Olorofim EUCAST Susceptibility Testing of Contemporary Moulds: *In Vitro* Activity and Improved Reproducibility

P412

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Objectives

Olorofim (F901318) is a novel antifungal orotomide compound for which we previously demonstrated good *in vitro* activity against various moulds.

This study sought to (a) investigate whether MIC variation observed for *A. fumigatus* (unimodal range <0.004–0.25 mg/L) was due to inherent MIC variation or true differences in susceptibility and (b) present EUCAST MICs for clinical mould isolates from 2018.

Materials & methods

MICs (mg/L) of olorofim were determined by EUCAST E.Def 9.3 using *A. fumigatus* ATCC 204305 for QC.

MIC variability study:

1. 15 *A. fumigatus* isolates selected from a previous olorofim study: five with low MIC: ≤0.002 mg/L (n=2) and 0.004 mg/L (n=3); five with middle MIC: 0.03 mg/L (n=2) and 0.06 mg/L (n=3); and five with high MIC: 0.125 mg/L (n=1) and 0.25 mg/L (n=4).
2. MICs were determined 10 times and read by observers blinded to the original MIC (ref.).

EUCAST MICs for 365 clinical moulds from 2018:

1. Olorofim activity of *A. fumigatus* was evaluated individually for itraconazole susceptible (MIC≤1 mg/L) and non-susceptible isolates (MIC>1 mg/L).
2. Statistical wildtype upper limits (highest MIC for organisms without phenotypically detectable acquired resistance mechanisms, WT-ULs) were determined using the ECOFFinder programme adopting 95%, 97.5% and 99% subset endpoints.
3. For *Fusarium* isolates, the 50%-MIC was also determined spectrophotometrically using 50% growth inhibition.

Results

MIC variability

The modal MIC for low/middle/high MIC isolates were as follows for repeated testing:
Observer 1 and 2: 0.03/0.03/0.016 mg/L
Observer 3: 0.03/0.03/0.03 mg/L

- The MICs for 14/15 isolates fell within two dilutions despite the broader MIC ranges observed previously (ref.) (Figure).
- For isolate #12 (high), 1/10 MICs fell one dilution below the two-dilution range when read by observer 1 and 2.
- For the control strain, 75/76 MICs fell within two dilutions. 1/76 MICs fell one dilution higher when read by observer 1.

