wide range within an organism group (as for *Scedosporium* spp.) or a single species complex (as for *Gibberella fujikuroi* species complex), emphasizing that susceptibility testing results are critically important to drive therapeutic choices.

Posaconazole and other azole derivatives inhibit the biosynthesis of the ergosterol, an essential component of the fungal cell membrane, by inhibiting the enzyme lanosterol 14α-demethylase. Currently, three posaconazole formulations are available for prophylaxis of invasive *Aspergillus* and *Candida* infections, namely an oral suspension (40 mg/mL), a delayed-release tablet (100 mg), and an intravenous formulation (18 mg/mL). While there is supporting evidence that posaconazole showed an exposure–response relationship in clinical studies, therapeutic drug monitoring of azole therapy is recommended to adjust antifungal dosing to ensure adequate exposure and improve the probability of optimal outcomes.[19,23] For patients receiving posaconazole suspension, a plasma trough of >0.7 mg/L is recommended during prophylaxis.[19] Even though posaconazole oral suspension has been used successfully in first-line treatment of IMI, it is limited by inconsistent oral absorption.[19,27] The introduction of extended-release tablets and the intravenous formulation of posaconazole more easily achieves increased serum drug levels and thus are preferred for the treatment of IMI.[19,28] Results of the pharmacokinetic analysis revealed that higher plasma concentrations were associated with greater response rates in invasive aspergillosis. Posaconazole plasma average concentration (C_{avg}) $\geq$1.25 mg/L at steady-state proved to be associated with 75% successful response rate.[29] Nevertheless, further studies are needed to address whether higher posaconazole levels are associated with toxicity and whether monitoring the plasma level is helpful or necessary for extended-release or intravenous formulations.[2]

In the present investigation, we evaluated the *in vitro* activity of posaconazole and other mould-active azoles against a large collection of filamentous fungal isolates causing invasive disease from worldwide hospitals. All isolates were identified to species or group level using MALDI-TOF and sequencing analysis. Differences among triazole agents were observed and, except for *Fusarium* spp., posaconazole demonstrated potent *in vitro* activity against all mould groups, including many uncommonly isolated species for which very limited susceptibility information is available to guide contemporary therapy. Overall, 88.4% of all 397 mould isolates tested were inhibited at the posaconazole MIC value of 1 mg/L. Additionally, 97.7% of *Aspergillus* isolates were inhibited by posaconazole at their respective ECVs when available or

determined by this study. Clinical studies of posaconazole for treatment of IMI should be considered based on these *in vitro* data.

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Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

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