28th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Madrid, Spain
21 – 24 April 2018

P0185 Stability of cefiderocol against clinically-significant broad-spectrum oxacillinases

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Background: The novel siderophore cephalosporin cefiderocol (S-649266) with potent activity against Gram-negative pathogens was recently developed (Shionogi & Co., Ltd). The purpose of this study was to evaluate the stability of this new molecule against a series of carbapenem-hydrolyzing ß-lactamases (CHDLs) encountered in Enterobacteriaceae and *Acinetobacter* spp., namely OXA-48, OXA-23, and OXA-40.

Materials/methods: The overexpression of the three carbapenemases OXA-23, OXA-40 and OXA-48 were obtained after cloning of their genes in expression vectors followed by expression in reference *E. coli* strains. The bla_{OXA-48} gene was cloned in pET24 vector that was transformed into *E. coli* strain BL21(DE3) pLysS. For the expression and purification of OXA-23 and OXA-40, the PCR-amplified genes were cloned in pGEX vector and transformed into *E. coli* strain TOP10. Production of the different enzymes was induced by adding IPTG 1mM for 3 h. Bacterial cultures were centrifuged and the pellets were resuspended in binding buffer. Sonicated pellets were concentrated using VIVASPIN columns (10k Da). The fractions were introduced into an AKTA prime collector using GEStrap column. Cefiderocol was provided in a lyophilized form and stored at -70°C prior use. Ampicillin, ticarcillin, chloramphenicol and imipenem were obtained from Sigma.

MICs were determined following the CLSI recommendations by broth microdilution, including the use of iron-depleted media for cefiderocol. MICs of ampicillin, ticarcillin, imipenem, and cefiderocol were measured for *E. coli* reference strains and isogenic *E. coli* strains containing recombinant vectors producing either OXA-48, or OXA-40, or OXA-23 ß-lactamases.

Results: Susceptibility testing results showed that the three carbapenemase genes, once expressed in *E. coli*, conferred resistance to ampicillin and ticarcillin, decrease susceptibility to imipenem, and did not affect at all the susceptibility to cefiderocol.

Kinetic parameters showed a significant hydrolysis of ampicillin, ticarcillin and to a lesser extend imipenem by the three enzymes that was consistent to previous findings. No hydrolysis of cefiderocol was detected for any of those enzymes.

Conclusions: Cefiderocol displayed an excellent activity against *E. coli* recombinant strains producing CHDLs. The activity of cefiderocol appears to be excellent, and the lack of hydrolysis observed for all the enzymes tested highlights its potential against CHDL producers.