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Emergence of echinocandin resistance in a patient with chronic pulmonary aspergillosis

Cristina Jimenez-Ortigosa*[1], Caroline Moore[2], David W. Denning[3], David Perlin[4]

[1]*Rutgers University; Public Health Research Institute*

[2]*University Hospital of South Manchester; Mycology Reference Centre Manchester*

[3]*Wythenshaw Hospital, Southmoor Hospital; Education & Research Centre*

[4]*New Jersey Medical School/Rutgers; Rutgers Biomedical and Health Sciences; Public Health Research Institute*

Background: Echinocandin drugs inhibit the β-(1,3)-glucan synthase (GS), which is responsible for the synthesis of β-(1,3)-glucan, an essential component of the fungal cell wall. The echinocandin micafungin exhibits high antifungal activity against *Aspergillus* spp. *in vitro* and is effective in clinical studies against aspergillosis. Micafungin is expected to increase the efficacy rate of treatment in patients with severe aspergillosis when used in combination with another antifungal drugs such as azoles. Mutations in *FKS* genes encoding GS enzymes have been linked to echinocandin resistance (ER) in *Candida* spp. To date, no ER *A. fumigatus* isolates showing a characteristic *fks* mutation have been recovered from patients after exposure to an echinocandin. The aim of this study was to analyse strains isolated from a patient with an aspergilloma and chronic pulmonary aspergillosis who first failed azole therapy and then was treated with micafungin. We assessed the presence of *FKS* mutations and their affect on inhibition of GS by different echinocandin drugs.

Material/methods: Susceptibility testing was performed on ten *A. fumigatus* isolates according to the recommendations of CLSI document M28-A2 for two echinocandins drugs (micafungin and caspofungin). *FKS1* gene was amplified and sequenced for the clinical isolates that showed high MECs for the echinocandins tested. GS was isolated by product entrapment and activity was assessed by glucan polymerization assay. IC_{50} values for GS inhibition were determined using a sigmoidal response curve. The 66 yo female patient has had a right upper lobe aspergilloma for many years and presented with life-threatening haemoptysis. She initially responded well to a sequence of triazoles, but then developed pan-azole resistance and further haemoptysis. She then received 2

years of 6x weekly micafungin 150mg daily, with terbinafine 500mg. Additional symptoms heralded failure of micafungin.

Results: Out of the ten isolates analysed, only one (24053B) showed an epidemiological cut off value (ECV) ≥ 0.5 µg/ml for echinocandins. The MECs of this isolate for micafungin and caspofungin were 2 µg/ml, between 16.6-66.6-fold higher MEC than the sensitive strain (ATCC13073). The *FKS1* gene was fully sequenced and a mutation leading to a F675S amino acid substitution was found in the hot spot 1. β-(1,3)-glucan synthase (GS) complexes were isolated from the isolate 24053B and the inhibition kinetic value (50% inhibitory concentrations [IC_{50}]) was obtained to confirm the ER phenotype. GS complexes isolated from the isolate 24053B yielded higher IC_{50} (>10000-fold) for all echinocandins tested relative to wild-type GS complexes.

Conclusions: The Fks1 point mutation F675S of the *A. fumigatus* isolate 24053B yielded a β-(1,3)-glucan synthase enzyme with highly reduced sensitivity to echinocandin drugs resulting in elevated MICs. To date, this is the first reported case of echinocandin resistance due to a point mutation in the *FKS1* gene in an *A. fumigatus* clinical isolate.