In vitro activity of olorofim against clinical isolates of Scedosporium species and Lomentospora prolificans using EUCAST and CLSI methodologies

Olga Rivero-Menendez , Manuel Cuenca-Estrella, Ana Alastruey-Izquierdo.

J Antimicrob Chemother. 2020 Dec 1;75(12):3582-3585.

OXFORD UNIVERSITY PRESS | Journal of Antimicrobial Chemotherapy

In vitro activity of olorofim against clinical isolates of *Scedosporium* species and *Lomentospora prolificans* using EUCAST and CLSI methodologies

Journal:	*Journal of Antimicrobial Chemotherapy*
Manuscript ID	JAC-2020-0913.R1
Manuscript Type:	Brief report
Date Submitted by the Author:	n/a
Complete List of Authors:	Rivero-menendez, Olga; Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Majadahonda Cuenca-Estrella, M.; Instituto de Salud Carlos III, Mycology Reference Laboratory, National Centre for Microbiology Alastruey-Izquierdo, Ana; Instituto de Salud Carlos III, Mycology Reference Laboratory, National Centre for Microbiology; Spanish Network for the Research in Infectious Diseases (REIPI RD16/CIII/0004/0003),
Keywords:	Scedosporium, Lomentospora, Antifungal activity, Antifungal resistance, In vitro susceptibility

In vitro activity of olorofim against clinical isolates of *Scedosporium* species and *Lomentospora prolificans* using EUCAST and CLSI methodologies

Olga RIVERO-MENENDEZ, Manuel CUENCA-ESTRELLA, Ana ALASTRUEY-IZQUIERDO*

Mycology Reference Laboratory, National Centre for Microbiology. Instituto de Salud Carlos III. Majadahonda, Madrid, Spain. Spanish Network for the Research in Infectious Diseases (REIPI RD16/CIII/0004/0003), Instituto de Salud Carlos III, Madrid, Spain.

*Corresponding author:

Ana Alastruey-Izquierdo

Mycology Reference Laboratory

Centro Nacional de Microbiología. Instituto de Salud Carlos III

28220. Majadahonda. Madrid. Spain.

Phone: +34-91 822 3784

Fax: 91 509 7966

E-mail: anaalastruey@isciii.es

Running title: Activity of olorofim to *Scedosporium* species and *Lomentospora prolificans*

Abstract

Objectives

To evaluate the *in vitro* activity of olorofim, a new broad-spectrum antifungal with a novel mechanism of action, against a collection of 123 Spanish clinical isolates belonging to five *Scedosporium* species and *Lomentospora prolificans*.

Methods

The activity of olorofim against *Scedosporium apiospermum* (n=30), *Scedosporium boydii* (n=30), *Scedosporium ellipsoideum* (n=10), *Scedosporium aurantiacum* (n=20), *Scedosporium dehoogii* (n=3) and *Lomentospora prolificans* (n=30) was compared with that of amphotericin B, voriconazole, isavuconazole and micafungin by performing EUCAST and CLSI reference methods for antifungal susceptibility testing.

Results

Amphotericin B and isavuconazole showed MICs ≥ 2 mg/L against all the species evaluated, and voriconazole was moderately active (GM, MIC_{50} and MIC_{90} values ≤ 2 mg/L) against all of them except *L. prolificans*. Micafungin was effective against *S. apiospermum* complex strains but exhibited elevated MECs against *S. dehoogii* and *S. aurantiacum*. Olorofim showed low MICs against all the *Scedosporium* strains tested (GM values were lower than 0.130 and 0.339 by EUCAST and CLSI, respectively, for all of the species), including those belonging to the multi-drug resistant species *L. prolificans*, for which GM values were 0.115 mg/L and 0.225 mg/L by EUCAST and CLSI, respectively, while for the rest of antifungals evaluated GMs were higher than 3.732 mg/L using both methodologies.

Conclusions

Olorofim displays a promising *in vitro* activity against *Scedosporium* and *L. prolificans* strains tested, some of which have reduced susceptibility against the antifungals that are currently in use.

Introduction

Aspergillus, *Candida* and *Cryptococcus* species are responsible for the largest number of invasive fungal infection cases,[1] which are being increasingly reported throughout the world. However, the epidemiology of these diseases has changed over the last years enhancing the appearance of emerging pathogens, like *Scedosporium* spp.[2]

Species such as *Scedosporium apiospermum* (which is identified as a species complex that includes *Scedosporium apiospermum sensu stricto* (*s.s.*), *Scedosporium boydii*, *Scedosporium angustum*, *Scedosporium ellipsoideum* and *Scedosporium fusoideum*), *Scedosporium aurantiacum*, *Scedosporium dehoogii* or *Lomentospora prolificans* (formerly *Scedosporium prolificans*) cause an invasive infection called scedosporiosis (or lomentosporiosis if caused by *L. prolificans*), which is considered life-threatening due to the reduced effectiveness that antifungals in clinical use have against it.[3,4] Consequently, there is an urgent need for the development of new antifungal agents with novel mechanisms of action that are active against these species and that help to address the limitations that available treatments have.

Olorofim is the first clinical compound from the novel antifungal class of the orotomides, and its mechanism of action is based on the inhibition of the dihydroorotate dehydrogenase (DHODH), an enzyme that catalyses one of the key steps in the pyrimidine biosynthesis pathway. This, consequently, compromises the synthesis of nucleic acids, proteins, the fungal cell wall and the fungal cell membrane. Even though mammalian cells carry a form of this enzyme, the activity of this new compound is highly fungal-specific, thus, providing an encouraging safety profile with reduced toxicity.[5] Its *in vitro* activity has been proved against a wide variety of pathogenic fungal species, like *Histoplasma capsulatum*, *Coccidioides posadasii*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Madurella mycetomatis* or those from the genera *Penicillium*, *Aspergillus* (including cryptic species and azole resistant *Aspergillus fumigatus*), *Fusarium* and dermatophytes, although olorofim is not active against *Candida* spp., *Cryptococcus* spp. or Mucorales.[5-11] It has also led to promising *in vivo* results when administered orally or intravenously in murine models of infection of aspergillosis and coccidiomycosis, and shows good distribution into tissues, including the brain.[5,8,11-13]

This new drug has also proved to have *in vitro* efficacy against *Scedosporium* and *Lomentospora* species [5,14-16] and has demonstrated marked improvements in survival in neutropenic mice with systemic infections caused by *S. apiospermum s.s.*, *S. boydii* and *L. prolificans*.[17] Nevertheless, only a low number of clinical strains from a few *Scedosporium* species have been *in vitro* tested, and further investigations are required to confirm olorofim's activity on isolates from distinct geographical areas. Therefore, the aim of the present study is to evaluate the *in vitro* activity of olorofim and comparator antifungal agents against a collection of clinical isolates of Spanish *Scedosporium* and *Lomentospora* species using the EUCAST and CLSI standardized reference methodologies for antifungal susceptibility testing. This study represents the first European assessment of the *in vitro* activity of olorofim against the *Scedosporium* genus.