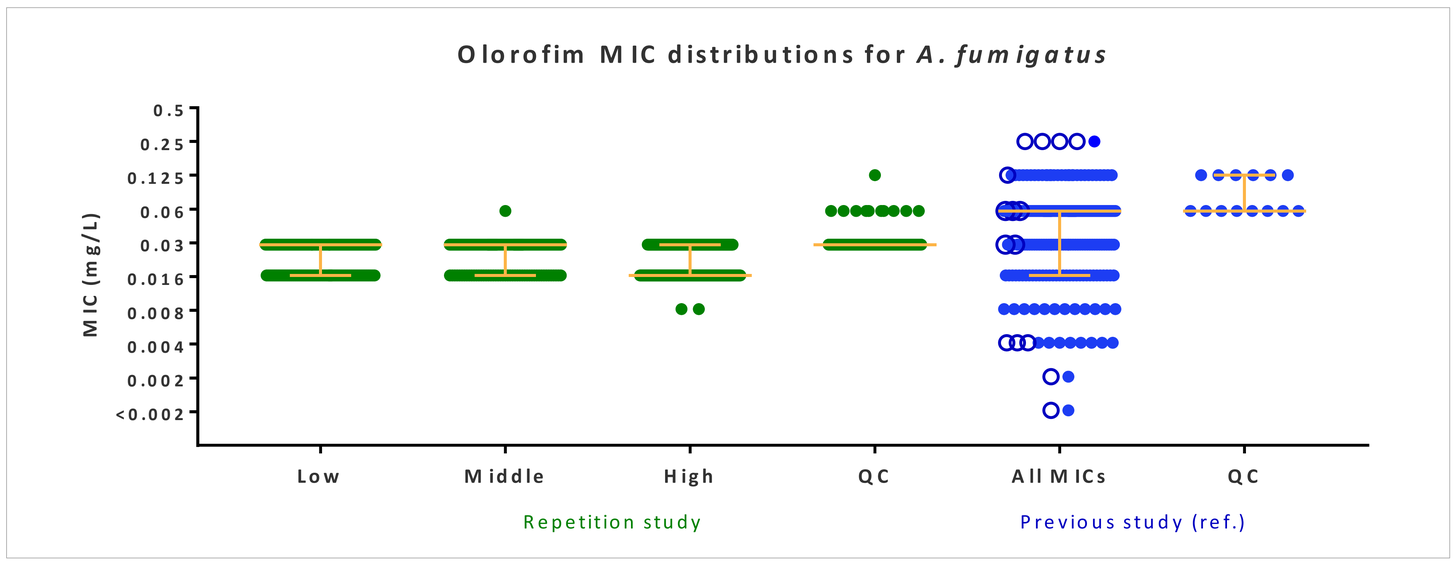

Figure. Olorofim MICs (mg/L) for the repetition study (green) and from the previous study (blue). All MICs: the 15 isolates tested in the repetition study (5 low MIC, 5 middle MIC and 5 high MIC) are shown as blue circles. QC used in both studies: ATCC 204305. No. of MICs: Low; 140, middle; 140, high; 196, QC rep. study; 76, and QC prev. study; 14. All MICs: 235 isolates tested once during prospective surveillance. The median with interquartile ranges is shown in yellow.

Contemporary MICs

Routine testing during 2018 for species represented by ≥15 isolates generated uniform Gaussian MIC distributions spanning ≤5 dilutions (Table).

- Modal MICs were 0.03-0.06 mg/L for all isolates except non-*proliferatum Fusarium* spp. (n=10).
- Modal MIC/WT-UL for *A. fumigatus* was 0.06/0.125 mg/L and was unaffected by itraconazole susceptibility and choice of ECOFFinder endpoint.

Olorofim displayed MICs of ≤0.25 mg/L against less common *Aspergillus* spp. except *A. montevidensis* (n=1. MIC>1 mg/L), as well as against dermatophytes and other moulds.

50%-MICs (mg/L) revealed partial inhibition for most *Fusarium* species: *F. dimerum* >8 (n=2), *F. oxysporum* 0.06 (n=1), *F. solani* complex MIC range 0.25-1, modal MIC 1 (n=7), and *F. proliferatum* 0.03 (n=1).

Table. Olorofim *in vitro* activity against 365 clinical mould isolates obtained during 2018.

	Olorofim MICs (mg/L)											
	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	>1	Range	Modal MIC	MIC_{50}	MIC_{90}
A. flavus complex (18)		7	8	3						(0.016-0.06)	0.03	0.03	0.06
A. fumigatus (220)		7	67	113	31	2				(0.016-0.25)	0.06	0.06	0.125
azole wild-type (179)		6	55	96	21	1				(0.016-0.25)	0.06	0.06	0.125
azole non-wild-type (41)		1	12	17	10	1				(0.016-0.25)	0.06	0.06	0.125
A. nidulans complex (4)			1	2	1					(0.03-0.125)			
A. niger complex (51)		1	8	23	17	2				(0.016-0.25)	0.06	0.06	0.125
A. terreus complex (32)	5	10	13	4						(0.008-0.06)	0.03	0.03	0.06
Other *Aspergillus* all (8)	2	4				1			1	(0.008->1)			
F. proliferatum (1)				1						(0.06)			
Other *Fusarium* (10)*									10	>1			
T. rubrum (12)	1	1	1	8		1				(0.008-0.25)	0.06	0.06	0.06
Other dermatophytes (2)**			1		1					(0.03-0.125)			
Other moulds (7)***	1	1	1	1	1	2				(0.008-0.25)			

*2 *F. dimerum*, 1 *F. oxysporum* and 7 *F. solani* complex. **1 *Microsporum gypseum* and 1 *T. interdigitale*. ***1 *Rasamsonia aegroticola*, 1 *Rasamsonia argillacea,* 3 *Scedosporium apiospermum,* and 2 *Scedosporium boydii*.

(Ref.: Jørgensen et al. 2018, AAC 62(8):1-10)

Conclusion

- We confirmed that Olorofim displays promising *in vitro* activity against most clinical moulds included in this study independent of azole susceptibility.
- Our repetition study demonstrated a uniform activity against isolates initially falling in the left, middle and right side of the MIC distribution obtained by testing all isolates once suggesting that technical variation accounted for the MIC variation initially observed.
- We hypothesize that the sources for technical variation may include: 1) the fact that tiny growth is observed in a few wells around the MIC, which may complicate visual endpoint determination, 2) that multiple batches of microdilution plates frozen for different time periods are used during a one-year surveillance programme in contrast to only a single batch of plates during our repetition study.

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