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Paper Poster Session
Colistin and polymyxin B pharmacokinetics

Population analysis of colistin resistance in carbapenemase producing Klebsiella pneumoniae (CP-Kp) isolates

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Background: Life threatening infections due to Carbapenemase producing *Klebsiella pneumoniae* (CP-Kp) isolates are an emerging clinical problem worldwide. This phenomenon has increased the interest in older drugs, such as colistin. Colistin exhibits *in vitro* and *in vivo* bactericidal activity against CP-Kp although resistance to colistin has been reported. We therefore, studied the development of resistance during colistin exposure simulated in an *in vitro* PK-PD model for a non-CP-Kp and a CP-Kp isolates.

Material/methods: Two *K. pneumoniae* clinical isolates were used, one non-CP-Kp with CLSI MIC 0.5 mg/l and one CP-Kp with colistin CLSI MICs 1 mg/l. One clinical dosing regimen of colistin were simulated in an in vitro PK-PD dialysis/diffusion closed model targeting fCmax of 4.5 mg/l q12. Drug levels were determined by a microbiological assay and bacterial growth by quantitative cultures. Resistant subpopulations were detected at 0h, 4h, 8h and 24h by spreading 50μl on Mueller–Hinton agar plates containing 0, 8, 16 and 32 mg/L colistin in order to detect subpopulations with different in vitro susceptibility. The MICs of a subset of subpopulations were assessed with CLSI broth microdilution method after 2 sequential subcultures on antibiotic-free medium.

Results: Within the first 2h of exposure, rapid killing with 4.37 and 3.66 $\log_{10}$ CFU/ml reduction was observed against the isolate with MIC 1 and 0.5 mg/l, respectively. For the isolate with MIC 1, regrowth was observed at 12h reaching a bacteriostasis at 24h whereas for the isolate with MIC 0.5 mg/l, regrowth was observed at 8h reaching 1 $\log_{10}$CFU/ml increase at 24h. For the isolate with the

MIC 1 mg/l, at 0h only subpopulations (1.77 log_{10}CFU/ml) grown on agar with 8 mg/l of colistin were found whereas at 8h subpopulations grown on agar media with 8, 16 and 32 mg/l of colistin were observed (1.3-3.3 log_{10}CFU/ml). On the contrary, for the isolate with MIC 0.5 mg/l, at 0h subpopulations grown on all agar media with 8, 16 and 32 mg/l of colistin were found (1.6-2.25 log_{10}CFU/ml) and persisted at 24h (1.6-3.44 log_{10}CFU/ml) The MICs of these subpopulations ranged from 16-64 mg/l.

Conclusions: Different colistin resistant subpopulations at different rates were found during exposure to colistin. The frequency of these subpopulations were different between the two strains tested. These differences may impact the pharmacodynamics of colistin.