Materials and methods

A total of 123 strains from different *Scedosporium* species and *L. prolificans* were tested. All strains were obtained from clinical samples (respiratory, cutaneous, oculars, optical, biopsies, abscess, blood cultures and wounds) in Spanish hospitals and identified to species level by standard microscopic morphology and by sequencing the Internal Transcribed Spacer Region of the rDNA and part of the β-tubulin gene following methods previously reported.[18]

Antifungal Susceptibility testing was performed following EUCAST reference method 9.3.2 [19] and CLSI M38A.[20] Antifungals used were olorofim (range 0.004-2 mg/L; F2G Limited, Manchester, United Kingdom), amphotericin B (range 0.03-16 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), voriconazole (range 0.015-8 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), isavuconazole (range 0.015-8 mg/L; Basilea Pharmaceutica, Basel, Switzerland) and micafungin (range 0.004-2 mg/L; Astellas Pharma Inc, Tokyo, Japan).

Aspergillus flavus ATCC 204304 and *A. fumigatus* ATCC 204305 were used as quality control strains in all test performed for both methods. MICs for amphotericin B, voriconazole, isavuconazole and olorofim, and minimal effective concentrations (MECs) for micafungin were visually read after 24, 48 and 72 hours of incubation at 35ºC in a humid atmosphere. Geometric mean (GM), MIC/MEC_{50} (MIC/MEC causing inhibition of

50% of the isolates tested) and MIC/MEC_{90} (MIC/MEC causing inhibition of 90% of the isolates tested) were determined. For calculation purposes, the MIC or MEC values that exceeded the maximum concentration tested were transformed to the next dilution (i.e. if MIC/MEC was >16 mg/L it was expressed as 32 mg/L) and values that were less than or equal to the minimum concentration tested were transformed to equal (i.e. if MIC/MEC was ≤0.03 mg/L it was expressed as 0.03 mg/L).

Results and discussion

Table 1 shows the GM, MIC/MEC_{50}, MIC/MEC_{90} and range for all the species tested for each antifungal susceptibility testing method at 48 hours of incubation by EUCAST and 72 hours of incubation by CLSI. MIC/MEC_{50} and MIC/MEC_{90} were only calculated for species that had ten or more isolates. Table S1 (available online as Supplementary data) displays MIC/MEC distributions for each species against all the antifungals tested.

Overall, olorofim was the only compound exhibiting low MICs (below 1mg/L) against all strains and species tested both by EUCAST and CLSI.

Regarding the *S. apiospermum* complex, all three species tested displayed high MIC values for amphotericin B and isavuconazole but lower MICs for voriconazole. MIC_{90}s for amphotericin B and isavuconazole were ≥16 mg/L using both methodologies, whereas voriconazole MIC_{90}s were 2 mg/L by both EUCAST and CLSI methods. These values are in agreement with those from previous reports on the activity of these antifungals against *Scedosporium* species,[3,16] which reflect that even though voriconazole is considered the first line treatment for scedosporiosis, its activity against some of the species that cause it is limited. Micafungin MEC_{90} values ranged from 0.25 mg/L to 0.5 mg/L both by EUCAST and CLSI for *S. apiospermum s.s.*, *S. boydii* and *S. ellipsoideum* strains, but olorofim was the most active drug against them, showing lower GM values than those of micafungin except for *S. apiospermum s.s.* and *S. ellipsoideum* strains by CLSI, for which they were similar. In addition, it is important to highlight that the activity for olorofim is determined as MIC while for micafungin it is assessed as MEC. These results are comparable to those from a previous study, in which olorofim yielded slightly higher MIC values against these species but was still active.[14]

Amphotericin B, isavuconazole and micafungin showed poor activity against the *S. aurantiacum* strains tested, while voriconazole exhibited similar MICs than those yielded

against *S. apiospermum* (GM values were 0.966 mg/L by EUCAST and 1.366 mg/L by CLSI for *S. aurantiacum*). Olorofim displayed the lowest MICs with GMs of 0.130 mg/L and 0.339 mg/L by EUCAST and CLSI, respectively, confirming results reported in previous studies.[14,16]

Scedosporium dehoogii strains tested were inhibited by olorofim (MICs ranged from 0.06 and 0.25 mg/L). Micafungin showed a similar activity (GMs of 0.250 mg/L), whereas voriconazole exhibited a moderate effectiveness and amphotericin B and isavuconazole were inactive against them, with GM values higher than 8 mg/L.

Regarding *L. prolificans*, olorofim was the only active compound against the strains tested with a MIC_{50} value of 0.12 mg/L by EUCAST and 0.25 mg/L by CLSI. The high MIC/MEC values displayed by the rest of the antifungals tested reasserted the fact that *L. prolificans* is an intrinsically multidrug-resistant species.[3] Nevertheless, the good activity yielded by olorofim in this and in previous studies [14-16] seems promising in order to develop an effective treatment against infections caused by this species, as even though the combination of voriconazole and terbinafine has been associated with positive outcomes in patients with invasive *L. prolificans* infections, overall mortality rates are still high.[21]

In addition to its good activity against *Scedosporium* and *L. prolificans* strains, olorofim has also been reported as being highly *in vitro* active against a broad number of *Aspergillus* species, including azole resistant *A. fumigatus* isolates both harboring *cyp51A* alterations and without known resistance mechanisms,[5-7] and those defined as cryptic that are usually multi-drug resistant.[8,10] The *in vitro* potential development of olorofim resistance has been studied in susceptible *A. fumigatus* isolates, proving that their spontaneous resistance rates are lower than those obtained with other antifungals, which would make olorofim a better therapy against infections caused by this species than azoles. This study also led to the description of a low frequency resistance mechanism to olorofim, as these strains harbored mutations in *pyrE* gene, which encodes the DHOH.[22] Besides, studies in murine models of invasive aspergillosis caused by susceptible and azole-resistant strains belonging to several *Aspergillus* species have also been successfully conducted, showing higher survival rates in mice treated with olorofim than with other antifungals.[8,12,13] This drug is currently undergoing phase II as an oral and intravenous formulation for the treatment of invasive aspergillosis and of

hard-to-treat rare mould infections, such as scedosporiosis, that lack of an appropriate therapy among the available antifungals. Nevertheless, its *in vitro* activity against some of the fungi that cause infections that are difficult to handle, like *Fusarium* species, is still not clear and requires further investigation, as olorofim seems to exhibit a species-dependent effectiveness against clinical isolates belonging to these genera.[7,23]

The results achieved in this study confirm the good *in vitro* activity of olorofim against *Scedosporium* and *Lomentospora* clinical isolates, in accordance with those obtained in previous studies in which this new compound was tested against Australian and USA clinical strains.[14,16] This suggests that the *in vitro* effectiveness of this drug is uniform among *Scedosporium*/*Lomentospora* strains from different geographic origins. It has also been recently confirmed in several *in vitro* cellular assays that olorofim compromises the growth and viability of *L. prolificans* and *S. apiospermum* even at low concentrations,[24] and that it has antibiofilm activity against *L. prolificans*.[15]

Although micafungin was moderately active against most of the *Scedosporium* strains tested, the role of echinocandins as monotherapy for scedosporiosis has not been clarified yet and this antifungal class has only been suggested as a treatment when combined with an azole drug.[3] CLSI showed, in general, higher MIC/MEC values (one or two dilutions) than EUCAST probably due to the longer incubation time (72h for CLSI versus 48h for EUCAST).

