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Can efflux confer high levels resistance to meropenem (MEM) in *Pseudomonas aeruginosa* (Pa) clinical isolates?

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Abstract (revised)

Objectives:
Carbapenems are increasingly used for treating infections due to multidrug-resistant *Pseudomonas aeruginosa* (MDR-Pa), with ensuing emergence of high level resistance (HLR) usually ascribed to the expression of carbapenemases. Upon screening a collection of Pa isolates (n=157) obtained from patients suffering of cystic fibrosis (CF), MICs ≥ 64 mg/L were observed for MEM in 19 strains that were negative by phenotypic detection of carbapenemase(s) (see Method). We therefore examined whether efflux could not be the cause of this HLR.

Methods:
MICs were measured by microdilution in CA-MHB following CLSI recommendations in the absence or in the presence of the wide-spectrum putative efflux pump inhibitor Phe-Arg-β-naphthylamide (PAβN; 20 mg/L [we checked for absence of direct toxicity to Pa at this concentration]). Carbapenemase(s) production was detected using the Carba NP test [Nordmann-Poirel] with imipenem as a substrate [1,2]. The 19 CF isolates were compared to 14 isolates obtained from patients suffering from hospital acquired pneumonia (HAP) and for which MEM showed similar MICs (≥ 64 mg/L). HAP-isolates were screened by multiplex PCR for 5 different genetic types of carbapenemases (NDM, OXA-48, IMP, KPC, VIM).

Results:
Out of the 19 isolates from CF patients, 8 showed a marked decrease of MIC (about 3 $\log_2$ dilutions) upon addition of PAβN. In contrast, no change was seen for the 14 strains from HAP patients that were positive for *vim-2* (metallo-β-lactamase).

Conclusions:
Active efflux can contribute to HLR resistance to MEM in Pa as evidenced here for isolates obtained from CF patients. Incomplete restoration of susceptibility may result from coexistence of other resistance mechanisms and/or incomplete inhibition of MEM efflux by PAβN. Since Pa efflux systems show a broad specificity of substrates, they may contribute to MDR phenotypes, making therapeutic options scarce for patients infected by these strains.

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Introduction & Aims

- Carbapenems are broad-spectrum antibiotics that are often used as first-line treatment for unresponsive infections due to multidrug-resistant *Pseudomonas aeruginosa* (MDR-Pa). Their increased use in this setting may promote the risk of emergence of high level resistance (HLR), which is usually ascribed to the expression of carbapenemases [3,4].
- Upon screening a collection of MDR-Pa obtained from patients suffering of cystic fibrosis (CF) and having received multiple antibiotic treatments, high MICs (≥ 64 mg/L) were observed for meropenem in strains that were negative for carbapenemases.
- Our aim was to evaluate a potential role of active efflux in the HLR phenotype of these strains.

Materials & Methods

Bacterial isolates. 14 strains collected form patients suffering from hospital-acquired pneumonia (HAP) and 8 strains collected from CF patients were selected from larger collections based on a preliminary screening for resistance to meropenem (MIC > 32 mg/L).

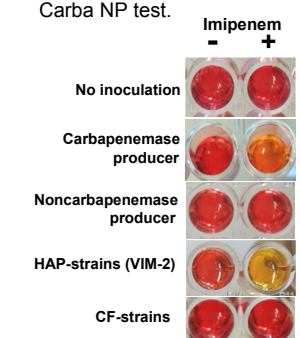
Carbapenemase(s) production. Phenotypic detection of carbapenemases was performed using Carba NP test [Nordmann-Poirel] with imipenem as a substrate [1,2]. In strains that were positive, the presence of carbapenemases genes (*ndm*, *oxa-48*, *imp*, *kpc*, *vim*) was established by multiplex PCR.

Susceptibility testing. MICs were determined by microdilution in cation-adjusted Muller Hinton broth following CLSI recommendations [5] and in the absence or presence of PAβN (20 mg/L). *P. aeruginosa* ATCC27853 served as quality control strain.

Data analysis. Susceptibility/resistance patterns were assessed using EUCAST interpretive criteria [6]. Statistical analyses were performed using GraphPad Instat v.3.10.

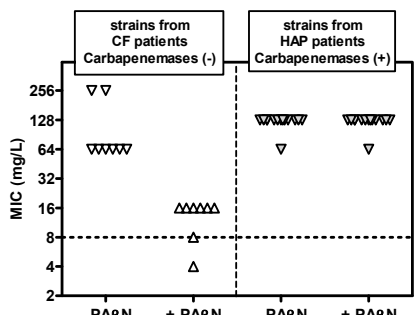
Results

Fig. 1: Phenotypic screening of carbapenemases using the Carba NP test.



➔ **All CF strains were negative while all HAP strains were positive (with presence of *vim-2* [metallo-β-lactamase] confirmed by PCR).**

Fig. 2: Influence of PAβN (20 mg/L) on the MIC of MEM (the horizontal dotted line shows the current EUCAST susceptibility breakpoint [5]).



➔ **MEM MICs were significantly decreased in the presence of PAβN for all CF strains but not for HAP strains (see values in Table 1).**

Table 1 : Change of MEM MIC with PAβN as a function of MIC w/o PAβN

Origin	Carba NP test	MIC (mg/L) geom. mean (range)	
		+ PAβN	- PAβN
Cystic fibrosis	- **(8/8)**	12 * (4-16)	91 (64-256)
Hospital acquired pneumonia	+ **(14/14)**	122 (64-128)	122 (64-128)

* mean $\log_2$ difference (95%CI): 2.8 (2.0-3.7); $p < 0.0001$

Conclusions

- *P. aeruginosa* strains from CF patients can display high level of resistance (HLR) to meropenem without detectable expression of carbapenemase(s).
- In these strains, addition of a wide spectrum efflux pump inhibitor (PAβN) markedly decreases their MICs, suggesting that efflux is primarily responsible for their HLR phenotype.
- However, the fact that MICs remain above the upper limit of the EUCAST wild type distribution suggest the coexistence of other resistance mechanisms and/or an incomplete efflux inhibition by PAβN.
- The comparison with strains from HAP patients highlights that efflux can contribute as effectively as carbapenemase(s) to high level resistance to meropenem.

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