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MexAB-OprM- and MexXY-Overproducing Mutants Are Very Prevalent among Clinical Strains of *Pseudomonas aeruginosa* with Reduced Susceptibility to Ticarcillin▽

Of the nine efflux systems of the RND (resistance nodulation cell division) family characterized so far in *Pseudomonas aeruginosa*, four confer significant resistance to antibiotics when overproduced. These systems, namely, MexAB-OprM (ABM), MexCD-OprJ (CDJ), MexEF-OprN (EFN), and MexXY (XY), are individually able to pump out multiple antipseudomonal compounds, including β-lactams (ABM, CDJ, XY), fluoroquinolones (ABM, CDJ, EFN, XY), and aminoglycosides (XY) (6). Multiresistant mutants overproducing these pumps have sporadically been described, but their prevalence in the clinical setting remains poorly known. This study was first designed to determine the proportion of ABM gain-of-efflux mutants in a collection of clinical *P. aeruginosa* isolates displaying reduced susceptibilities to ticarcillin (TIC). As resistance to TIC is frequently associated with resistance to aminoglycosides and to fluoroquinolones, we also assessed the expression of CDJ, EFN, and XY efflux systems in the isolates.

In 2004, 450 clinically relevant (noncystic fibrosis-associated) isolates of *P. aeruginosa* were collected from 450 patients hospitalized in 15 French hospitals (30 isolates from each center). The MICs of TIC, amikacin (AMK), and ciprofloxacin (CIP) were determined with the conventional agar dilution method (5), allowing the selection of a subset of 170 isolates (38%) exhibiting increased resistance to TIC (MICs ≥ 32 μg/ml). Their relative expression levels for the *mexB*, *mexC*, *mexE*, and *mexY* genes were measured in duplicate by quantitative real-time PCR, with *rpsL* as the housekeeping gene (1, 2). Based on previous studies (2, 3), typical MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY gain-of-efflux mutants expressed *mexB*, *mexC*, *mexE*, and *mexY* at least 2, 100, 100, and 4 times more than the wild-type reference strain PAO1, respectively.

Very high proportions of ABM (46%) and XY (58%) overproducers were found among the selected isolates, with no fewer than 28% of isolates overexpressing the two systems simultaneously. As expected, overproduction of CDJ or EFN, which is associated with hypersusceptibility to β-lactam antibiotics (8), was absent (no CDJ mutant) or rare (one EFN mutant) in this series. Overproduction of ABM was observed in isolates with low-level resistance to TIC (MICs, 32 to 64 μg/ml) as well as in highly resistant strains (MICs ≥ 512 μg/ml) (Table 1). Similarly, overproduction of ABM and/or XY was associated with low-level resistance to CIP (MICs, 0.25 to 0.5 μg/ml) but was also very common (up to 100% for MexXY) in more-resistant strains (MICs ≥ 2 μg/ml) (7) (Table 1). Finally, overproduction of XY was observed in a large proportion of isolates displaying either low-level (MICs, 8 to 16 μg/ml) or high-level (MICs ≥ 32 μg/ml) resistance to AMK. Since when upregulated, systems ABM and XY provide only moderate resistance to their respective substrates (MICs increased two- to eightfold) (6), it is likely that additional mechanisms were also present in the most resistant strains (e.g., production of β-lactamases or aminoglycoside-modifying enzymes, alteration of fluoroquinolone targets).

Efforts have been made over the past years for the development of efflux pump inhibitors active on the Mex pumps in *P. aeruginosa* (4). Our present results highlight the urgent need for inhibitors targeting with priority the two most prevalent efflux systems in clinical isolates, namely, ABM and XY.

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TABLE 1. Prevalence of isolates overexpressing *mexAB-oprM* and *mexXY* in relation to levels of resistance to TIC, CIP, or AMK

Drug	Value (*n*)[a] at indicated MIC (μg/ml)										
TIC	16[b]	32 (39)	64 (49)	128 (34)	256 (17)	512 (4)	1,024 (5)	2,048 (5)	≥2,048 (17)		
% ABM		31 (12)	51 (25)	59 (20)	47 (8)	25 (1)	60 (3)	20 (1)	53 (9)		
Range of *mexB* expression[c]		2.1–5.7	2.0–52.8	2.0–20.0	2.8–7.8	2.6	2.0–4.3	3.1	2.2–4.6		
CIP	<0.125 (5)	0.125[b] (10)	0.25 (22)	0.5 (16)	1 (14)	2 (13)	4 (8)	8 (11)	16 (18)	32 (42)	64 (9)	>64 (2)
% ABM	0 (0)	10 (1)	45 (10)	63 (10)	850 (7)	62 (8)	63 (5)	45 (5)	44 (8)	48 (20)	56 (5)	0 (0)
% XY	20 (1)	0 (0)	27 (6)	47 (7)	36 (5)	69 (9)	50 (4)	55 (6)	83 (15)	88 (37)	100 (9)	0 (0)
AMK	1 (6)	2 (19)	4[b] (57)	8 (34)	16 (25)	32 (10)	64 (10)	128 (7)	>128 (2)			
% XY	17 (1)	32 (6)	54 (31)	56 (19)	64 (16)	100 (10)	80 (8)	86 (6)	100 (2)			
Range of *mexY* expression[c]	53.9	4.2–17.5	4.7–82.0	4.0–42.1	4.4–52.9	6.3–45.2	4.4–21.5	11.5–49.7	4.4–42.3		

[a] *n*, number of isolates.
[b] MIC for the wild-type reference strain PAO1.
[c] Mean values for two independent experiments.

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