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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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In vitro activities of antifungal drugs against *Rhinocladiella mackenziei*, an agent of fatal brain infection

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Sir,

Cerebral phaeohyphomycosis is a rare disease with a mortality of up to 70% despite combinations of surgical and antifungal therapy.[1] This infection is predominantly caused by *Exophiala dermatitidis*, *Cladophialophora bantiana* and *Rhinocladiella mackenziei* (order Chaetothyriales, family Herpotrichiellaceae), although organisms from other orders including Pleosporales, Sordariales, Xylariales and Helotiales can also cause infection. *Rhinocladiella* is a genus of black yeast-like fungi and can cause human infections ranging from mild cutaneous lesions to fatal brain infections.[2] *R. mackenziei* (formerly *Ramichloridium mackenziei*) is an extremely neurotropic fungus, and infections that invade the CNS cause death in most instances.[1,2] The infection is restricted largely to the Middle East, especially Saudi Arabia and Kuwait, although sporadic cases involving visitors or immigrants from endemic areas have been diagnosed in the UK and the USA. No clear epidemiological factors other than area of residence link these patients. Most *R. mackenziei* infections are brain abscesses in patients with no predisposing factors or immunodeficiency.[1,2] Symptoms included headache, neurological deficits and seizures. Pathogenesis may be haematogenous from a subclinical pulmonary focus, although it remains unclear why this fungus preferentially causes CNS disease in immunocompetent individuals.[2] Treatment regimens, which involve mostly amphotericin B, itraconazole and 5-flucytosine, are associated with a poor outcome in both animal and clinical studies.[3] *R. mackenziei* demonstrates *in vitro* resistance to amphotericin B but susceptibility to triazoles such as itraconazole.[3–5] Experimental *R. mackenziei* cerebral infection in mice indicates that posaconazole is superior to either amphotericin B or itraconazole for treatment of this condition.[3]

Here we describe the *in vitro* activity of a new triazole antimycotic, isavuconazole, and seven comparators against 10 clinical isolates of *R. mackenziei*. Strains of *R. mackenziei* (nine from cerebral abscesses, one from a liver abscess) were obtained from the CBS-KNAW Fungal Biodiversity Centre (Utrecht, The Netherlands) and identified as *R. mackenziei* by sequencing of the internal transcribed spacer regions of rDNA. Antifungal susceptibility testing was performed in microdilution plates according to CLSI guidelines[6] after 96 h of incubation at 35°C. Amphotericin B (Bristol–Myers Squibb, Woerden, The Netherlands), fluconazole (Pfizer Central

Table 1. Source, clinical data and susceptibility (mg/L) of *R. mackenziei* strains ($n=10$)

CBS no.	Other collection no.	Site of infection	Sex	Age (years)	Risk factor	Therapy	Outcome	Country	MIC (mg/L)							
									AMB	FLC	ITC	VOR	POS	ISA	CAS	ADF
CBS 650.93	NCPF 2808	brain abscess	F	55	–	AMB, 5FC, KET	died	Saudi Arabia	8	32	0.063	0.5	0.031	0.25	4	2
CBS 367.92	NCPF 2738	brain abscess	F	60	–	–	–	Israel	8	16	0.125	1	0.016	0.25	8	8
CBS 368.92	UTMB 3170	brain abscess	M	–	–	–	died	Israel	8	32	0.125	2	0.031	0.5	8	4
CBS 102587	NCPF 2810	brain abscess	M	70	bowel surgery	AMB, 5FC	died	Saudi Arabia	8	32	0.25	2	0.063	1	8	2
CBS 102588	NCPF 2780	hepatic abscess	M	55	renal transplant	AMB	died	Qatar	8	32	0.125	1	0.031	0.5	4	2
CBS 102590	NCPF 2853	brain abscess	M	–	–	–	died	UAE	8	32	0.25	1	0.036	0.25	8	2
CBS 102591	NCPF 7123	brain abscess	F	67	diabetes mellitus	AMB	died	Saudi Arabia	8	16	0.125	0.5	0.016	0.5	8	8
CBS 102592	NCPF 7460	brain abscess	M	65	Hodgkin's lymphoma	AMB	died	Saudi Arabia	16	32	0.25	2	0.031	1	4	8
CBS 109634	–	brain abscess	M	56	hepatitis B infection	AMB	died	Egypt	8	32	0.125	1	0.031	0.5	4	2
CBS 102589	NCPF 2779	brain abscess	F	32	renal transplant	–	–	Oman	2	64	0.125	0.25	0.031	1	4	1

F, female; M, male; AMB, amphotericin B; FLC, fluconazole; ITC, itraconazole; VOR, voriconazole; POS, posaconazole; ISA, isavuconazole; CAS, caspofungin; ADF, anidulafungin; 5FC, 5-flucytosine; KET, ketoconazole; CBS, Centraalbureau voor Schimmelcultures; NCPF, National Collection of Pathogenic Fungi; UTMB, University of Texas Medical Branch.