Further development of olorofim is warranted in order to complete the clinical trial phases for the treatment of infections caused by *Scedosporium* and *L. prolificans*, which would take the process of finding a suitable therapy for these diseases that are hard to treat with currently available antifungals one step forward.

Acknowledgements

These data were previously published as a paper poster in the Eighth Trends in Medical Mycology (TIMM) congress, Belgrade, Serbia, 2017. Poster number: P317.

We thank Cristina de Armentia and Teresa Merino for their technical assistance.

Funding

This work was supported by F2G Ltd (UK). The funders had no role in the study design, data collection, analysis, decision to publish, or preparation/content of the manuscript.

Olga Rivero-Menendez is supported by a fellowship from the Fondo de Investigación Sanitaria (grant FI14CIII/00025).

Transparency declarations

M. C.-E. has received grant support from Astellas, bioMérieux, Gilead Sciences, Merck Sharp & Dohme, Pfizer, Schering Plough, Soria Melguizo, Ferrer International, CIDARA, F2G, Basilea, Amplyx and Scynexis. A. A.-I. has received research grants or honoraria as a speaker or advisor from Astellas, Gilead Sciences, MSD, Pfizer, F2G, Amplyx and Scynexis outside the submitted work. All other authors: none to declare.

References

1 Bongomin F, Gago S, Oladele RO, *et al*. Global and multi-national prevalence of fungal diseases-estimate precision. *J Fungi* 2017; **3**: 57.

2 Lass-Florl C, Cuenca-Estrella M. Changes in the epidemiological landscape of invasive mould infections and disease. *J Antimicrob Chemother* 2017; **72**: i5-i11.

3 Lackner M, de Hoog GS, Verweij PE, *et al.* Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrob Agents Chemother* 2012; **56**: 2635-2642.

4 Seidel D, Meißner A, Lackner M, *et al*. Prognostic factors in 264 adults with invasive *Scedosporium* spp. and *Lomentospora prolificans* infection reported in the literature and FungiScope®. *Crit Rev Microbiol* 2019; **45**: 1-21.

5 Oliver JD, Sibley GEM, Beckmann N, *et al.* F901318 represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase. *Proc Natl Acad Sci USA* 2016; **113**: 12809-12814.

6 Buil JB, Rijs A, Meis JF, *et al*. *In vitro* activity of the novel antifungal compound F901318 against difficult-to-treat *Aspergillus* isolates. *J Antimicrob Chemother* 2017; **72**: 2548-2552.

7 Jorgensen KM, Astvad KMT, Hare RK, *et al*. EUCAST determination of olorofim (F901318) susceptibility of mold species, method validation, and MICs. *Antimicrob Agents Chemother* 2018; **62**: e00487-18.

8 Lackner M, Birch M, Naschberger V, *et al*. Dihydroorotate dehydrogenase inhibitor olorofim exhibits promising activity against all clinically relevant species within *Aspergillus* section Terrei. *J Antimicrob Chemother* 2018; **73**: 3068-3073.

9 Lim W, Eadie K, Konings M, *et al*. *Madurella mycetomatis*, the main causative agent of eumycetoma, is highly susceptible to olorofim. *J Antimicrob Chemother* 2020; **75**: 936-941.

10 Rivero-Menendez O, Cuenca-Estrella M, Alastruey-Izquierdo A. *In vitro* activity of olorofim (F901318) against clinical isolates of cryptic species of *Aspergillus* by EUCAST and CLSI methodologies. *J Antimicrob Chemother* 2019; **74**: 1586-1590.

11 Wiederhold NP, Najvar LK, Jaramillo R, *et al*. The orotomide olorofim is efficacious in an experimental model of central nervous system coccidioidomycosis. *Antimicrob Agents Chemother* 2018; **62**: e00999-18.

12 Negri CE, Johnson A, McEntee L, *et al*. Pharmacodynamics of the novel antifungal agent F901318 for acute sinopulmonary aspergillosis caused by *Aspergillus flavus*. *J Infect Dis* 2018; **217**: 1118-1127.

13 Seyedmousavi S, Chang YC, Law D, *et al*. Efficacy of olorofim (F901318) against *Aspergillus fumigatus*, *A. nidulans*, and *A. tanneri* in murine models of profound neutropenia and chronic granulomatous disease. *Antimicrob Agents Chemother* 2019; **63**: e00129-19.

14 Biswas C, Law D, Birch M, *et al*. *In vitro* activity of the novel antifungal compound F901318 against Australian *Scedosporium* and *Lomentospora* fungi. *Med Mycol* 2018; **56**: 1050-1054.

15 Kirchoff L, Dittmer S, Weisner AK, *et al*. Antibiofilm activity of antifungal drugs, including the novel drug olorofim, against *Lomentospora prolificans*. *J Antimicrob Chemother* 2020. In press.

16 Wiederhold NP, Law D, Birch M. Dihydroorotate dehydrogenase inhibitor F901318 has potent *in vitro* activity against *Scedosporium* species and *Lomentospora prolificans*. *J Antimicrob Chemother* 2017; **72**: 1977-1980.

17 Seyedmousavi A, Chang YC, Law D, *et al*. *In vivo* efficacy of olorofim against systemic infection caused by *Scedosporium apiospermum*, *Pseudallescheria boydii*, and *Lomentospora prolificans* in neutropenic CD-1 mice. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P415. *J Fungi* 2019; **5**: 409-410.

18 Alastruey-Izquierdo A, Mellado E, Pelaez T, *et al*. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). *Antimicrob Agents Chemother* 2013; **57**: 3380-3387.

19 Arendrup MC, Meletiadis J, Mouton JW, *et al*. *EUCAST Definitive Document E.DEF 9.3.2: Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Conidia Forming Moulds*. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9.3.2_Mould_testing_definitive_revised_2020.pdf.

20 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi—Second Edition: M38-A2*. 2008.

21 Jenks JD, Seidel D, Cornely OA, *et al*. Voriconazole plus terbinafine combination antifungal therapy for invasive *Lomentospora prolificans* infections: analysis of 41

patients from the FungiScope® registry 2008–2019. *Clin Microbiol Infect* 2020; **26**: 784.e1-784.e5.

22 Buil J, Oliver J, Law D, *et al*. Molecular mechanism and frequency of olorofim resistance in *Aspergillus fumigatus*. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P054. *J Fungi* 2019; **5**: 129-130.

23 Wiederhold N, Patterson H, Birch M, *et al*. Evaluation of the *in vitro* activity of olorofim against *Fusarium* species. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P417. *J Fungi* 2019; **5**: 411-412.

24 Du Pré S, Law D, Sibley G, *et al*. The effect of the orotomide antifungal olorofim on the growth and viability of *Scedosporium* and *Lomentospora* species. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P423. *J Fungi* 2019; **5**: 414-415.

Table 1. MIC values and ranges for amphotericin B, voriconazole, isavuconazole and olorofim, and MEC values for micafungin against *Scedosporium* species and *Lomentospora prolificans*, as determined by the CLSI and EUCAST broth microdilution methods.

