Imipenem Resistance in *Pseudomonas aeruginosa* PAO: Mapping of the OprD2 Gene

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Carbapenem antibiotics have been shown to penetrate the outer membrane of *Pseudomonas aeruginosa* through a unique porin protein, OprD2. We mapped the OprD2 gene by conjugation using plasmid FP2 and by transduction using phage F116L. This gene maps between 71 and 75 min on the PAO1 chromosome.

Imipenem resistance (Imp') in *Pseudomonas aeruginosa* is usually dissociated from resistance to other β-lactams (6, 13). We (6, 7) and others (1, 4, 10) have shown that imipenem-specific resistance is due to loss of a specific outer membrane protein (Opr) with a molecular weight of 45,000 to 47,000. This Opr, recently identified by Trias and Nikaido as porin D2, is a porin which facilitates the penetration of carbapenem antibiotics across the outer membrane of *P. aeruginosa* (11).

The purpose of this study was to map the chromosomal genes responsible for imipenem resistance in a genetically well-characterized strain of *P. aeruginosa*, PAO1.

(References: 1-13)

*Bacterial strains, bacteriophages, and plasmids.* All bacterial strains used in this study are listed in Table 1. Phage F116L was used for transduction (3). Plasmid FP2 was used in conjugation experiments (3).

*Media.* Bacteria were grown in Luria-Bertani (LB) medium (Difco, Detroit, Mich.). Vogel-Bonner minimal medium (12) without citrate was used to select recombinants in conjugation experiments. A stock solution of glucose was added at a final concentration of 50 mM. The concentration of amino acids was 1 mM. *Pseudomonas* minimal medium supplemented with 0.4% glucose (5) was used for transduction. Amino acids were used at a concentration of 25 μg/ml.

*Isolation of mutants.* Semispoorous imipenem-resistant mutants were obtained by plating 10⁹ cells each of strains PAO1 and PAO381 onto LB plates containing 10 μg of imipenem per ml.

*Genetic crosses.* Plate matings were performed as described by Haas et al. (2). Transductions were carried out by the method of Miller and Ku (5).

*Chemicals.* Imipenem was a gift from Merck Sharp and Dohme (Rahway, N.J.).

**MICs.** MICs were determined by an automated method (Vitek Systems, St. Louis, Mo.) (9). Table 2 lists the β-lactam susceptibilities of the parent strains (PAO381 and PAO1), four imipenem-resistant mutants (PAO1.1, PAO1.2, PAO1.3, and PAO1.4) picked at random for further study and then cloned on imipenem-containing agar, and PJQ382. Four of these five mutants were selectively resistant to imipenem, while one (PAO1.2) was broadly resistant.

**Outer membrane proteins.** Outer membrane proteins were isolated and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (6). As seen in Fig. 1, the imipenem-resistant mutants (lanes 3 and 5 through 8) show reduction or deletion of a 47-kDa outer membrane protein compared with the parent PAO381 and PAO1 strains.

**Mapping of mutations conferring imipenem resistance.** Imp' variants (resistance frequency, 10⁻⁷⁻¹) of *P. aeruginosa* PAO381 [leu-10 FP2"(Hg")] were isolated by plating on overnight culture on LB agar containing imipenem (10 μg/ml). When imipenem-resistant PJQ382 was mated with recipient PAO222 [his-4 lys-12 met-28 trp-6 proA82 FP"], 50% of the *his-4* selected recombinants were imipenem resistant. When the same donor was mated with recipient PAO1042 [pur-67 cys-59 thr-9001 proB65 FP"], 100% of the *proB"* recombinants were imipenem resistant. Other chromosomal markers tested (e.g., *his-4*, *met-28*, *trp-6*, *proA*, and *cys-59*) did not yield significant numbers of imipenem-resistant colonies. Thus, imipenem resistance appears to map in a late region of the PAO1 chromosome, linked to the *proB* locus (Fig. 2).

Cell-free lysates of the generalized transducing phage F116L were prepared with PJQ382 and used to transduce PAO222 and PAO1042. The transfer of amino acid prototroph-
phy was used as the primary criterion of selection for transductants. At least 100 and usually 200 transductants were screened for coinheritance of imipenem resistance by replica plating on LB plates containing 10 μg of imipenem per ml. The frequency of cotransduction of Imp with proB65 was 7.2%, and the frequency of cotransduction with ilv-226 was 1.5%. No cotransduction with met-28 or thr-9001 was detected. These data indicate that the Imp gene lies between proB and ilv-226 and is more closely linked to proB than to ilv-226.

Since our initial report describing loss of a specific outer membrane protein in clinical isolates of *P. aeruginosa* displaying selective resistance to imipenem (6), a compelling body of evidence has emerged to support the hypothesis that carbapenems traverse the outer membrane of this organism through a unique porin. This evidence includes the following points. (i) Experiments in other laboratories have produced similar data on outer membrane protein profiles in clinical isolates (4) and laboratory mutants (1) of imipenem-resistant *P. aeruginosa*. (ii) Two groups of investigators have described an imipenem-specific permeability barrier in resistant strains (8, 11). (iii) Trias and Nikaido recently identified the 47-kDa outer membrane protein as porin D2 (11). The normal substrate of this porin may be basic amino acids (11).

The purpose of this study was to map the gene controlling imipenem resistance on the PAO chromosome. To our knowledge, this is the first outer membrane protein gene in this organism to be mapped.

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### REFERENCES


