Prevalence of Mex-mediated resistance in *Pseudomonas aeruginosa* from patients with ventilator-associated pneumonia in 4 Belgian hospitals

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ABSTRACT

Objectives: Efflux mediated resistance is difficult to detect in *Pseudomonas aeruginosa* (Pa) by routine susceptibility testing. Yet, it may confer cross-resistance to unrelated classes of drugs and contribute to selection of other resistance mechanisms. Our aim was to determine the prevalence of Mex efflux pumps in Pa isolates obtained from patients with ventilator-associated pneumonia (VAP).

Methods: Pa isolates were collected as pairs from each patient (first isolate before initiation of antibiotic treatment [pre]; second isolate, after 5 to 10 days of treatment [post]). In three hospitals (A-C), isolates were randomly collected from all eligible patients; in the 4th hospital (D), isolates were selected on the basis of interpretative reading of the susceptibility tests and compatible resistance phenotypes. mexA and mexX transcription levels were quantified by real time PCR; mexC and mexE transcription was detected by semi-quantitative PCR (their basal expression being undetectable in wild-type strains). Isolates typing was performed by fAFLP.

Results: The table shows the number of isolates in which overexpression of mex genes was detected (pre and post) by hospital. DNA-based typing globally confirmed the clonality of the sucessive isolates in each patient, and excluded the occurence of epidemic strains in the non-selected isolates.

Hospital (nb of pairs)	Nb of strains showing gene overexpression							
	mexA		*mexC*		*mexE*		*mexX*	
	pre	post	pre	post	pre	post	pre	post
Random sampling								
A (n=9)	0	1	1	0	0	0	4	7
B (n=7)	0	1	5	6	2	3	1	3
C (n=6)	0	0	0	0	0	0	0	0
Total (n= 22)	0	2	6	6	2	3	5	10
Strains collected on the basis of compatible resistance phenotype								
D (n=17)	2	8	4	5	0	4	7	9

A: AZ-Vrije Universiteit Brussels; B: CHU Saint-Pierre; C: Hôpital Erasme; D: Clinique universitaire UCL Mont-Godinne

Conclusions: A variable prevalence of Mex efflux pumps is found before treatment in isolates from patients selected at random, and increases in several cases following antibiotic exposure. In non-randomly collected samples, prevalence was very high, confirming the value of the interpretive algorithms used to detect mechanisms of efflux resistance. These data highlight the need of detecting efflux-mediated resistance in Pa clinical isolates originating from hospitalised ICU patients.

INTRODUCTION

Among resistance mechanisms present in *P. aeruginosa* (Pa), active efflux is of particular interest because it can confer cross-resistance to unrelated classes of antibiotics and favor the selection of other resistance mechanisms (1,2). Yet, it is difficult to evidence by routine susceptibility testing.

AIMS OF THE STUDY

- To compare the prevalence of Mex efflux pumps in Pa isolates obtained from patients with ventilator-associated pneumonia (VAP) from 3 University hospitals of the Brussels Region (see map).

- To examine whether the expression of these efflux pumps is modified upon antibiotic treatment
- To confront these data with those obtained for Pa isolates obtained from a 4th hospital (Mt Godinne) located in another Region (Wallonia) and selected based on antibiotic resistance patterns suggestive of resistance by efflux.

RESULTS

Prevalence of efflux pump gene expression in isolates collected before (A) or after (P) antibiotic treatment

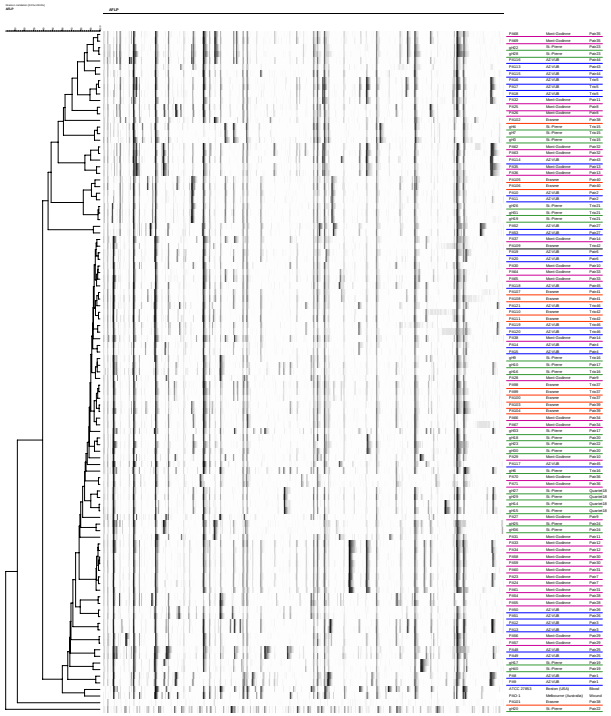
mexC and mexE: positive or negative detection