Research, Sandwich, Kent, UK), voriconazole (Pfizer), anidulafungin (Pfizer), itraconazole (Janssen Research Foundation, Beerse, Belgium), posaconazole (Schering-Plough, Kenilworth, NJ, USA), isavuconazole (Basilea Pharmaceutica International Ltd, Basel, Switzerland) and caspofungin (Merck Sharp & Dohme BV, Haarlem, The Netherlands) were obtained as reagent grade powders. The antifungal agents were dispensed into microdilution trays at a final concentration of 0.016–16 mg/L for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin, 0.063–64 mg/L for fluconazole and 0.008–8 mg/L for isavuconazole and anidulafungin. Conidial inocula were prepared under biosafety laboratory (level 3) regulations from ≤2 week potato dextrose agar cultures by gently scraping the surface of mature colonies with a sterile cotton swab moistened with sterile physiological saline containing Tween 40 (0.05%). Supernatants were adjusted spectrophotometrically (530 nm) to a percentage transmission in the range 68–71 (corresponding to $1.5–4\times10^6$ cfu/mL). Quality control strains *Paecilomyces variotii* ATCC 22319, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were included in each assay run. The MIC endpoint for amphotericin B, itraconazole, voriconazole, posaconazole and isavuconazole, determined with the aid of a magnifying mirror, corresponded to the lowest drug concentration resulting in wells without any recognizable growth; the MIC endpoint for fluconazole was defined as the minimum drug concentration resulting in a prominent reduction (≥50%) of growth. For the echinocandins, MICs were determined microscopically as the lowest concentration of caspofungin or anidulafungin leading to formation of small, rounded, compact hyphal forms rather than the long unbranched hyphal clusters seen in growth controls devoid of antimycotic.

The origin, CBS identification numbers, clinical data and MIC values for the *R. mackenziei* isolates surveyed are summarized in Table 1. MIC ranges for amphotericin B (2–16 mg/L), voriconazole (0.25–2 mg/L) and anidulafungin (1–8 mg/L) ranged over 3 $\log_2$ dilution steps, whereas MIC ranges for the remaining azoles ranged over 2 $\log_2$ dilution steps, and the MIC range for caspofungin (4–8 mg/L) ranged over 1 $\log_2$ dilution step. Fluconazole had high MICs, indicating poor activity against this pathogen, while the lowest MIC values were seen for posaconazole (MIC_{90} 0.063 mg/L). Drug treatment of human *R. mackenziei* cerebral phaeohyphomycosis, according to several case reports, has been unsuccessful despite aggressive therapy with amphotericin B and/or itraconazole, with or without 5-flucytosine and irrespective of surgical intervention;[1–4] only a single patient is reported to have survived an *R. mackenziei* cerebral infection, with pronounced radiological and clinical improvement after switching therapy from a combination of liposomal amphotericin B, 5-flucytosine and itraconazole to posaconazole.[5] This sole surviving patient relapsed on itraconazole and displayed symptoms of progressive disease after several months of treatment with voriconazole, though when posaconazole therapy was resumed his condition improved significantly.[5] Treatment of *R. mackenziei* cerebral infection with posaconazole is supported by *in vitro* results and data from a murine infection model,[3] in which posaconazole prolonged the survival of mice and reduced the brain fungal burden compared with itraconazole and amphotericin B. Its apparently good penetration into the CNS,[7] combined with excellent *in vitro* data and activity in animal models,

supports use of posaconazole for this difficult to treat infection. Most melanized fungi appear to be non-susceptible to echinocandins, probably due to a reduced presence of β-glucan in their cell walls,[8] and we found poor activity of caspofungin and anidulafungin against *R. mackenziei* (MIC_{90} 8 mg/L). Posaconazole and itraconazole (MIC_{90} 0.25 mg/L) demonstrated the best *in vitro* activities, followed by isavuconazole (MIC_{90} 1 mg/L), though clinical experience strongly suggests that itraconazole is not a suitable choice for treating an *R. mackenziei* cerebral infection.[4,5] The new drug isavuconazole may be a promising alternative for this indication, but its activity against *R. mackenziei* needs to be confirmed in animal models.

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