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O0265 Multicentre evaluation of XTT assay for echinocandin susceptibility testing of Aspergillus spp. with the EUCAST E. Def. 9.3 method

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Background: Microscopic determination of the minimal effective concentration (MEC) of echinocandins against *Aspergillus* spp. is subjective, time consuming and of doubtful clinical correlation (Arendrup AAC 2008). This multicentre study evaluated a recently developed colorimetric method for in vitro antifungal susceptibility testing of echinocandins against *Aspergillus* spp. using inhibition of metabolic activity as endpoint.

Materials/methods: Forty *Aspergillus* wild-type (10 each of *A. fumigatus, A. terreus*, *A. niger*, and *A. flavus*) and 4 fks mutant *A. fumigatus* isolates were tested. Anidulafungin, caspofungin and micafungin MECs were determined microscopically after 48h of incubation following EUCAST E.Def 9.3 using a reverse microscope. The metabolic activity was determined using the colorimetric XTT assay. Fifty µl containing 400 mg/l of XTT and 6.25 µM of MEN were added to each well after 24h incubation and the colorimetric MIC was determined after additional 1-2h of incubation as the lowest drug concentration corresponding to <10%, 25% or 50% absorbance at 450/630 nm compared to that of the drug-free control. The levels of agreement (within 2 twofold dilutions) between MEC and XTT MICs were calculated for each centre and drug. The reproducibility of XTT method between 1h and 2h of incubation and two replicates were calculated. Finally, the overall agreement among the three centres was calculated.

Results: T Significant inter-strain and inter-centre variation in colour production was found. However, all resistant isolates converted XTT even at high concentrations resulting in >50% colour at the highest concentration of echinocandins in all three centres. The 50% XTT conversion provided the best agreement among the centres. The levels of agreement between MEC and XTT MIC for all centres were 85-95% for caspofungin, 58-85% for anidulafungin and 65-80% for micafungin after 2h of incubation. The overall agreement of XTT MICs between the two centres that standardised inoculum via filtration (11 nm) and cell counting was high for all three echinocandins (88-98%). The reproducibility of XTT method between replicates and incubation times was >70%.

Conclusions: The XTT assay may improve antifungal susceptibility testing of echinocandins against *Aspergillus* spp. when standardized inoculum was used. The XTT assay performed excellently in detecting resistant isolates.