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## Leading article

# Of *Pseudomonas*, porins, pumps and carbapenems

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The carbapenems, imipenem and meropenem, are anomalous $\beta$-lactams in their antipseudomonal behaviour, as well as in their $\beta$-lactamase stability. In the case of imipenem, MICs for *Pseudomonas aeruginosa* isolates are unrelated to those of other $\beta$-lactams, whereas a strong relationship exists between the MICs of different penicillins and cephalosporins for most *Pseudomonas* isolates.[1–4] This observation is partly because imipenem MICs are independent of derepression of the chromosomal AmpC $\beta$-lactamase, whereas this factor strongly co-determines the MICs of penicillins and cephalosporins.[5] More critically, the MICs of imipenem are unaffected by the broad-spectrum intrinsic resistance expressed by many *P. aeruginosa* isolates, whereas this mechanism strongly co-determines the MICs of penicillins, cephalosporins and several unrelated drug classes, including quinolones, tetracyclines and chloramphenicol.[1–4]

Intrinsic resistance is expressed to a variable degree by all *P. aeruginosa* isolates, which are less susceptible than Enterobacteriaceae to most antibiotics. This behaviour was long thought to depend on impermeability,[1,2] but is now found to involve an interplay of impermeability with multi-drug efflux, mediated principally by MexA-MexB-OprM.[3,4,6,7] The MexB protein is a broad-spectrum pump, located in the cytoplasmic membrane; the OprM protein is a pore-forming protein that provides an exit portal through the outer membrane; and the MexA protein physically links these components.

Upregulation of MexA-MexB-OprM arises at high frequency as a result of the *nalB* mutation at the *mexR* locus[8–10] and raises the MICs of penicillins, cephalosporins, quinolones, tetracycline and chloramphenicol (Table), but not those of imipenem. *nalB* mutants may be selected *in vitro*, or during therapy with fluoroquinolones, penicillins and cephalosporins,[11,12] and were the predominant phenotype selected when carbenicillin and ticarcillin were the mainstays of antipseudomonal treatment.[13] The broad range of resistance conferred by upregulation is intriguing, and it is postulated that MexA-MexB-OprM has a natural role in removing amphipathic substances, which otherwise invade and disorganize the cytoplasmic membrane.[14] Such a role would favour the evolution of a very broad substrate specificity. The lack of activity against imipenem may be because this carbapenem lacks any lipophilic phenyl or heterocyclic side chain(s).

The advantage of evading intrinsic resistance is tempered by the fact that imipenem readily selects resistant mutants of *P. aeruginosa*, which are found to lack OprD protein (initially called D2 porin).[15] The primary role of this protein is in the passive uptake of basic amino acids across the outer membrane, but it forms pores that are also permeable to carbapenems, though not to other $\beta$-lactams.[16] Its loss raises the imipenem MICs from 1–2 mg/L, as for typical *P. aeruginosa*, to 8–32 mg/L, thus conferring clinical resistance. The MICs of non-carbapenems are unaffected. The resistance demands continued expression of the chromosomal AmpC $\beta$-lactamase, whether inducible or derepressed,[17] but this aspect is peripheral in the present context. Carmeli *et al.*[18] noted that selection of resistant *P. aeruginosa* during imipenem therapy was substantially more frequent than selection of ceftazidime-, piperacillin- or ciprofloxacin-resistant mutants, and Calandra *et al.*[19] found a 17% rate of resistance emerging during imipenem treatment of *P. aeruginosa* infections.

The interactions of meropenem with these resistance mechanisms differ from those of imipenem, with implications that are provoking debate. The MICs of meropenem are 0.12–0.5 mg/L for typical strains of *P. aeruginosa*, and *either* upregulation of MexA-MexB-OprM *or* loss of OprD raises them to 2–4 mg/L,[5,20] thereby reducing susceptibility without conferring resistance relative to either BSAC or NCCLS breakpoints. It is inferred that meropenem, like

