

Emergence and Dissemination of BEL-1-Producing *Pseudomonas aeruginosa* Isolates in Belgium[∇]

Clavulanic acid-inhibited Ambler class A extended-spectrum β -lactamases (ESBLs) are only rarely reported in *Pseudomonas aeruginosa* (2, 5, 7). BEL-1 is a novel chromosomally encoded ESBL located on a class 1 integron (In120) and recently detected in a *P. aeruginosa* clinical strain isolated at one Belgian hospital located in Flanders (6).

Here, we report the dissemination of BEL-1-producing *P. aeruginosa* isolates in several hospitals located in different geographic areas of Belgium.

Between May and November 2006, we identified five *P. aeruginosa* strains originating from patients hospitalized in five different cities (Brussels, Charleroi, Huy, Namur, and Yvoir) which showed high-level resistance to ticarcillin (no inhibition zone around the disk) and intermediate or demonstrable resistance to ceftazidime and aztreonam by the disc diffusion method according to the guidelines of the CLSI (formerly NCCLS) (1). This phenotypic resistance profile initially suggested the association with a penicillinase and a partially depressed cephalosporinase. The five patients had severe underlying diseases, had all been hospitalized for a long period (Table 1), and had previously received broad-spectrum antimicrobial therapy. All of the isolates were resistant to ticarcillin; four were either resistant or borderline susceptible to piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, and aztreonam but susceptible to imipenem and meropenem by Etest MIC determination (Table 1). One isolate (62062) dis-

played high-level resistance to all β -lactams, including carbapenems. A positive synergy test result was usually observed with ceftazidime/ceftazidime-clavulanate double-combination disks (Neosensitabs; Rosco, Taastrup, Denmark), but it was not easily detected by double-disk tests (10- to 15-mm disk-to-disk edges) with ceftazidime, cefepime, aztreonam, and ticarcillin-clavulanic acid (SirScan discs, I2A; SirScan, Montpellier, France) (data not shown).

PCR targeting various ESBL genes (*bla*_{TEM}, *bla*_{VEB}, *bla*_{PER}, *bla*_{GES}, *bla*_{BEL-1}, *bla*_{OXA} of groups 1, 2, and 3, *bla*_{OXA-20}, and *bla*_{OXA-18}) was positive for the *bla*_{BEL-1} gene (forward primer, BEL-1FW [5'-CGACAATGCCGCAGCTAACC-3']; reverse primer, BEL-1RV [5'-CAGAAGCAATTAATAACGCCC-3']) for all five isolates. Sequencing of the variable region of class 1 integrons obtained for these strains (3) revealed that all isolates harbored at least two integrons. The first contained an *aadA6* gene followed by *orfD* identical to part of In51 described by Naas et al. (4) and identical to variable region DQ091179 found in a Chinese *P. aeruginosa* isolate. The second revealed four gene cassettes (*aacA4*, *bla*_{BEL-1}, *smr2*, and *aadA5*) with an organization identical to that already identified by Poirel et al. (6). Isoelectrofocusing confirmed the presence of a unique band at pI 7.3 matching the value for BEL-1 (6).

Interestingly, strain 62062 (MIC of meropenem, >32 μ g/ml) also contained a third integron harboring a *bla*_{VIM-1}-like metallo- β -lactamase gene. Pulsed-field gel electrophoresis

TABLE 1. Case history and MIC data for selected antimicrobial agents of *P. aeruginosa* clinical isolates expressing BEL-1 β -lactamase

Parameter	Result for isolate no.				
	21-7048	0605-07125	2532578	6110831	62062
City of origin	Huy	Brussel	Namur	Yvoir	Charleroi
Age (yr)	73	33	78	59	60
Ward	Intensive care	Trauma	Intensive care	General medicine	Intensive care
Hospitalization date (mo/day/yr)	5/1/06	11/2/05	9/20/06	11/3/06	6/14/06
Date of isolation (mo/day/yr)	5/28/06	5/15/06	10/7/06	11/8/06	6/29/06
Site of isolation	Blood	Urine	Endotracheal aspirate	Endotracheal aspirate	Blood
Underlying disease	COPD, ^a stroke	Multiple injuries, tetraplegic, urinary catheter	COPD, stroke	COPD, diabetes	Lung carcinoma, diabetes
Antimicrobial MIC (μ g/ml)					
Piperacillin-tazobactam	48	48	32	64	>256
Ceftazidime	16	16	12	24	256
Cefepime	24	16	16	24	256
Aztreonam	64	32	32	96	64
Imipenem	1.5	0.5	1	0.75	>32
Meropenem	4	0.5	1.5	4	>32
Amikacin	64	96	48	48	64
Ciprofloxacin	>32	>32	4	8	>32

^a COPD, chronic obstructive pulmonary disease.

(PFGE) analysis revealed that all isolates displayed profiles showing 80 to 95% genomic similarity (data not shown) and which were similar to the PFGE profile of the BEL-1-producing *P. aeruginosa* index strain isolated in Roeselaere in May 2004 (6).

Our results indicate the current dissemination of *bla*_{BEL-1} in Belgium and suggest the spread of an epidemic *P. aeruginosa* strain across several hospitals in different parts of the country. Moreover, the true prevalence of this resistance mechanism could be underestimated since BEL-1-producing *P. aeruginosa* isolates do apparently often display relatively low-level MICs to most expanded-spectrum β -lactams and also because ESBLs are not easily detected in *P. aeruginosa* in routine laboratories. The emergence of BEL-1 in *P. aeruginosa* isolates in Belgium may constitute a rising concern for the future and should prompt a nationwide surveillance effort in order to address the degree of this dissemination in Belgian hospitals.

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