		Test method									
		Antifungal test at 48 hours of incubation by EUCAST and 72 hours of incubation by CLSI									
		EUCAST					CLSI				
Species (no. tested)		AMB	VRC	ISC	MCF	OFM	AMB	VRC	ISC	MCF	OFM
Scedosporium apiospermum (30)	GM	4.000	0.933	8.187	0.166	0.050	2.764	1.414	10.079	0.133	0.173
	MIC/MEC_{50}	4	1	8	0.25	0.06	2	1	16	0.12	0.25
	MIC/MEC_{90}	16	2	16	0.25	0.12	16	2	16	0.25	0.25
	Range	0.5 - 32	0.5 - 2	2 - 16	0.015 – 0.5	0.015 - 0.12	0.25 - 16	0.5 - 8	1 - 16	0.03 - 1	0.03 – 0.5
Scedosporium boydii (30)	GM	12.126	0.706	6.063	0.214	0.040	6.650	0.977	7.639	0.136	0.127
	MIC/MEC_{50}	16	0.5	8	0.25	0.03	8	1	8	0.12	0.12
	MIC/MEC_{90}	32	2	16	0.5	0.12	32	2	16	0.5	0.25
	Range	0.5 - 32	0.12 - 16	0.5-16	0.06 - 4	0.007 - 0.25	0.5 - 32	0.5 - 2	1 - 16	0.03 – 0.5	0.06 – 0.5
Scedosporium ellipsoideum (10)	GM	19.698	1.000	8.000	0.130	0.052	14.929	1.320	16.000	0.150	0.186
	MIC/MEC_{50}	16	1	8	0.12	0.06	16	1	16	0.12	0.25
	MIC/MEC_{90}	32	2	16	0.25	0.12	32	2	16	0.25	0.5
	Range	8 - 32	0.5 - 2	2-16	0.06 – 0.25	0.015 – 0.5	2 - 32	1 - 2	16 - 16	0.12 – 0.25	0.06 - 1
Scedosporium aurantiacum (20)	GM	22.627	0.966	9.514	3.249	0.130	7.210	1.366	14.420	3.357	0.339

	MIC/MEC_{50}	32	1	8	4	0.12	8	2	16	4	0.5
	MIC/MEC_{90}	32	2	16	4	0.25	16	2	16	4	1
	Range	8 - 32	0.5 - 4	4 - 16	0.25 - 4	0.03 – 0.25	2 - 32	0.5 - 2	8 - 16	0.12 - 4	0.06 – 1
Scedosporium dehoogii (3)	GM	32.000	0.794	10.079	0.250	0.095	20.159	1.000	8.000	0.250	0.250
	Range	32 - 32	0.5 - 1	4 - 16	0.25 – 0.25	0.06 – 0.12	16 - 32	1 - 1	8 - 8	0.25 – 0.25	0.25 – 0.25
Lomentospora prolificans (30)	GM	24.818	12.699	15.635	3.732	0.115	17.959	16.000	15.635	3.732	0.225
	MIC/MEC_{50}	32	16	16	4	0.12	32	16	16	4	0.25
	MIC/MEC_{90}	32	16	16	4	0.25	32	16	16	4	0.5
	Range	2 - 32	4 - 16	8 - 16	0.5 - 4	0.03 – 0.25	2 - 32	16 - 16	8 - 16	0.5 - 4	0.06 – 0.5

AMB, amphotericin B; VRC, voriconazole; ISC, isavuconazole; MCF, micafungin; OFM, olorofim.

In vitro activity of olorofim against clinical isolates of *Scedosporium* species and *Lomentospora prolificans* using EUCAST and CLSI methodologies

Olga RIVERO-MENENDEZ, Manuel CUENCA-ESTRELLA, Ana ALASTRUEY-IZQUIERDO*

Mycology Reference Laboratory, National Centre for Microbiology. Instituto de Salud Carlos III. Majadahonda, Madrid, Spain. Spanish Network for the Research in Infectious Diseases (REIPI RD16/CIII/0004/0003), Instituto de Salud Carlos III, Madrid, Spain.

*Corresponding author:

Ana Alastruey-Izquierdo

Mycology Reference Laboratory

Centro Nacional de Microbiología. Instituto de Salud Carlos III

28220. Majadahonda. Madrid. Spain.

Phone: +34-91 822 3784

Fax: 91 509 7966

E-mail: anaalastruey@isciii.es

Running title: Activity of olorofim to *Scedosporium* species and *Lomentospora prolificans*

Abstract

Objectives

To evaluate the *in vitro* activity of olorofim, a new broad-spectrum antifungal with a novel mechanism of action, against a collection of 123 Spanish clinical isolates belonging to five *Scedosporium* species and *Lomentospora prolificans*.

Methods

The activity of olorofim against *Scedosporium apiospermum* (n=30), *Scedosporium boydii* (n=30), *Scedosporium ellipsoideum* (n=10), *Scedosporium aurantiacum* (n=20), *Scedosporium dehoogii* (n=3) and *Lomentospora prolificans* (n=30) was compared with that of amphotericin B, voriconazole, isavuconazole and micafungin by performing EUCAST and CLSI reference methods for antifungal susceptibility testing.

Results

Amphotericin B and isavuconazole showed MICs ≥ 2 mg/L against all the species evaluated, and voriconazole was moderately active (GM, MIC_{50} and MIC_{90} values ≤ 2 mg/L) against all of them except *L. prolificans*. Micafungin was effective against *S. apiospermum* complex strains but exhibited elevated MECs against *S. dehoogii* and *S. aurantiacum*. Olorofim showed low MICs against all the *Scedosporium* strains tested (GM values were lower than 0.130 and 0.339 by EUCAST and CLSI, respectively, for all of the species), including those belonging to the multi-drug resistant species *L. prolificans*, for which GM values were 0.115 mg/L and 0.225 mg/L by EUCAST and CLSI, respectively, while for the rest of antifungals evaluated GMs were higher than 3.732 mg/L using both methodologies.

Conclusions

Olorofim displays a promising *in vitro* activity against *Scedosporium* and *L. prolificans* strains tested, some of which have reduced susceptibility against the antifungals that are currently in use.

Introduction

Aspergillus, *Candida* and *Cryptococcus* species are responsible for the largest number of invasive fungal infection cases,[1] which are being increasingly reported throughout the world. However, the epidemiology of these diseases has changed over the last years enhancing the appearance of emerging pathogens, like *Scedosporium* spp.[2]

Species such as *Scedosporium apiospermum* (which is identified as a species complex that includes *Scedosporium apiospermum sensu stricto* (*s.s.*), *Scedosporium boydii*, *Scedosporium angustum*, *Scedosporium ellipsoideum* and *Scedosporium fusoideum*), *Scedosporium aurantiacum*, *Scedosporium dehoogii* or *Lomentospora prolificans* (formerly *Scedosporium prolificans*) cause an invasive infection called scedosporiosis (or lomentosporiosis if caused by *L. prolificans*), which is considered life-threatening due to the reduced effectiveness that antifungals in clinical use have against it.[3,4] Consequently, there is an urgent need for the development of new antifungal agents with novel mechanisms of action that are active against these species and that help to address the limitations that available treatments have.

