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Nationwide Investigation of Extended-Spectrum β-Lactamases, Metallo-β-Lactamases, and Extended-Spectrum Oxacillinases Produced by Ceftazidime-Resistant *Pseudomonas aeruginosa* Strains in France[▽]

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A nationwide study aimed to identify the extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs), and extended-spectrum oxacillinases (ES-OXAs) in a French collection of 140 clinical *Pseudomonas aeruginosa* isolates highly resistant to ceftazidime. Six ESBLs (PER-1, $n = 3$; SHV-2a, $n = 2$; VEB-1a, $n = 1$), four MBLs (VIM-2, $n = 3$; IMP-18, $n = 1$), and five ES-OXAs (OXA-19, $n = 4$; OXA-28, $n = 1$) were identified in 13 isolates (9.3% of the collection). The prevalence of these enzymes is still low in French clinical *P. aeruginosa* isolates but deserves to be closely monitored.

Pseudomonas aeruginosa could potentially become resistant to any of the antibiotics used to treat Gram-negative nosocomial infections. The development of resistance to β-lactams in this opportunistic pathogen results from mutations leading to stable overexpression of intrinsic cephalosporinase AmpC, overproduction of efflux systems, reduced permeability, acquisition of transferable genes coding for a variety of secondary β-lactamases, or a combination of these mechanisms (21). A growing number of Ambler class A extended-spectrum β-lactamases (ESBLs), class B carbapenemases (metallo-β-lactamases [MBLs]), and class D extended-spectrum oxacillinases (ES-OXAs) have been reported in clinical strains of *P. aeruginosa* (14, 18, 19, 34, 40). The present multicenter study gave a snapshot of these acquired enzymes in a French collection of 140 *P. aeruginosa* isolates highly resistant to ceftazidime.

During a 1-month period (June 2007), 85 hospital laboratories participating in the surveillance networks affiliated with ONERBA (Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques) collected nonredundant strains of *P. aeruginosa* resistant to ceftazidime (Caz^r) (as defined by the Comité de l'Antibiogramme de la Société Française de Microbiologie [CA-SFM] in 2006 [12]), except those obtained from screening samples and cystic fibrosis patients. The susceptibility tests were performed in each laboratory according to their routine testing methods. All isolates showing an inhibition zone of <15 mm around the ceftazidime-containing disk (30 μg) or with a MIC of ceftazidime of >32 μg/ml were sent to a central laboratory for further investigation. In addition, the total number of patients with at least one clinical specimen positive for *P. aeruginosa* as well as the number of hospitalization days was recorded in each participating center during the study period. The central laboratory confirmed bacterial identification by using API32GN strips (bioMérieux, Craponnes, France) and determined the MICs of eight antipseudomonal antibiotics by the conventional 2-fold dilution method in agar (26). The β-lactamase contents of the strains were first analyzed by isoelectric focusing (IEF) (23) and then confirmed by gene sequencing with consensus primers targeting the bla_{TEM}, bla_{PSE}, bla_{SHV}, bla_{PER}, bla_{VEB}, bla_{GES}, bla_{BEL}, $bla_{CTX\text{-}M}$, bla_{VIM}, bla_{SPM}, $bla_{OXA\text{-}I}$ group, $bla_{OXA\text{-}II}$ group, $bla_{OXA\text{-}III}$ group, and $bla_{OXA\text{-}18}$ genes (1, 3, 5, 6, 24, 25, 28, 30–32, 35, 38). Genes bla_{IMP}, bla_{GIM}, and $bla_{OXA\text{-}9}$, respectively, were also specifically amplified with primers IMP2004-A and IMP2004-B (5′-ACAYGGYTTGG TTGTTCTTG-3′ and 5′-GGTTTAAYAAAACAACCACC-3′, respectively), GIM-A and GIM-B (5′-GGAGTATATCTT CATACCTCC-3′ and 5′-TTCCAACTTTGCCATGCCCC-3′, respectively), and OXA9A and OXA9B (5′-CCGAGAGATC GCACATACAA-3′ and 5′-CCCATCAACACGGGTAATT C-3′, respectively). Class 1 integrons were amplified in the isolates producing ESBLs, MBLs, and ES-OXAs with consensus primers (20) for content analysis and bla_{ESBL}, bla_{MBL}, and $bla_{ES\text{-}OXA}$ localization. Purified amplicons were sequenced on both strands, and their nucleotide sequences were compared and aligned with reference sequences using the NCBI BLAST program (2). Clonality of the Caz^r isolates was investigated by pulsed-field gel electrophoresis (PFGE) of DraI macrorestricted genomic DNA (36, 37).

