P2174 EUCAST reference testing of rezafungin susceptibility: impact of choice of plastic plates

Maiken C. Arendrup*1,2,3, Karin Meinike Jørgensen1, Rasmus Krøger Hare1, Manuel Cuenca-Estrella4, Oscar Zaragoza4

1 Unit for Mycology, Statens Serum Institute, Copenhagen, Denmark, 2 Dept Clin Microbiology, Rigshospitalet, Copenhagen, Denmark, 3 Dept Clinical Medicine, Copenhagen University, Copenhagen, Denmark, 4 Mycology Reference Laboratory, Instituto de Salud Carlos III, Madrid, Spain

Background: Rezafungin is a new long-acting echinocandin currently undergoing Phase 3 clinical trials. Epidemiological cut-off values are necessary for clinical breakpoint setting but have not been established, in part due to an unexplained interlaboratory variation observed particularly for C. albicans. Here we investigated if the choice of microtitre susceptibility testing (AFST) trays contributed to interlaboratory variability of rezafungin. Anidulafungin was included as comparator.

Materials/methods: EUCAST E.Def 7.3.1 AFST using tissue/cell-culture treated (TC-plates) and untreated polystyrene plates (UT-plates) from four manufacturers was performed. Six control strains (C. albicans CNM-CL-F8555, ATCC 64548 and ATCC 64550, C. krusei CNM-CL-3403 and ATCC 6258, and C. parapsilosis ATCC 22019) were tested repetitively (yielding 520 MICs). Five to six wild-type and four to five FKS mutant clinical isolates of C. albicans, C. glabrata, C. krusei, and C. tropicalis and 5 wild-type C. parapsilosis were subsequently tested (580 MICs).

Results: Repetitive MICs for QC strains fell within 2/3 dilutions for rezafungin in 82%/100% and for anidulafungin in 90%/98% of the cases. The modal MIC for rezafungin and collated C. albicans control strain distributions were 0.016 mg/L across TC-plates but 0.03 mg/L across UT-plates. The modal anidulafungin MICs were 0.004 mg/L and 0.016 mg/L for TC-plates versus UT-plates. The difference was most pronounced with Falcon plates (TC-plates: rezafungin MICs 0.008-0.016 mg/L versus UT-plates: 0.016-0.125 mg/L) but not observed for C. krusei and C. parapsilosis.

For rezafungin, 11 MICs for mutants overlapped with the MIC range for wild-type isolates (TC-plates on 4 occasions; UT-plates on 7 occasions). For anidulafungin, overlaps were observed on 5 occasions (all UT-plates). Most overlaps (n=5 for rezafungin; n=3 for anidulafungin) were caused by a C. tropicalis harbouring a F650L alteration and C. glabrata harbouring a D666Y alteration (n=2 for rezafungin; n=1 for anidulafungin). On 12 occasions the MIC of mutant isolates were at the highest MIC of the wild-type range.

Conclusions: Intralaboratory variation was low for both compounds and all plates. Treated plates resulted in lower MICs most profoundly for C. albicans, for Falcon plates, and more for anidulafungin than rezafungin. Standardisation of plate choice for EUCAST AFST would help minimise interlaboratory variation.