**OPRD MUTATIONS IN IMIPENEM-RESISTANT CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA**

Jean-Paul Pirnay\textsuperscript{1,2}, Daniel De Vos\textsuperscript{3,1}, Dimitris Mossialos\textsuperscript{1}, Alain Vanderkelen,\textsuperscript{2} Martin Zizi,\textsuperscript{2} and Pierre Cornelis\textsuperscript{1*}

Laboratory of Microbial Interactions, Vrije Universiteit Brussel, Flanders Interuniversity Institute of Biotechnology, B-1640 Sint-Genesius-Rode,\textsuperscript{1} Science Department, Belgian Military Medical Service, Queen Astrid Military Hospital, B-1120 Neder-Over-Heembeek,\textsuperscript{2} and Department of Infectious Diseases, Innogenetics N.V., B-9052 Ghent,\textsuperscript{3} Belgium

*Pseudomonas aeruginosa* is intrinsically resistant to a broad range of commonly used antibiotics. The potency of (β-lactams) antibiotics is limited by a rather impermeable outer membrane, which works in synergy with active multi-drug efflux mechanisms, and inducible β-lactamases (from chromosomal or plasmid-borne genes). Carbapenems are not prone to inactivation by extended spectrum β-lactamases and penetrate across the outer membrane of *P. aeruginosa* through a specialized porin, protein D2 (OprD), which allows selective penetration of basic amino acids, small peptides containing these amino acids, and carbapenems, their structural analogs. Prolonged treatment of *P. aeruginosa*-infected patients with imipenem has often allowed for the emergence of imipenem-resistant mutants. These resistant strains have either lost OprD or have strongly reduced OprD levels due to a nfxC-type of quinolone-resistant mutation (*mexT*) which represses *oprD* expression and activates the *mexEF-oprN* multidrug efflux operon. Recently Ochs et al. (1) suggested that OprD production is influenced by more than one mechanism of repression. Yoneyama and Nakae (2) studied the elimination of OprD in 23 independent imipenem-resistant mutants from a strain harboring a plasmid carrying cloned *oprD* and having a mutation in chromosomal *oprD*. They found that all 23 mutant plasmids could be divided into two groups, both carrying a specific deletion in the *oprD* gene. One group had an 11-bp deletion in the coding region, generating a frameshift mutation and a premature termination codon. Another had a 1,204-bp deletion covering the initiation codon and putative Shine-Dalgarno sequences. All isolates produced immunologically undetectable levels of OprD. Although only plasmid-borne *oprD* was analyzed, it was suggested that elimination of OprD from most imipenem resistant *P. aeruginosa* isolates is due to efficient selection of *oprD* deletion mutants. In this study we report and discuss the possible mechanisms by which resistance to imipenem emerged in 17 non-clonal, imipenem-resistant *P. aeruginosa* clinical isolates. We report here that loss of OprD was the predominant reason of imipenem resistance, and that in 10 isolates, OprD loss was caused by a chromosomal *oprD* mutation. But, all mutations were unique, and different from the two deletions previously described.