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**Table.** Effects of mutations affecting resistance to $\beta$-lactam antibiotics in *P. aeruginosa*

| Mutational event | Effect on susceptibility or resistance to | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ureidopenicillins | carboxypenicillins | ceftazidime | imipenem | meropenem | quinolones | tetracycline | chloramphenicol |
| Derepression of AmpC $\beta$-lactamase (mutation at *ampD*) | R | r | R | – | – | – | – | – |
| Upregulation of MexA-MexB-OprM (*nalb* mutation at *mexR*) | r/R | R | r/R | – | r | R | RR | RR |
| Loss of OprD | – | – | – | R | r | – | – | – |
| Upregulation of MexE-MexF-OprN (*nfxc* mutation, also downregulating *oprD*) | – | – | – | r/R | r | R | – | – |
| Loss of OprD plus upregulation of MexA-MexB-OprM | R | R | R | R | R | R | RR | RR |

r, susceptibility reduced, often without frank resistance; R, resistance usually conferred; RR, natural resistance of *P. aeruginosa* increased to these drugs; –, no effect on MICs.

imipenem, can use the OprD pathway to enter the pseudomonal cell but that, unlike imipenem, it is recognized and ejected by MexB-mediated efflux, presumably because of its 2′ heterocyclic side chain.

These differences between imipenem and meropenem allow arguments for either compound as 'the less likely to cause resistance in *P. aeruginosa*'. The case for imipenem[21] argues that, although it selects OprD$^-$ mutants, these have a narrow-spectrum insensitivity and remain fully susceptible to other drugs, whereas meropenem resistance co-depends on upregulation of MexA-MexB-OprM, a mechanism that compromises fluoroquinolones as well as other $\beta$-lactams. The case for meropenem[17,22] is that substantive resistance is much harder to achieve than to imipenem, since two mutations (loss of OprD and upregulation of MexA-MexB-OprM) are needed rather than one. The likelihood that a cell will simultaneously undergo both mutations is *c.* $<10^{-14}$, whereas imipenem-resistant mutants, lacking OprD, emerge at *c.* $10^{-7}$. Although formal analyses of the risk of meropenem resistance emerging during therapy are lacking, there are far fewer case reports than for imipenem, even after 6 years of use. Moreover, there is a greater facility to raise the dosage with meropenem, overcoming low-level resistance.

The stronger case is surely that for meropenem, primarily because of the high frequency of emergent imipenem resistance reported by Carmeli *et al.*[18] If imipenem is preferred as an antipseudomonal carbapenem, there is a strong risk that resistance will emerge and that a subsequent antibiotic will be needed. This, in turn, will exert its own selection pressure. In general the risk of obtaining multi-resistant mutants in such sequential usage is greater than where a drug requiring a double mutation is used in the first instance against a drug-naive bacterial population.[23]

Several caveats should be noted. First, in the UK at least, resistance to either carbapenems remains rare in *P. aeruginosa*.[24] Secondly, the present arguments relate only to the therapy of *P. aeruginosa* infections. Thirdly, upregulation of MexA-MexB-OprM and loss of OprD are not the sole routes to carbapenem resistance in *P. aeruginosa* (although they are considerably the most prevalent). Upregulation of another efflux system, MexE-MexF-OprN is associated with raised MICs of both carbapenems as well as fluoroquinolones,[25] although it is unclear whether this pump itself recognizes carbapenems or whether the association reflects co-regulation of MexE-MexF-OprN with OprD.[26] This infrequent mechanism, mediated by the *nfxc* mutation, is sometimes selected by quinolones but rarely, if ever, by carbapenems. Finally, although still rare, metallo-$\beta$-lactamases of the IMP and VIM families are increasing sources of resistance to carbapenems in *P. aeruginosa*.[27] Isolates of *P. aeruginosa* with IMP-1 enzyme are widely scattered, but infrequent, in Japan,[28] and a major outbreak of VIM-producers was reported in Greece.[29] IMP and VIM enzymes can confer high-level resistance to all $\beta$-lactams,

including both carbapenems, and may become a significant problem in the future. At present, though, these enzymes are rare, and future increases in their prevalence require *de novo* spread of strains or genes. In contrast, upregulation of MexA-MexB-OprM and loss of OprD arise by simple mutation, can occur in any *P. aeruginosa* strain and are widely relevant to the choice of antipseudomonal carbapenem.

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