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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 ascribed to carbapenemase expression [1]. Upon screening of a collection of *Pseudomonas aeruginosa (Pa)* isolates from patients suffering of cystic fibrosis, MICs ≥ 64 mg/L for meropenem were observed in carbapenemase(s) - negative strains.

❑ 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).

❑ Meropenem (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; 20 mg/L [no toxicity at this concentration]).

❑ Carbapenemases (VIM, IMP, NDM, OXA-48, KPC), ESBLs blaBEL (BEL-1 to 3), PER (PER-1 to 5, 7), GES (GES-1 to 18), VEB (VEB-1 to 7), CTX-M (1, 2, 9), blaTEM, blaSHV, and blaOXA (1, 2, 9, 10, 18, 20, 23, 24, 30, 58, 198) , and AmpC expression was assessed by molecular techniques (PCR) and/or phenotypic tests (double disk for metallo-β-lactamases ; ESBL NDP and Carba NP tests [3]).

❑ *oprD2* gene and its promoter were sequenced.

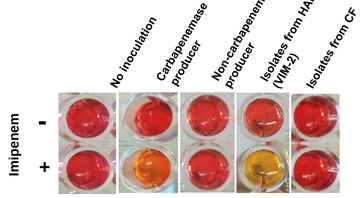
❑ *mexA*, *mexX* and *mexC* transcripts were quantified by qPCR.

References

1. Boyle DP and Zembower TR. Urol Clin North Am. 2015 Nov; 42(4):493-505.
2. Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
3. Poirel L *et al.*, J Clin Microbiol. 2015 Sep; 53(9):3003-8.
4. European Committee on Antimicrobial Suscpetibility Testing. Breakpoint tables for interpretation of MICs and zone diameters – version 6.0. 2016.

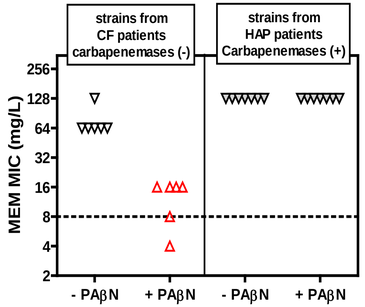
Results

Figure 1: Phenotypic screening of carbapenemases in meropenem-resistant strains using the Carba NP test [3].



➔ Carbapenemase phenotypic detection returned negative results for CF strains but positive results for HAP strains (Fig. 1), with presence of bla_{VIM-2} [metallo-β-lactamase gene] confirmed by PCR.

Figure 2: Influence of the efflux inhibitor (PAβN) on meropenem MIC in CF vs. HAP strains (the horizontal dotted line shows the current meropenem EUCAST susceptibility breakpoint [4]).



➔ Meropenem MICs were decreased of 2 to 4 $\log_2$ dilutions in the presence of PAβN for all CF strains but not for HAP strains (Fig. 2).

Table 1: Molecular characterization of CF and HAP clinical isolates: expression of efflux systems, porin alterations, expression of β-lactamases.

Clinical isolates (with patient's identification code and date of collection)	Expression of genes encoding efflux pumps (relative to PAO1)			OprD2 porin sequencing				β-lactamase
	mexA	*mexX*	*mexC*	no amino acid (WT=443)	Amino acid changes in protein sequence	Loops affected	Insertions/deletions in *oprD2* gene	
CF (DAF69 - 09/09/10)	2.2	0.8	0.0	117	D43N, S57E, S59R, D118STOP	L1	Deletion of 2 nt (312-313)	AmpC
CF (DAF69 – 04/10/10)	3.7	1.7	2.3					
CF (DAF69 – 19/10/10)	2.3	1.2	0.2	295	D43N, S57E, S59R, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296STOP	L1, L4, L5, L6	-	
CF (DAF69 – 26/10/10)	14.3	3.1	2.7					
CF (DAF69 – 09/11/10)	6.9	2.4	1.7					
CF (132 - 08/07/12)	0.3	3.5	0.7	228	D43N, S57E, S59R, change in reading frame → 229STOP	L1	Deletion of 1 nt (410)	
HAP (DS – 26/12/05)	2.3	1.0	1.0	441	V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, R310E, G312R, A315G, L347M, S403A, **R412P*** (new mutation in L8), Q424E	L2, L3, L4, L5, L6, L7, L8	Shortened loop 7	VIM-2
HAP (DS – 26/01/06)	4.3	2.8	1.4					
HAP (DS – 13/02/06)	6.5	4.3	13.6					
HAP (OG - 08/04/06)	6.4	9.6	5.4	276	V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, W277STOP	L2, L3, L4, L5	-	
HAP (OG – 02/05/06)	2.8	1.6	0.9					
HAP (ND – 10/08/06)	3.4	7.5	1.2					
HAP (ND – 11/09/06)	3.7	4.8	1.3					

➔ All clinical isolates showed an increase in transcription levels of *mexA*, *mexX* and/or *mexC*, and mutations in *oprD2* gene leading to truncated OprD2 porins.

➔ All CF isolates were derepressed for AmpC cephalosporinases.

➔ All HAP isolates expressed VIM-2 metallo-β-lactamase.

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.**

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