PP4.028 Development and multicentre evaluation of a screening method for echinocandin susceptibility testing of *Aspergillus* spp.

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INTRODUCTION AND PURPOSE

Antifungal susceptibility testing of *Aspergillus* spp. against echinocandins relies on the microscopic determination of the minimal effective concentration (MEC) with broth microdilution method which is difficult-to-perform, time consuming and subjective even for trained staff.

We therefore developed and evaluated in a multicentre study the performance of an echinocandin screening agar method for susceptibility testing of *Aspergillus* spp.

METHODS

Isolates. A total of 40 wild-type *Aspergillus* isolates (10 *A. fumigatus*, 10 *A. flavus*, 10 *A. terreus* and 10 *A. niger*) and 4 non wild-type *A. fumigatus* isolates with high MECs with (AFS678P MUS/794-homo, AFS678P MUS/765-hetero, DPLMD24053) or without (DPLDS55985) known fks mutants were used (kindly provided by Dr. D. Perlin). All isolates were tested blindly at three centers using the EUCAST E.Def 9.3 and the 4-well agar screening methods, against caspofungin, micafungin and anidulafungin.

EUCAST E.Def 9.3. The MECs were determined with EUCAST E.Def 9.3 after 48h of incubation as the lowest drug concentration where aberrant mycelia were observed.

4-well agar screening method. 20 µL of 1-2 McFarland inocula in 0.1% Tween20 were added to each well of the 4-well screening plates containing either caspofungin, micafungin, anidulafungin and drug free control. After inoculum was fully absorbed, plates were incubated for 48 h at 37°C. The formation of fluffy colonies like in the drug free control was recorded for each well after 24h and 48h for the wild-type and the non wild-type isolates.

Optimal concentrations finding and shelf life studies. The optimal concentrations of each echinocandin were evaluated in preliminary studies using extensive range of concentrations (0.001-4 mg/L) and monitor colonial growth qualitatively (fluffy vs. compact appearance, aerial mycelia around the colony) and quantitatively (diameter of colony). Stability of 4-well plates was assessed up to 3 months using QC isolates *C. krusei* ATCC6258, *C. parapsilosis* ATCC22019 and *A. fumigatus* NIH4215.

RESULTS

MEC. The median (range) MECs were 0.5 (0.125-1) mg/L of caspofungin, 0.03 (0.002-0.125) mg/L of micafungin and 0.015 (0.001-0.125) mg/L of anidulafungin for the 40 wild-type *Aspergillus* isolates, and >16 mg/L for all three echinocandins against the non-wild type isolates (DPLMD24053 had a MEC of 2-4 mg/L).

RESULTS

Optimal concentrations and shelf life. Prevention of fluffy colony formation similar to the ones observed in drug free media and by non wild-type *A. fumigatus* isolates was best obtained using 1 mg/L of caspofungin, 0.25 mg/L of anidulafungin and 0.12 mg/L of micafungin. The 4-well plates were stable up to 3 months with the same colonial appearance of *Aspergillus* isolates.

Detection of non wild-type strains. All centres successfully detected all non wild-type isolates by the absence of fluffy colony formation after 24h on echinocandin-containing agars (Figure). All wild-type isolates failed to produce such colonies after 24h. Some wild-type isolates (8/40 for centre 1, 3/40 for centre 2, 0/40 for centre 3) produced a small (colony diameter <8 cm) or irregular halos around the colonies after 48h but they were clearly different than the colonies grew on the drug free control (colony diameter >11 cm).

CAS:caspofungin, MIC:micafungin, ANI:anidulafungin, GC:growth control
^a fluffy colonies, ^b compact colonies

CONCLUSIONS

The 4-well plate agar screening method is suitable for antifungal susceptibility screening of *Aspergillus* isolates and could be combined with the azole-screening method in order to detect azole- and echinocandin-resistant isolates.

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