Influence of antibiotic treatments on gene expression of RND efflux pumps in successive isolates of *Pseudomonas aeruginosa* collected from patients with nosocomial pneumonia hospitalized in Intensive Care Units from Belgian Teaching Hospitals.

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Abstract (revised)

Background and Objective: Emergence of resistance during therapy is a worrisome problem that must be addressed in life-threatening infections such as nosocomial pneumonia (NP). The contribution of efflux in this phenomenon remains largely undefined because undetected in routine. Our aim was to evaluate the impact of antibiotic treatment on the expression of genes coding for RND efflux pumps in P. aeruginosa (P.a) isolated from patients with NP.

Methods: Samples: 62 pairs of P.a. collected from NP pts in 5 ICUs (a) at the time of NP diagnosis (day 0) and (b) during treatment (day x). Analyses: clonality delineated by REP-PCR and phylogenetic analysis (DiversilabTM; > 95% identity). Gene expression evaluated by Real Time PCR (mex Q-Test Kit, Coris BioConcept) for mexA (constitutively expressed) and mexX (inducible with low expression level in WT strains), and by PCR on cDNA for mexC and mexE (repressed in WT strains). Antipseudomonal antibiotics prescribed: as from the clinical records of each patient.

Results: The table shows (i) a high prevalence of genes encoding RND efflux pumps in the initial isolates with mexA and mexX, alone or in combination, being most prevalent, (ii) a further, highly significant increase of prevalence between day 0 and day x when considering all genes globally (Fisher's Exact Test 2-sided P=0.0028) or mexA, mexX and mexA+mexX only (Chi-squared Test for Independence P=0.024). The most used antibiotics were piperacillin-tazobactam (26 pts), amikacin (22 pts), meropenem (20 pts), cefepime (19 pts), and ciprofloxacin (6 pts), but in combination for 69 % of pts. No simple correlation could be established between overexpression of a specific gene pump and the corresponding antibiotics administered to the patient. However, the predominant overexpressions seen (mexA, mexX) matched well with the global use of the anti-pseudomonal antibiotics at the population level (see note in Table for substrate specificities).

Table: overexpression of genes coding for Mex pumps (total isolates: 62 pairs)

day of collection	none	*mexA*	*mexX*	*mexA+mexX*	others [a]
0 [b]	37	7	6	6	6
x [c]	20	13	12	9	8

[a] *mexC*, *mexE*, *mexA+mexC*, *mexX+mexE*, *mexX+mexC+mexE*, *mexA+mexX+mexC+mexE*

[b] before initiation of the treatment

[c] during treatment (last sample available; mean: 23 days; median: 17.5 days; extremes: 1-123 days)

Preferential substrates: MexAB-OprM: β-lactams and fluoroquinolones; MexXY-OprM: aminoglycosides, cefepime, fluoroquinolones.

Conclusion: The study points to a high initial prevalence of genes coding for efflux mechanisms towards the main antipseudomonal antibiotics in P.a. isolates from patients with NP and its further increase during treatment. An early detection of the genomic and functional overexpression of these efflux transporters may be useful for both epidemiological and therapeutic purposes. These observations also point to the interest of developing efflux inhibitors as complementary therapeutic agents.

Introduction

Nosocomial pneumonia in intensive care units (ICU) is a common complication of critical illness and is associated with significant attributable morbidity and mortality, including prolongation of mechanical ventilation and increased risk of death [1]. *P. aeruginosa* (PA) is a major cause of nosocomial pneumonia in ICU and is able to rapidly develop resistance to multiple classes of antibiotics even during therapy [2-3]. Efflux pumps are of particular concern in this respect since they are associated with multiresistance phenotypes. The most characterized efflux systems in PA include (i) MexAB-OprM and MexXY-OprM that are constitutively expressed at a basal level but can be overexpressed upon antibiotic exposure, and (ii) MexCD-OprJ and MexEF-OprN that are not expressed in the absence of inducer [4].

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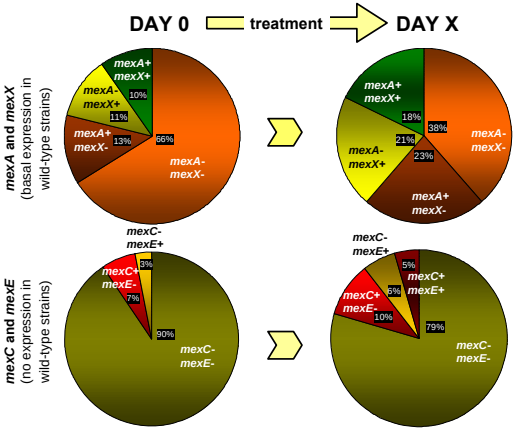
Results

