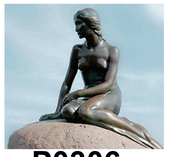

P0306

Temocillin is not substrate for OprD2 porin from *Pseudomonas aeruginosa*

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Introduction & Aims

❑ Resistance to beta-lactams in *P. aeruginosa* (*Pa*) can be mediated by a decreased bacterial outer membrane permeability (e.g., loss or modification of the OprD2 porin or overexpression of efflux pumps), associated or not with overproduction of AmpC cephalosporinases, extended-spectrum beta-lactamases (ESBLs) or carbapenemases. Temocillin (TMO; 6-alpha-methoxy-ticarcillin), a beta-lactam stable against most beta-lactamases (including most ESBLs, AmpC and KPC-type carbapenemases), is proposed as a sparing drug for carbapenems. Temocillin, however, is usually reported as devoid of useful activity against *P. aeruginosa*, which we showed to be due to active efflux by the constitutively-expressed MexAB-OprM pump [1]. Yet, we showed that a subset of strains (~ 20 %) collected from cystic fibrosis (CF) patients harbored natural mutations in *mexA* or *mexB* genes, which restored their susceptibility to temocillin, suggesting a potential therapeutic interest in this specific population [2].

❑ Our aim was to determine whether temocillin is substrate for the OprD2 porin, for which mutations or loss of expression are known to confer high resistance to carbapenems in *P. aeruginosa*.

Methods

MICs were determined by microdilution in Muller Hinton broth, according to CLSI guidelines [3], using as test organisms
(a) PA14 and its OprD2 defective mutant (PA14ΔOprD2) and
(b) a porin-deficient *E.coli* (K-12 W3110: ΔompFΔompC) transformed with either a pB22 empty vector or a pB22-OprD2 construct coding for the *P. aeruginosa* OprD2 under the control of the PBAD promoter of the arabinose operon.

Acknowledgments

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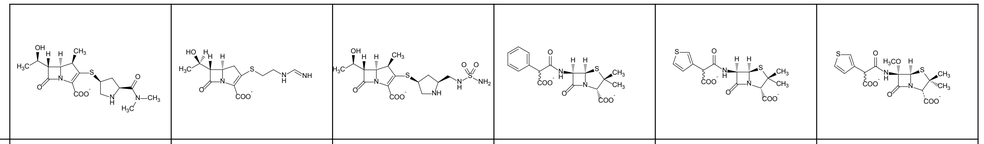
Results

Table 1 : MICs (mg/L) for carbapenems and carboxypenicillins in PA14 WT versus its OprD2 defective mutant

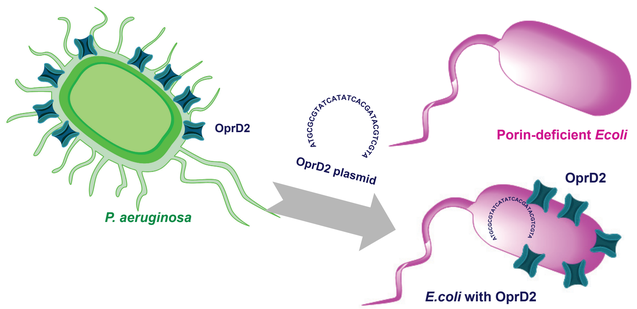


Pa strains	Meropenem	Imipenem	Doripenem	Carbenicillin	Ticarcillin	Temocillin
PA14 (wt)	0.5	0.25	0.25	64	32	256
PA14 ΔOprD2	8	2	2	64	32	256

➔ **MICs of carbapenems were higher in the OprD2 defective mutant of PA14 while the MICs of temocillin, as that of other carboxypenicillins, remained unchanged.**

Table 2 : MICs (mg/L) for carbapenems and carboxypenicillins in porin-deficient *E.coli* over-expressing OprD2

	Antibiotic MIC (mg/L)	
	1. Empty vector	+++OprD2
Meropenem	1	0.125
Imipenem	0.25	0.032
Doripenem	0.5	0.064
Carbenicillin	>2048	>2048
Ticarcillin	>2048	>2048
Temocillin	32	32



➔ **MICs of carbapenems were lower in *E.coli* over-expressing OprD2 than in the strain transformed by the empty vector.**
➔ **On the contrary, MICs of carboxypenicillins, including temocillin, were the same, whether oprD2 was expressed or not.**

Conclusions

❑ Temocillin, as other carboxypenicillins, is not a substrate for OprD2.

❑ Testing susceptibility to temocillin in carbapenem-resistant strains isolated from CF patients (including those with mutations or loss of expression of OprD2 porin) could be useful.

Reference

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2. Chalhoub *et al.*, "Comparative in vitro activity of temocillin and other β - lactams against Pseudomonas aeruginosa isolated from cystic fibrosis patients" (poster P02, ESCMID Conference on Reviving Old Antibiotics, Vienna, Austria, 22-24 October, 2014), http://www.facm.ucl.ac.be/posters/2014/ESCMID-old-antibiotics-2014/Chalhoub-et-al-temocillin-cystic-fibrosis-Vienna-2014.pdf
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