Incidence of *P. aeruginosa* infections. Eighty-five hospital laboratories from 70 cities in France were enrolled in the study (Fig. 1). With 58,022 beds, the participating hospitals accounted for a total annual activity of 17 million hospital days. The total catchment area population was 8 million people, which corresponds to 13% of the French population. Public (university-affiliated or general) hospitals accounted for 95% of the hospital beds. During the 1-month study, the participating centers isolated 2,326 nonredundant isolates of *P. aerugi-*

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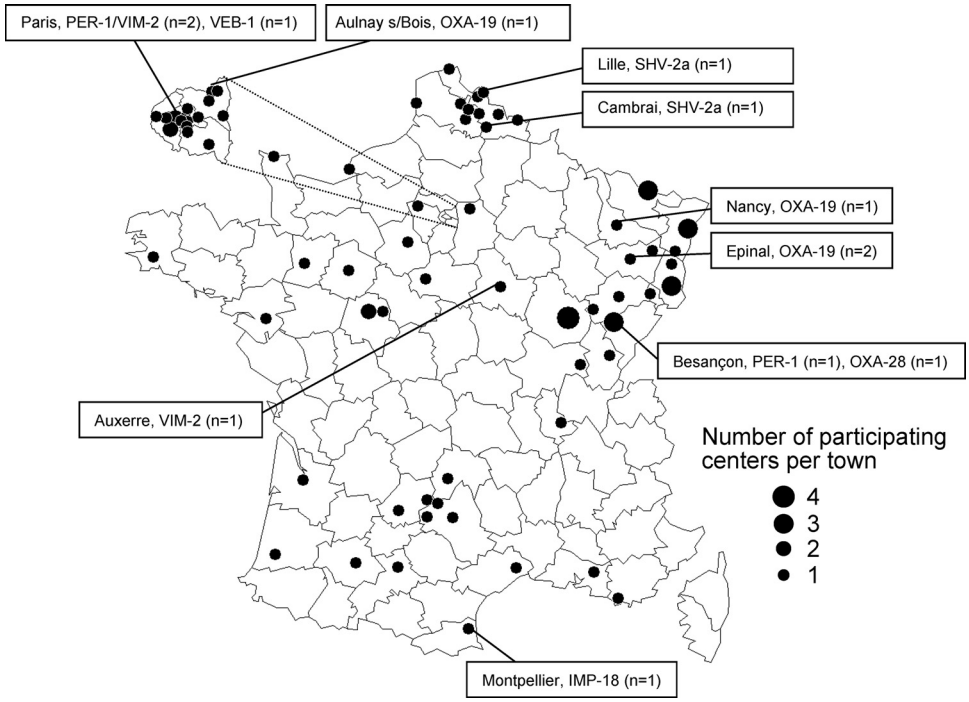

FIG. 1. Map of France, showing the 85 sites included in the study and the localization of isolates of *P. aeruginosa* producing ESBLs, MBLs, and ES-OXAs. An enlarged map of the Ile-de-France region is provided at the upper left. Labels indicate the town of isolation, the nature of the enzymes, and the number of isolates.

nosa, giving an attack rate of 0.76 cases per 100 admissions or a global incidence of 1.58 per 1,000 patient days. One hundred forty of these isolates (6.0%) appeared to be resistant to ceftazidime (MIC of >32 μg/ml). The resistance rates were similar between the university-affiliated (6.4%) and general (5.3%) hospitals, for a global incidence of Caz^r *P. aeruginosa* isolates of 0.095 per 1,000 patient days.

