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Multicentre validation of a EUCAST method for the antifungal susceptibility testing of microconidia-forming Dermatophytes.

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Background: Terbinafine resistance is increasingly reported in *Trichophyton rubrum* and *Trichophyton interdigitale* rendering susceptibility testing important particularly in non-responding cases. We performed a multicentre evaluation of a recently proposed modified EUCAST method implementing medium supplemented with chloramphenicol and cycloheximide (CC) to avoid contamination.

Materials/methods: A blinded panel of wild-type and squalene epoxidase (SQLE) target gene mutant *T. rubrum* and *T. interdigitale* strains were distributed to 10 European laboratories. Susceptibility to terbinafine, itraconazole, voriconazole and amorolfine) were performed according to the E.Def 9.3.1 method with and without addition of chloramphenicol and cycloheximide (final concentrations 50 mg/L and 300 mg/L, respectively). Plates were incubated at 25 °C (one laboratory used 30 °C) for 5-7 days until sufficient growth. MICs were determined visually (ignoring trailing growth for itraconazole) and spectrophotometrically with 90% and 50% endpoints yielding a total of 7,829 MICs. *A. flavus* ATCC 204304 and *A. flavus* CNM-CM1813 were included as controls.

Results: 100%/96% (voriconazole) and 84%/84% (itraconazole) MIC determinations fell within the QC ranges for the two QC strains, respectively, and 96%/92% terbinafine MICs fell in a 0.25-1 mg/L 3 two-fold-dilution range suggesting a high interlaboratory reproducibility. Across the six methods, the number of terbinafine MEs varied from 2 (2.6%) to 5 (6.6%) for *T. rubrum* and between 0 and 2 (2.0%) for *T. interdigitale* (lowest for the CC-method (2.6%-4.4%/ 0-1% for *T. rubrum/T. interdigitale*). The difference between the modes for the wt and mutant population were ≥7 two-fold-dilutions in all cases (Table). If excluding a I121M/V237I *T. rubrum* mutant, and two mixed *T. interdigitale* strains, the number of VMEs were CC visual: *T. rubrum:* 1/77 (1.3%), CC spec-90%: 3/68 (4.4%) and CC spec-50%: 1/76 (1.3%), and none for *T. interdigitale*. The activity of voriconazole, itraconazole and amorolfine were quite uniform against *T. rubrum* and *T. interdigitale,* but unacceptably wide MIC ranges were found for the visual and spec-90% inhibition methods for itraconazole (data not shown).

Conclusions: Although none of the laboratories perform dermatophyte testing at a regular basis an acceptable interlaboratory agreement and good separation between SQLE wt and mutants were found, suggesting a robust performance of the proposed method.