Cooperation between active efflux and porin alteration is sufficient to confer high-level resistance to meropenem in *Pseudomonas aeruginosa* clinical isolates

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**Introduction & Purpose**

- Carbapenems are used for treating infections caused by multidrug-resistant Gram-negative bacteria, which may promote the risk of emergence of high-level resistance usually associated with carbapenemase expression [1]. Upon screening of a collection of *Pseudomonas aeruginosa* (PA) isolates from patients suffering from cystic fibrosis, MICs ≥ 64 mg/L for meropenem were observed in carbapenemase(-) negative strains.

- Six meropenem resistant (MEM-R) strains isolated from clinically-confirmed cystic fibrosis (CF) cases were compared to seven MEM-R strains collected from patients suffering from hospital-acquired pneumonia (HAP) (Table 1).

- Our aim was to examine whether activity of efflux pumps, alterations of porins and expression of other β-lactamase(s) than carbapenemase(s) could explain the high-level resistance to meropenem in these strains.

**Methods**

- Six meropenem resistant (MEM-R) strains isolated from clinically-confirmed cystic fibrosis (CF) cases were compared to seven MEM-R strains collected from patients suffering from hospital-acquired pneumonia (HAP) (Table 1).

- Memopenem (MEM) MICs were measured by microdilution in CA-MHB according to CLSI [2] in the absence or presence of the efflux pump inhibitor Phe-Arg-β-Naphthylamide (PAβN) at the concentration used.

- Carbapenemases (VIM, IMP, NDM, OXA-48, KPC), ESBLs, blaBELL, (BEL-1 to 3), PER (PER-1 to 5), GES (GES-1 to 18), VEB (VEB-1 to 7), CTX-M (2, 3, 9), blatEM, blatSHV, and blaoXA (1, 2, 9, 10, 18, 20, 23, 30, 58, 198), and AmpC expression was assessed by molecular techniques (PCR) and/or phenotypic tests (double disk for metallo-β-lactamases).

- β-lactamase expression was assessed by molecular techniques (PCR) and/or phenotypic tests (double disk for metallo-β-lactamases ; ESBL NDM and Carbapen MIC tests (3)).

- oprD2 gene and its promoter were sequenced.

**Results**

- Carbapenemase phenotypic detection returned negative results for CF strains but positive results for HAP strains (Fig. 1), with presence of blaMEX-2 (metallo-β-lactamase gene) confirmed by PCR.

- Meropenem MICs were decreased of 2 to 4 log dilutions in the presence of PAβN for all CF strains but not for HAP strains (Fig. 2).

- Complete restoration of susceptibility upon in vitro addition results from the coexistence of OprD2 mutations, AmpC production and/or possibly incomplete inhibition of MEM efflux by PAβN at the concentration used.

- As active efflux can confer cross-resistance to other antipseudomonal agents, i.e. other β-lactams or quinolones for example, determining the mechanism of resistance to meropenem is recommended in clinical settings in order to optimize the antibiotic therapy.

**Conclusions**

- Antibiotic exclusion from bacteria by concomitant efflux and reduced uptake is as effective as carbapenemases to confer high-level resistance to meropenem in strains expressing AmpC.

- Incomplete restoration of susceptibility upon PAβN addition results from the coexistence of OprD2 mutations, AmpC production and/or possibly also incomplete inhibition of MEM efflux by PAβN at the concentration used.

- As active efflux can confer cross-resistance to other antipseudomonal agents, i.e. other β-lactams or quinolones for example, determining the mechanism of resistance to meropenem is recommended in clinical settings in order to optimize the antibiotic therapy.

**References**


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