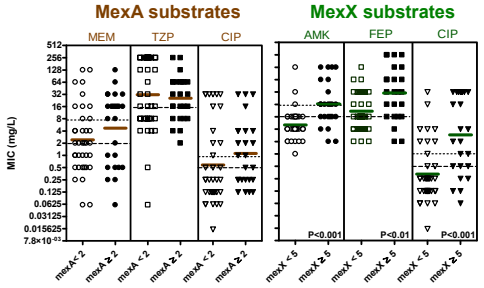
Prevalence of efflux pumps in 62 pairs of *Pseudomonas aeruginosa* collected from ICU patients



Prevalence of *mexA*⁺ (≥ 2 x basal level) and *mexX*⁺ (≥ 5 x basal level) or both, and of *mexC*⁺ or *mexE*⁺ (or both) before (day 0) and during (day x) treatment

- **The prevalence of *mexA*⁺ and *mexX*⁺ was already high in first isolates.**
- **For all genes tested, the number of isolates with overexpression increased during treatment (P < 0.05, Fisher's Exact Test 2-sided).**

Relationship between MIC of typical substrates and overexpression of the corresponding efflux system



MIC of antibiotics substrates of MexA (left) or MexX (right) in the last strains obtained during treatment (Day X), categorized according to the level of expression of the corresponding gene (*mexA* : ≥ 2 x basal level; *mexX* : ≥ 5 x basal level). Solid tick lines: geometric means; dotted lines : EUCAST S and R breakpoints

- **Overexpression of *mexA* was not associated with statistical increase of MICs for corresponding substrates**
- **Overexpression of *mexX* was associated with significant increases of MIC of typical substrates, with geometric means MICs moving from < the EUCAST S to > the EUCAST R breakpoint for amikacin and ciprofloxacin**

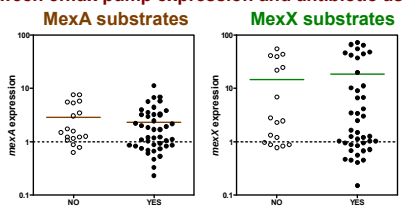
Relationship between efflux pump expression and antibiotic usage in patients

Antipseudomonal antibiotics received by the patients during treatment

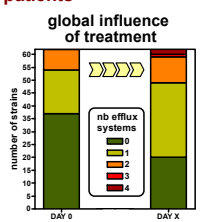
Antibiotic	no. patients
Piperacillin-tazobactam (TZP)	26
Amikacin (AMK)	22
Meropenem (MEM)	20
Cefepime (CEF)	19
Ciprofloxacin (CIP)	6

69% combinations

- **There is a relation between antibiotic usage and pump expression, but not specifically linked to antibiotics that are substrates.**



Expression level of *mexA* or *mexX* in strains isolated at day X from patients having received at least one of the drugs substrates of the corresponding pump between day 0 and day X



Number of efflux systems detected at day 0 and day X

Aim of the study

To evaluate the impact of antibiotic treatment on the expression of genes coding for RND efflux pumps in PA isolated as pairs (at the time of diagnosis and after treatment) from patients with nosocomial pneumonia in 5 Belgian hospital over the 2004 and 2008 period.

Methods

Bacteria: 124 strains of *P. aeruginosa* corresponding to 62 pairs collected from patients admitted in ICU with a diagnosis of nosocomial pneumonia. First isolate (Day 0): obtained at the time of the diagnostic; second isolate (Day X): last isolate obtained during treatment.

MICs: determined by geometric microdilution in cation-adjusted Muller-Hinton broth with PA ATCC 27853 and PAO1 used as a quality control. Susceptibility assessed according to EUCAST Breakpoints.

Clonality: Phylogenetic analysis performed by REP-PCR (DiversilabTM; > 95% identity).

Pump expression: Gene expression evaluated by Real Time PCR (mex Q-Test Kit, Coris BioConcept) for *mexA* (constitutively expressed) and *mexX* (inducible with low expression level in WT strains), and by PCR on cDNA for *mexC* and *mexE* (repressed in WT strains).

Conclusions

- High initial prevalence of genes coding for efflux mechanisms towards the main antipseudomonal antibiotics in PA isolates from patients suffering from nosocomial pneumonia.
- Further increase of this prevalence during treatment.
- Early detection of the genomic and functional overexpression of efflux transporters may be useful for both epidemiological and therapeutic purposes.

References

1. Parker *et al.* (2008). Ventilator-associated pneumonia caused by multidrug-resistant organisms or *Pseudomonas aeruginosa*: prevalence, incidence, risk factors, and outcomes. *J Crit Care*. 23:18-26
2. Lister *et al.* (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev*. 22(4):582-610.
3. Strateva T, Yordanov D. (2009). *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. *J Med Microbiol*. 58:1133-48.
4. Schweizer (2003) Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genet Mol Res*. 2(1):48-62.

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