Secondary β-lactamases in Caz^r *P. aeruginosa*. The β-lactamases detected in the 140 Caz^r isolates are indicated in Table 1. Six ESBLs, four MBLs, and five ES-OXAs were identified in 13 isolates, for an overall prevalence of 9.3% of the 140 Caz^r isolates and 0.6% of the total isolates. Table 2 gives the resistance levels to antipseudomonal compounds and characteristics of the isolates producing these enzymes.

In our series, the overall prevalence of *P. aeruginosa* strains producing ESBLs or MBLs remains relatively low, far below that observed in some Asian countries (4, 10), Latin America (9), or Turkey (27) but in concordance with those observed in previous studies in France (7, 8, 13, 15–17, 22, 29, 39).

However, we showed here an unexpected proportion of *P. aeruginosa* strains producing ES-OXAs (OXA-19 and OXA-28). As expected, all of the $bla_{\text{ES-OXA}}$ genes were borne by class 1 integrons (Table 2) (33). Some new extended-spectrum oxacillinases have recently been described in several European countries (19, 33). Altogether, these data suggest the possible emergence of this class of enzymes in *P. aeruginosa*. bla_{VIM} and bla_{VEB} genes are usually carried on class 1 integrons (34, 40). However, $bla_{\text{VIM-2}}$, in isolates P19 and P22, and $bla_{\text{VEB-1}}$, in isolate P151, have not been associated with such genetic determinants.

Genotyping. One hundred thirty-seven isolates (3 isolates were nontypeable using PFGE) clustered in 113 PFGE patterns as follows: 98 unique patterns, 12 patterns including isolates from two patients, 1 pattern including isolates from 3 patients, 1 pattern including isolates from 4 patients, and 1 pattern including isolates from 8 patients. In most cases, the clonally related isolates were recovered from the same hospital

TABLE 1. Secondary β-lactamases detected in the collected *P. aeruginosa* isolates

Secondary β-lactamase(s)[a]	No. of isolates
None	110
TEM-2	5
PSE-1	4
OXA-19	3
OXA-56	3
OXA-10	2
OXA-9	2
SHV-2a	2
PSE-1, OXA-10	1
OXA-19, OXA-2	1
OXA-28	1
PER-1	1
VEB-1a, OXA-10	1
VIM-2	1
IMP-18	1
OXA-30, PSE-1, **VIM-2**, **PER-1**	1
OXA-10, PSE-1, **VIM-2, PER-1**	1

[a] Secondary β-lactamases with an extended spectrum are shown in boldface type. In a given strain, the β-lactamases are ordered according to decreasing pI.

TABLE 2. Epidemiological data, clonal lineages, and resistance phenotypes of *P. aeruginosa* isolates producing ESBLs, MBLs, and ES-OXAs