Olorofim is the first clinical compound from the novel antifungal class of the orotomides, and its mechanism of action is based on the inhibition of the dihydroorotate dehydrogenase (DHODH), an enzyme that catalyses one of the key steps in the pyrimidine biosynthesis pathway. This, consequently, compromises the synthesis of nucleic acids, proteins, the fungal cell wall and the fungal cell membrane. Even though mammalian cells carry a form of this enzyme, the activity of this new compound is highly fungal-specific, thus, providing an encouraging safety profile with reduced toxicity.[5] Its *in vitro* activity has been proved against a wide variety of pathogenic fungal species, like *Histoplasma capsulatum*, *Coccidioides posadasii*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Madurella mycetomatis* or those from the genera *Penicillium*, *Aspergillus* (including cryptic species and azole resistant *Aspergillus fumigatus*), *Fusarium* and dermatophytes, although olorofim is not active against *Candida* spp., *Cryptococcus* spp. or Mucorales.[5-11] It has also led to promising *in vivo* results when administered orally or intravenously in murine models of infection of aspergillosis and coccidiomycosis, and shows good distribution into tissues, including the brain.[5,8,11-13]

This new drug has also proved to have *in vitro* efficacy against *Scedosporium* and *Lomentospora* species [5,14-16] and has demonstrated marked improvements in survival in neutropenic mice with systemic infections caused by *S. apiospermum s.s.*, *S. boydii* and *L. prolificans*.[17] Nevertheless, only a low number of clinical strains from a few *Scedosporium* species have been *in vitro* tested, and further investigations are required to confirm olorofim's activity on isolates from distinct geographical areas. Therefore, the aim of the present study is to evaluate the *in vitro* activity of olorofim and comparator antifungal agents against a collection of clinical isolates of Spanish *Scedosporium* and *Lomentospora* species using the EUCAST and CLSI standardized reference methodologies for antifungal susceptibility testing. This study represents the first European assessment of the *in vitro* activity of olorofim against the *Scedosporium* genus.

Materials and methods

A total of 123 strains from different *Scedosporium* species and *L. prolificans* were tested. All strains were obtained from clinical samples (respiratory, cutaneous, oculars, optical, biopsies, abscess, blood cultures and wounds) in Spanish hospitals and identified to species level by standard microscopic morphology and by sequencing the Internal Transcribed Spacer Region of the rDNA and part of the β-tubulin gene following methods previously reported.[18]

Antifungal Susceptibility testing was performed following EUCAST reference method 9.3.2 [19] and CLSI M38A.[20] Antifungals used were olorofim (range 0.004-2 mg/L; F2G Limited, Manchester, United Kingdom), amphotericin B (range 0.03-16 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), voriconazole (range 0.015-8 mg/L; Sigma-Aldrich Quimica, Madrid, Spain), isavuconazole (range 0.015-8 mg/L; Basilea Pharmaceutica, Basel, Switzerland) and micafungin (range 0.004-2 mg/L; Astellas Pharma Inc, Tokyo, Japan).

Aspergillus flavus ATCC 204304 and *A. fumigatus* ATCC 204305 were used as quality control strains in all test performed for both methods. MICs for amphotericin B, voriconazole, isavuconazole and olorofim, and minimal effective concentrations (MECs) for micafungin were visually read after 24, 48 and 72 hours of incubation at 35ºC in a humid atmosphere. Geometric mean (GM), MIC/MEC_{50} (MIC/MEC causing inhibition of

50% of the isolates tested) and MIC/MEC_{90} (MIC/MEC causing inhibition of 90% of the isolates tested) were determined. For calculation purposes, the MIC or MEC values that exceeded the maximum concentration tested were transformed to the next dilution (i.e. if MIC/MEC was >16 mg/L it was expressed as 32 mg/L) and values that were less than or equal to the minimum concentration tested were transformed to equal (i.e. if MIC/MEC was ≤0.03 mg/L it was expressed as 0.03 mg/L).

Results and discussion

Table 1 shows the GM, MIC/MEC_{50}, MIC/MEC_{90} and range for all the species tested for each antifungal susceptibility testing method at 48 hours of incubation by EUCAST and 72 hours of incubation by CLSI. MIC/MEC_{50} and MIC/MEC_{90} were only calculated for species that had ten or more isolates. Table S1 (available online as Supplementary data) displays MIC/MEC distributions for each species against all the antifungals tested.

Overall, olorofim was the only compound exhibiting low MICs (below 1mg/L) against all strains and species tested both by EUCAST and CLSI.

Regarding the *S. apiospermum* complex, all three species tested displayed high MIC values for amphotericin B and isavuconazole but lower MICs for voriconazole. MIC_{90}s for amphotericin B and isavuconazole were ≥16 mg/L using both methodologies, whereas voriconazole MIC_{90}s were 2 mg/L by both EUCAST and CLSI methods. These values are in agreement with those from previous reports on the activity of these antifungals against *Scedosporium* species,[3,16] which reflect that even though voriconazole is considered the first line treatment for scedosporiosis, its activity against some of the species that cause it is limited. Micafungin MEC_{90} values ranged from 0.25 mg/L to 0.5 mg/L both by EUCAST and CLSI for *S. apiospermum s.s.*, *S. boydii* and *S. ellipsoideum* strains, but olorofim was the most active drug against them, showing lower GM values than those of micafungin except for *S. apiospermum s.s.* and *S. ellipsoideum* strains by CLSI, for which they were similar. In addition, it is important to highlight that the activity for olorofim is determined as MIC while for micafungin it is assessed as MEC. These results are comparable to those from a previous study, in which olorofim yielded slightly higher MIC values against these species but was still active.[14]

Amphotericin B, isavuconazole and micafungin showed poor activity against the *S. aurantiacum* strains tested, while voriconazole exhibited similar MICs than those yielded

against *S. apiospermum* (GM values were 0.966 mg/L by EUCAST and 1.366 mg/L by CLSI for *S. aurantiacum*). Olorofim displayed the lowest MICs with GMs of 0.130 mg/L and 0.339 mg/L by EUCAST and CLSI, respectively, confirming results reported in previous studies.[14,16]

Scedosporium dehoogii strains tested were inhibited by olorofim (MICs ranged from 0.06 and 0.25 mg/L). Micafungin showed a similar activity (GMs of 0.250 mg/L), whereas voriconazole exhibited a moderate effectiveness and amphotericin B and isavuconazole were inactive against them, with GM values higher than 8 mg/L.