mexA and mexX: ratio with expression level in a wild-type strain

☐ increased expression; ■ increased expression during treatment

hospital	strains (A-P)	mexA		mexC		mexE		mexX	
		A	P	A	P	A	P	A	P
AZ-VUB	PA9-8	1	1	-	-	-	-	1	3
	PA11-10	1	1	-	-	-	-	7	7
	PA13-12	1	1	-	-	-	-	2	4
	PA15-14	1	1	-	-	-	-	1	1
	PA18-16	1	1	-	-	-	-	42	59
	PA20-19	2	1	+	-	-	-	7	3
	PA49-48	1	1	-	-	-	-	6	6
	PA51-50	1	1	-	-	-	-	2	36
	PA53-52	1	10	-	-	-	-	1	42
	TOTAL (n=9)	**0**	**1**	**1**	**0**	**0**	**0**	**4**	**7**
St Pierre	GH3-7	1	1	-	-	+	-	1	1
	GH6-16	1	1	-	-	-	-	1	1
	GH10-33	1	1	-	-	-	-	1	5
	GH14-29	2	6	-	+	-	+	1	1
	GH17-40	1	1	-	+	+	+	1	1
	GH18-30	2	2	+	+	-	+	1	1
	GH19-31	2	2	+	+	-	-	1	13
	GH20-23	1	1	+	+	-	+	1	1
	GH22-28	2	3	+	+	+	-	6	11
	GH25-36	2	2	+	-	-	-	1	8
	TOTAL (n=10)	**0**	**1**	**5**	**6**	**3**	**4**	**1**	**4**
Erasme	PA98-100	1	1	-	-	-	-	1	1
	PA101-102	1	1	-	-	-	-	1	1
	PA103-104	1	1	-	-	-	-	1	1
	PA105-106	1	1	-	-	-	-	1	1
	PA107-108	1	1	-	-	-	-	1	1
	PA109-111	1	1	-	-	-	-	1	2
	TOTAL (n= 6)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mt Godinne	MG249-274	1	1	+	+	-	-	1	1
	MG298-324	1	5	-	-	-	-	6	1
	MG386-376	1	3	-	-	-	-	1	5
	MG403-411	4	7	-	-	-	+	2	1
	MG684-699	1	6	-	+	-	-	1	1
	MG747-762	1	2	-	+	-	-	1	2
	MG782-785	2	8	-	-	-	-	10	9
	MG849-905	1	7	-	-	-	-	1	8
	MG309-331	1	4	-	-	-	-	61	101
	MG565-612	2	2	+	-	-	+	3	1
	MG653-660	3	3	+	+	-	-	2	3
	MG391-397	2	2	+	-	-	-	3	4
	MG353-372	2	9	-	-	-	-	348	293
	MG591-609	9	6	+	+	-	-	16	14
	MG603-650	2	2	-	-	-	+	2	2
	MG838-851	1	1	-	-	-	-	81	92
	MG856-865	2	2	-	-	-	-	56	71
	TOTAL (n= 17)	**2**	**8**	**4**	**5**	**0**	**3**	**7**	**9**

Genotyping of the strains using fAFLP



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METHODS

- **Collection of strains**: Pa isolates were collected as pairs from each patient (first isolate before initiation of antibiotic treatment [A, *ante*]; second isolate, after 5 to 10 days of treatment [P, *post*]). In 3 hospitals, isolates were collected consecutively from all eligible patients; in the 4th hospital, isolates were selected on the basis of antibiograms compatible with resistance mediated by active efflux.
- **Genotypic detection of *mex* genes expression levels:** mexA and mexX transcription levels were quantified by real time PCR; mexC and mexE transcription was detected by semi-quantitative PCR (their basal expression being undetectable in wild-type strains) (3).
- **Genotyping of the strains:** isolate typing was performed by fAFLP (4).

CONCLUSIONS

- In samples collected at random, a variable prevalence of Mex efflux pumps is found in clinical isolates collected before treatment.
- In samples collected based on antibiograms, prevalence is very high, confirming the value of the interpretive algorithms used to detect mechanisms of efflux resistance.
- In both cases, the prevalence increases following antibiotic exposure.
- DNA-based typing globally confirmed the clonality of the sucessive isolates in each patient, and excluded the occurence of epidemic strains in the 3 hospitals where isolates were not selected .
- These data highlight the need of detecting efflux-mediated resistance in *P. aeruginosa* clinical isolates originating from hospitalised ICU patients.

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