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Abstract (poster session)

**Resistance and virulence of Pseudomonas aeruginosa mutants overproducing the MexXY/OprM efflux pump**

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Objectives: Constitutive overproduction of the active efflux system MexXY/OprM (XY/M) is a major cause of resistance to aminoglycosides (AGs), ciprofloxacin (CIP), and cefepime (FEP) in clinical strains of P. aeruginosa. In agrZ mutants, upregulation of the mexXY operon results from mutations in mexZ, the local repressor gene of mexXY. In this study, we describe a novel type of mutants named agrW1 exhibiting allelic mutations in the genes encoding 23S ribosomal RNA. Methods: From reference strain PAO1, two spontaneous multidrug resistant mutants, PAOW1 and KJD9, were selected in vitro on amikacin and dihydrostreptomycin, respectively. DNA sequencing, gene cloning, gene replacement, and RT-qPCR experiments were performed according to standard protocols. Whole genome sequences (6.3 Mbp) and transcriptomic data were obtained by using the Illumina and Affymetrix technologies, respectively. Bacterial cytotoxicity was assessed on a murine macrophage cell line by measuring extracellular lactate dehydrogenase activities. Results: The two mutants were found to share a similar resistance profile to AGs (MIC of amikacin 32 µg/ml), CIP (1 µg/mL), and FEP (16 µg/mL). Comparison of the genomic sequences of the mutants to that of PAO1 revealed single mutations (C1262G in PAOW1, C2121A in KJD9) in 1 of the 4 alleles encoding 23S rRNA. In addition to mexXYZ, 65 and 80 genes were up- or down-regulated (>= 2-fold) in PAOW1 and KJD9, respectively compared with the parent strain. Interestingly, 40 genes of the Type 3 Secretion System (SST3) were significantly (from 2.6 to 9.7) overexpressed in both mutants. Consistent with these data, the bacteria were 2-fold more cytotoxic on murine macrophages at 2 h postinfection than wild-type PAO1. Gene deletion experiments demonstrated that the mutations were responsible for both the activation of XY/M and of T3SS. Interestingly, slightly different subsets of genes were differentially expressed between the two mutants. Conclusion: For the first time in P. aeruginosa, this work reveals a coregulation between drug resistance (XY/M) and virulence (T3SS) through single mutations in 23S rRNA genes. Recently, the relevance of such a regulation was established by the identification of agrW1 mutants in a collection of multidrug resistant clinical strains.