Regarding *L. prolificans*, olorofim was the only active compound against the strains tested with a MIC_{50} value of 0.12 mg/L by EUCAST and 0.25 mg/L by CLSI. The high MIC/MEC values displayed by the rest of the antifungals tested reasserted the fact that *L. prolificans* is an intrinsically multidrug-resistant species.[3] Nevertheless, the good activity yielded by olorofim in this and in previous studies [14-16] seems promising in order to develop an effective treatment against infections caused by this species, as even though the combination of voriconazole and terbinafine has been associated with positive outcomes in patients with invasive *L. prolificans* infections, overall mortality rates are still high.[21]

In addition to its good activity against *Scedosporium* and *L. prolificans* strains, olorofim has also been reported as being highly *in vitro* active against a broad number of *Aspergillus* species, including azole resistant *A. fumigatus* isolates both harboring *cyp51A* alterations and without known resistance mechanisms,[5-7] and those defined as cryptic that are usually multi-drug resistant.[8,10] The *in vitro* potential development of olorofim resistance has been studied in susceptible *A. fumigatus* isolates, proving that their spontaneous resistance rates are lower than those obtained with other antifungals, which would make olorofim a better therapy against infections caused by this species than azoles. This study also led to the description of a low frequency resistance mechanism to olorofim, as these strains harbored mutations in *pyrE* gene, which encodes the DHOH.[22] Besides, studies in murine models of invasive aspergillosis caused by susceptible and azole-resistant strains belonging to several *Aspergillus* species have also been successfully conducted, showing higher survival rates in mice treated with olorofim than with other antifungals.[8,12,13] This drug is currently undergoing phase II as an oral and intravenous formulation for the treatment of invasive aspergillosis and of

hard-to-treat rare mould infections, such as scedosporiosis, that lack of an appropriate therapy among the available antifungals. Nevertheless, its *in vitro* activity against some of the fungi that cause infections that are difficult to handle, like *Fusarium* species, is still not clear and requires further investigation, as olorofim seems to exhibit a species-dependent effectiveness against clinical isolates belonging to these genera.[7,23]

The results achieved in this study confirm the good *in vitro* activity of olorofim against *Scedosporium* and *Lomentospora* clinical isolates, in accordance with those obtained in previous studies in which this new compound was tested against Australian and USA clinical strains.[14,16] This suggests that the *in vitro* effectiveness of this drug is uniform among *Scedosporium/Lomentospora* strains from different geographic origins. It has also been recently confirmed in several *in vitro* cellular assays that olorofim compromises the growth and viability of *L. prolificans* and *S. apiospermum* even at low concentrations,[24] and that it has antibiofilm activity against *L. prolificans*.[15]

Although micafungin was moderately active against most of the *Scedosporium* strains tested, the role of echinocandins as monotherapy for scedosporiosis has not been clarified yet and this antifungal class has only been suggested as a treatment when combined with an azole drug.[3] CLSI showed, in general, higher MIC/MEC values (one or two dilutions) than EUCAST probably due to the longer incubation time (72h for CLSI versus 48h for EUCAST).

Further development of olorofim is warranted in order to complete the clinical trial phases for the treatment of infections caused by *Scedosporium* and *L. prolificans*, which would take the process of finding a suitable therapy for these diseases that are hard to treat with currently available antifungals one step forward.

Acknowledgements

These data were previously published as a paper poster in the Eighth Trends in Medical Mycology (TIMM) congress, Belgrade, Serbia, 2017. Poster number: P317.

We thank Cristina de Armentia and Teresa Merino for their technical assistance.

Funding

This work was supported by F2G Ltd (UK). The funders had no role in the study design, data collection, analysis, decision to publish, or preparation/content of the manuscript.

Olga Rivero-Menendez is supported by a fellowship from the Fondo de Investigación Sanitaria (grant FI14CIII/00025).

Transparency declarations

M. C.-E. has received grant support from Astellas, bioMérieux, Gilead Sciences, Merck Sharp & Dohme, Pfizer, Schering Plough, Soria Melguizo, Ferrer International, CIDARA, F2G, Basilea, Amplyx and Scynexis. A. A.-I. has received research grants or honoraria as a speaker or advisor from Astellas, Gilead Sciences, MSD, Pfizer, F2G, Amplyx and Scynexis outside the submitted work. All other authors: none to declare.

References

1 Bongomin F, Gago S, Oladele RO, *et al*. Global and multi-national prevalence of fungal diseases-estimate precision. *J Fungi* 2017; **3**: 57.

2 Lass-Florl C, Cuenca-Estrella M. Changes in the epidemiological landscape of invasive mould infections and disease. *J Antimicrob Chemother* 2017; **72**: i5-i11.

3 Lackner M, de Hoog GS, Verweij PE, *et al.* Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrob Agents Chemother* 2012; **56**: 2635-2642.

4 Seidel D, Meißner A, Lackner M, *et al*. Prognostic factors in 264 adults with invasive *Scedosporium* spp. and *Lomentospora prolificans* infection reported in the literature and FungiScope®. *Crit Rev Microbiol* 2019; **45**: 1-21.

5 Oliver JD, Sibley GEM, Beckmann N, *et al.* F901318 represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase. *Proc Natl Acad Sci USA* 2016; **113**: 12809-12814.

6 Buil JB, Rijs A, Meis JF, *et al*. *In vitro* activity of the novel antifungal compound F901318 against difficult-to-treat *Aspergillus* isolates. *J Antimicrob Chemother* 2017; **72**: 2548-2552.

7 Jorgensen KM, Astvad KMT, Hare RK, *et al*. EUCAST determination of olorofim (F901318) susceptibility of mold species, method validation, and MICs. *Antimicrob Agents Chemother* 2018; **62**: e00487-18.

8 Lackner M, Birch M, Naschberger V, *et al*. Dihydroorotate dehydrogenase inhibitor olorofim exhibits promising activity against all clinically relevant species within *Aspergillus* section Terrei. *J Antimicrob Chemother* 2018; **73**: 3068-3073.

9 Lim W, Eadie K, Konings M, *et al*. *Madurella mycetomatis*, the main causative agent of eumycetoma, is highly susceptible to olorofim. *J Antimicrob Chemother* 2020; **75**: 936-941.

10 Rivero-Menendez O, Cuenca-Estrella M, Alastruey-Izquierdo A. *In vitro* activity of olorofim (F901318) against clinical isolates of cryptic species of *Aspergillus* by EUCAST and CLSI methodologies. *J Antimicrob Chemother* 2019; **74**: 1586-1590.

11 Wiederhold NP, Najvar LK, Jaramillo R, *et al*. The orotomide olorofim is efficacious in an experimental model of central nervous system coccidioidomycosis. *Antimicrob Agents Chemother* 2018; **62**: e00999-18.

12 Negri CE, Johnson A, McEntee L, *et al*. Pharmacodynamics of the novel antifungal agent F901318 for acute sinopulmonary aspergillosis caused by *Aspergillus flavus*. *J Infect Dis* 2018; **217**: 1118-1127.

13 Seyedmousavi S, Chang YC, Law D, *et al*. Efficacy of olorofim (F901318) against *Aspergillus fumigatus*, *A. nidulans*, and *A. tanneri* in murine models of profound neutropenia and chronic granulomatous disease. *Antimicrob Agents Chemother* 2019; **63**: e00129-19.

14 Biswas C, Law D, Birch M, *et al*. *In vitro* activity of the novel antifungal compound F901318 against Australian *Scedosporium* and *Lomentospora* fungi. *Med Mycol* 2018; **56**: 1050-1054.

15 Kirchoff L, Dittmer S, Weisner AK, *et al*. Antibiofilm activity of antifungal drugs, including the novel drug olorofim, against *Lomentospora prolificans*. *J Antimicrob Chemother* 2020. In press.

16 Wiederhold NP, Law D, Birch M. Dihydroorotate dehydrogenase inhibitor F901318 has potent *in vitro* activity against *Scedosporium* species and *Lomentospora prolificans*. *J Antimicrob Chemother* 2017; **72**: 1977-1980.