Isolate	Origin	β-Lactamase(s) (pI)[a]	Isolation site	MIC (μg/ml)[b]								PFGE pattern
				Tic	Tzp[c]	Caz	Fep	Atm	Ipm	Amk	Cip	
P9	Epinal	**OXA-19** (7.5)	Wound	512	128	256	64	16	16	**16**	64	A
P11	Epinal	**OXA-19** (7.5)	Wound	256	**64**	256	32	16	8	**8**	32	A
P122	Nancy	**OXA-19** (7.5)	Sputum	256	**64**	256	32	16	8	**8**	64	A
P66	Aulnay sous Bois	**OXA-2** (7.7), **OXA-19** (7.6)	Blood	512	**32**	64	16	16	**1**	128	128	C
P174	Besançon	**OXA-28** (7.8)	Blood	128	128	256	32	32	**4**	**16**	128	E
P19	Paris	OXA-30 (7.2), PSE-1 (5.7), VIM-2 (5.6), PER-1 (5.3)	Urine	>512	256	256	128	128	128	64	256	F
P22	Paris	OXA-10 (6.3), PSE-1 (5.7), VIM-2 (5.6), PER-1 (5.3)	Urine	>512	256	256	128	256	128	64	256	F
P170	Besançon	PER-1 (5.1)	Urine	512	128	256	64	128	**4**	32	64	G
P60	Cambrai	SHV-2a (7.4)	Sputum	>512	128	64	64	32	**1**	32	32	H
P102	Lille	SHV-2a (7.4)	Urine	>512	128	64	64	32	16	128	32	H
P151	Paris	VEB-1a (7.3), OXA-10 (6.3)	ETA[e]	>512	256	>512	>512	>512	32	128	512	I
P67	Auxerre	**VIM-2** (5.6)	Urine	>512	**32**	128	64	32	512	32	128	J
P85	Montpellier	**IMP-18**[d]	Wound	>512	128	>512	256	16	64	>512	128	K

[a] *bla* genes borne by class 1 integrons are indicated in boldface type. The gene cassettes and order for these isolates are as follows, with GenBank accession numbers given in parentheses: for P9, P11, and P122, *aacA4* and bla_{OXA-19} (FJ906752); for P66, bla_{OXA-2} and bla_{OXA-19}; for P174, *aacA4* and bla_{OXA-28} (FJ374756); for P67, bla_{VIM-2}; and for P85, *dfrA22* and bla_{IMP-18}.

[b] Tic, ticarcillin; Tzp, piperacillin-tazobactam; Caz, ceftazidime; Fep, cefepime; Atm, aztreonam; Ipm, imipenem; Amk, amikacin; Cip, ciprofloxacin. Drug susceptibility according to current NCCLS/CLSI breakpoints (26) is shown in boldface type: for Tic, MICs of ≤64 μg/ml; for Tzp, MICs of ≤64 μg/ml; for Caz, MICs of ≤8 μg/ml; for Fep, MICs of ≤8 μg/ml; for Atm, MICs of ≤8 μg/ml; for Ipm, MICs of ≤4 μg/ml; for Amk, MICs of ≤16 μg/ml; and for Cip, MICs of ≤1 μg/ml. The susceptible reference strain *P. aeruginosa* ATCC 27853 was used as the internal quality control.

[c] MIC of piperacillin with a fixed concentration (4 μg/ml) of tazobactam.

[d] The pI value for IMP-18 has not been reported in the literature. IEF experiments for this isolate showed a smear between pI 5.0 and 7.5.

[e] ETA, endotracheal aspiration.

or from hospitals in the same region. Regarding the isolates producing ESBLs, MBLs, or ES-OXAs, genotypic analysis revealed that a clone (PFGE pattern A) producing OXA-19 had spread in two hospitals (Nancy and Epinal, France, 70 km apart). The spread of this clone has been described in a recent publication (11). A second clone (PFGE pattern F), producing both PER-1 and VIM-2, was isolated in different wards of the same university hospital in Paris, France, while a third clone (PFGE pattern H), producing SHV-2a, was detected in two other hospitals in the north of France (Lille and Cambrai, 68 km apart) (Fig. 1).

Since most ES-OXAs are poorly inhibited by clavulanate, used in screening tests (14), *Pseudomonas aeruginosa* strains expressing these enzymes remain difficult to recognize in routine testing and require genotypic methods. ES-OXAs have been described to occur sporadically, but their spread in the clinical setting remains poorly understood and probably underestimated. Our data stress the need for a simple and reliable routine test able to detect ESBLs, MBLs, and ES-OXAs produced by clinical *P. aeruginosa* strains. This test will be helpful to rapidly implement control measures for preventing the spread of multidrug-resistant strains harboring emerging resistance mechanisms.

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