P1123
Poster Session IV
Pseudomonas sp. and Burkholderia sp.: knowing the mechanics of resistance

RAPID EMERGENCE OF COLISTIN RESISTANT PSEUDOMONAS AERUGINOSA MUTANTS

A. Nogues[1], A. Bolard[1], P. PlÈsiat[1], **K. Jeannot**[1]

[1]Centre National de Référence Résistance aux Antibiotiques, CHRU Jean Minjoz Université de Franche-Comté, Besançon, France

Objectives. Aerosolized polymyxins B and E (colistin) are more and more often used to treat acute pulmonary infections caused by multidrug (MDR) and extensively-drug (XDR) resistant Gram-negative bacilli. However, despite the strong and straightforward bactericidal effect of these antimicrobials, resistant mutants of *P. aeruginosa* are sometimes reported to emerge under particular clinical circumstances. The goal of the present study was to characterize two colistin highly resistant mutants selected by aerosol therapy in a non cystic fibrosis patient.

Methods. Three *P. aeruginosa* strains were isolated from two distinct tracheal aspirates in a 70-year-old man presenting an exacerbation of pulmonary secretions in a context of lung adenocarcinoma. The patient was treated with 2 MUI of aerosolized colistin methane sulfonate (CMS) twice/day for 7 days. The clonality of the bacterial isolates was established by MLVA. Antibiotic MICs were determined by the broth macro-dilution and agar dilution methods according to EUCAST guidelines. Bacterial growth was recorded spectrophotometrically in Mueller Hinton broth. Mutations in genes *pmrB, parRS, phoQ, cprRS and colRS* were searched by BLAST alignment analysis after by DNA sequencing.

Results. MLVA confirmed that the three isolates were clonally related. The isolate (T0) recovered prior to colistin therapy appeared to be resistant to carbapenems (imipenem, 16 mg/mL), ceftazidime (16 mg/L), ciprofloxacin (>16 mg/L), and aminoglycosides (tobramycin 32 mg/mL) but fully susceptible to colistin (MIC=1mg/L). Twenty-four hours after treatment, two mutants (T1, T2) showing an increased resistance to colistin (16 and 32 mg/L, respectively) were isolated from a same respiratory sample. Surprisingly, both of them exhibited a higher susceptibility to ß-lactams (ceftazidime 1 mg/L; imipenem 2 mg/L) than T0. Sequencing of the genes known to contribute to colistin resistance development in *P. aeruginosa* revealed the existence of a large deletion (30 amino acid residues) in sensor PhoQ (T1, T2), and several amino-acid substitutions in response regulator ParR of the most resistant isolate (T2). Both post-therapy isolates T1 and T2 turned out to grow more poorly than T0 in Mueller Hinton broth.

Conclusion. Despite an excellent *in vitro* activity and a good pulmonary diffusion, colistin cannot prevent the emergence of resistant mutants *in vivo*. Though easily obtained *in vitro* (data not shown) such mutants are rarely isolated in acute pulmonary infections likely because of the impaired fitness that is associated with colistin resistance. Accumulation of mutations in the two-component regulatory systems that control the expression of LPS modification accounts of the acquisition of high levels of resistance to polymyxins in clinical strains of *P. aeruginosa.*