17 Seyedmousavi A, Chang YC, Law D, *et al*. *In vivo* efficacy of olorofim against systemic infection caused by *Scedosporium apiospermum*, *Pseudallescheria boydii*, and *Lomentospora prolificans* in neutropenic CD-1 mice. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P415. *J Fungi* 2019; **5**: 409-410.

18 Alastruey-Izquierdo A, Mellado E, Pelaez T, *et al*. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). *Antimicrob Agents Chemother* 2013; **57**: 3380-3387.

19 Arendrup MC, Meletiadis J, Mouton JW, *et al*. *EUCAST Definitive Document E.DEF 9.3.2: Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Conidia Forming Moulds*. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9.3.2_Mould_testing_definitive_revised_2020.pdf.

20 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi—Second Edition: M38-A2*. 2008.

21 Jenks JD, Seidel D, Cornely OA, *et al*. Voriconazole plus terbinafine combination antifungal therapy for invasive *Lomentospora prolificans* infections: analysis of 41

patients from the FungiScope® registry 2008–2019. *Clin Microbiol Infect* 2020; **26**: 784.e1-784.e5.

22 Buil J, Oliver J, Law D, *et al*. Molecular mechanism and frequency of olorofim resistance in *Aspergillus fumigatus*. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P054. *J Fungi* 2019; **5**: 129-130.

23 Wiederhold N, Patterson H, Birch M, *et al*. Evaluation of the *in vitro* activity of olorofim against *Fusarium* species. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P417. *J Fungi* 2019; **5**: 411-412.

24 Du Pré S, Law D, Sibley G, *et al*. The effect of the orotomide antifungal olorofim on the growth and viability of *Scedosporium* and *Lomentospora* species. In: *Abstracts of the 9th Trends in Medical Mycology, Nice, France, 2019.* Abstract P423. *J Fungi* 2019; **5**: 414-415.

Table 1. MIC values and ranges for amphotericin B, voriconazole, isavuconazole and olorofim, and MEC values for micafungin against *Scedosporium* species and *Lomentospora prolificans*, as determined by the CLSI and EUCAST broth microdilution methods.

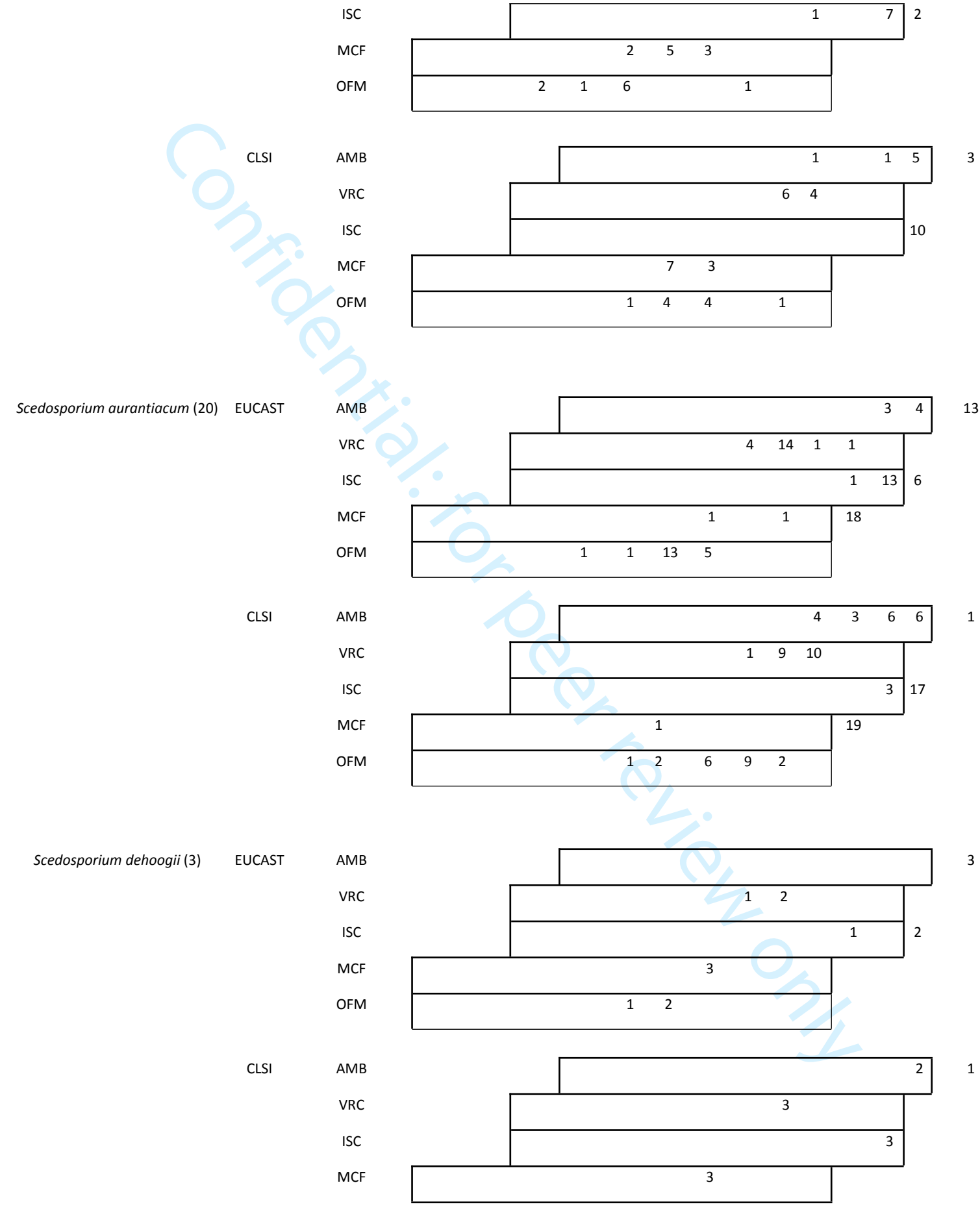
		Test method									
		Antifungal test at 48 hours of incubation by EUCAST and 72 hours of incubation by CLSI									
		EUCAST					CLSI				
Species (no. tested)		AMB	VRC	ISC	MCF	OFM	AMB	VRC	ISC	MCF	OFM
Scedosporium apiospermum (30)	GM	4.000	0.933	8.187	0.166	0.050	2.764	1.414	10.079	0.133	0.173
	MIC/MEC_{50}	4	1	8	0.25	0.06	2	1	16	0.12	0.25
	MIC/MEC_{90}	16	2	16	0.25	0.12	16	2	16	0.25	0.25
	Range	0.5 - 32	0.5 - 2	2 - 16	0.015 – 0.5	0.015 - 0.12	0.25 - 16	0.5 - 8	1 - 16	0.03 - 1	0.03 – 0.5
Scedosporium boydii (30)	GM	12.126	0.706	6.063	0.214	0.040	6.650	0.977	7.639	0.136	0.127
	MIC/MEC_{50}	16	0.5	8	0.25	0.03	8	1	8	0.12	0.12
	MIC/MEC_{90}	32	2	16	0.5	0.12	32	2	16	0.5	0.25
	Range	0.5 - 32	0.12 - 16	0.5-16	0.06 - 4	0.007 - 0.25	0.5 - 32	0.5 - 2	1 - 16	0.03 – 0.5	0.06 – 0.5
Scedosporium ellipsoideum (10)	GM	19.698	1.000	8.000	0.130	0.052	14.929	1.320	16.000	0.150	0.186
	MIC/MEC_{50}	16	1	8	0.12	0.06	16	1	16	0.12	0.25
	MIC/MEC_{90}	32	2	16	0.25	0.12	32	2	16	0.25	0.5
	Range	8 - 32	0.5 - 2	2-16	0.06 – 0.25	0.015 – 0.5	2 - 32	1 - 2	16 - 16	0.12 – 0.25	0.06 - 1
Scedosporium aurantiacum (20)	GM	22.627	0.966	9.514	3.249	0.130	7.210	1.366	14.420	3.357	0.339

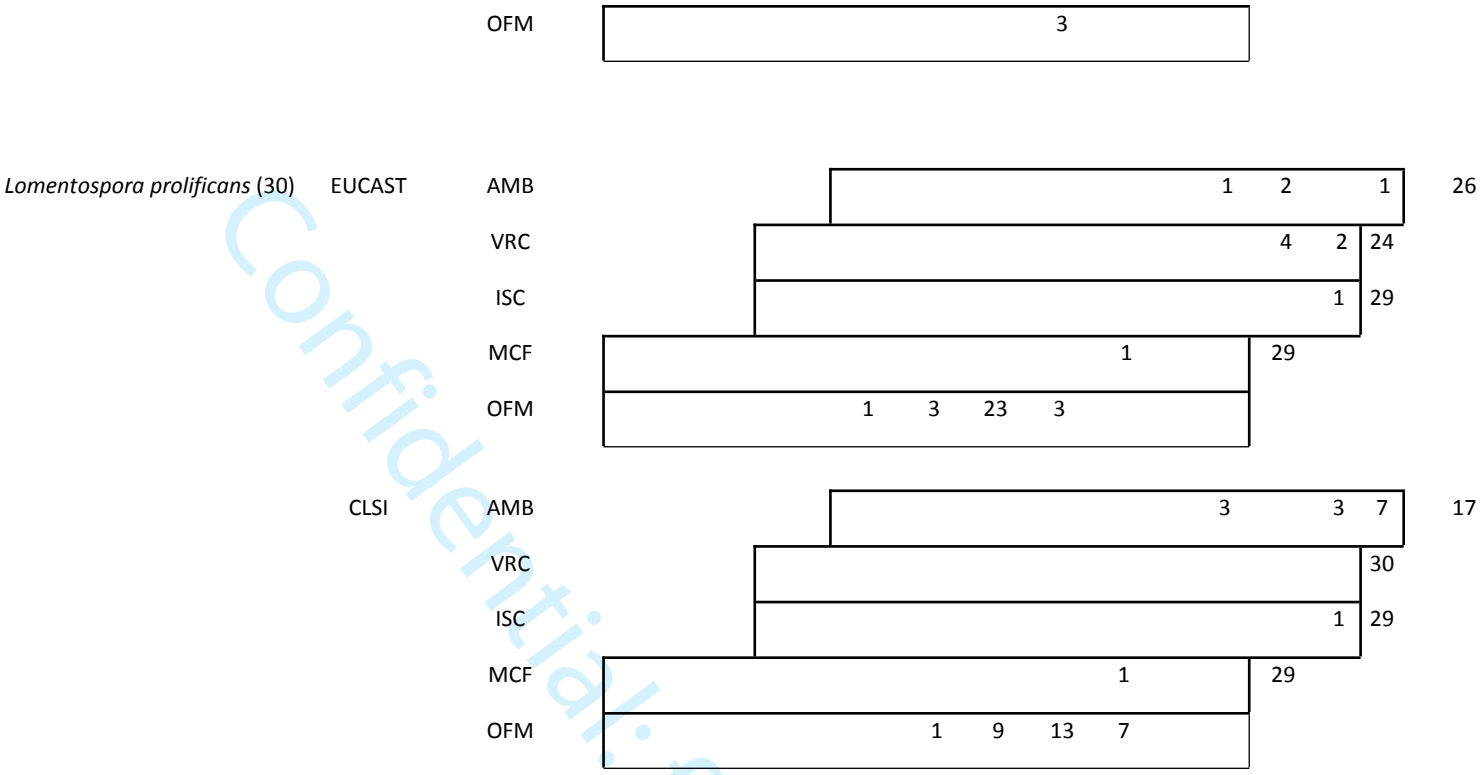
	MIC/MEC_{50}	32	1	8	4	0.12	8	2	16	4	0.5
	MIC/MEC_{90}	32	2	16	4	0.25	16	2	16	4	1
	Range	8 - 32	0.5 - 4	4 - 16	0.25 - 4	0.03 – 0.25	2 - 32	0.5 - 2	8 - 16	0.12 - 4	0.06 – 1
Scedosporium dehoogii (3)	GM	32.000	0.794	10.079	0.250	0.095	20.159	1.000	8.000	0.250	0.250
	Range	32 - 32	0.5 - 1	4 - 16	0.25 – 0.25	0.06 – 0.12	16 - 32	1 - 1	8 - 8	0.25 – 0.25	0.25 – 0.25
Lomentospora prolificans (30)	GM	24.818	12.699	15.635	3.732	0.115	17.959	16.000	15.635	3.732	0.225
	MIC/MEC_{50}	32	16	16	4	0.12	32	16	16	4	0.25
	MIC/MEC_{90}	32	16	16	4	0.25	32	16	16	4	0.5
	Range	2 - 32	4 - 16	8 - 16	0.5 - 4	0.03 – 0.25	2 - 32	16 - 16	8 - 16	0.5 - 4	0.06 – 0.5

AMB, amphotericin B; VRC, voriconazole; ISC, isavuconazole; MCF, micafungin; OFM, olorofim.

Table S1. MIC/MEC distributions obtained after testing the susceptibility of *Scedosporium* and *Lomentospora prolificans* strains against olorofim and other antifungal comparators using EUCAST and CLSI methodologies.

Species (no. tested)	Test method	Antifungal agent	No. of isolates at MIC/MEC (mg/L)													
			0.004	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Scedosporium apiospermum (30)	EUCAST	AMB								4	2	6	5	6	5	2
		VRC								8	17	5				
		ISC										2	4	15	9	
		MCF			1		2	11	14	2						
		OFM			5	8	8	9								
	CLSI	AMB							1	3	4	8	5	5	4	
		VRC								1	15	13		1		
		ISC									1	1	1	11	16	
		MCF				1	5	16	6	1	1					
		OFM				1	1	12	14	2						
Scedosporium boydii (30)	EUCAST	AMB								1		2	4	5	6	12
		VRC						1	1	16	9	2			1	
		ISC								1		2	10	11	6	
		MCF					1	10	16	2			1			
		OFM		1	6	9	9	4	1							
	CLSI	AMB								1	2	6	2	7	8	4
		VRC								6	19	5				
		ISC									1	1	4	17	7	
		MCF				1	5	15	6	3						
		OFM					6	17	6	1						
Scedosporium ellipsoideum (10)	EUCAST	AMB												1	5	4
		VRC								1	8	1				





AMB, amphotericin B; VRC, voriconazole; ISC, isavuconazole; MCF, micafungin; OFM, olorofim.