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Beta-lactamases Among Ceftazidime-resistant *Pseudomonas aeruginosa* from Five European Countries: High Prevalence of Oxacillinases and VIM Enzymes in the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To evaluate the beta-lactamase (BL)-encoding genes carried by ceftazidime- and/or carbapenem-resistant *P. aeruginosa* (PSA) strains collected from five European countries displaying high beta-lactam resistance rates, including Belgium, Greece, Poland, Russia (3 cities; Smolensk, Tomsk and Yekaterinburg) and Ukraine.

Methods: A total of 139 ceftazidime-resistant PSA recovered from five European countries during 2011 were evaluated for the presence of BL-encoding genes: bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{GES}, bla_{VEB}, bla_{PER}, bla_{BEL}, bla_{PSE}, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-2}-, bla_{OXA-10}- and $bla_{OXA-1/30}$-group, bla_{OXA-18} and bla_{OXA-45}. Detection of BL was performed by a commercial nucleic acid based microarray and a combination of PCRs. All amplicons were sequenced.

Results: Among the 139 PSA, 100 (71.9%) produced VIM metallo-beta-lactamases (MBLs). VIM-2 was the most prevalent (87 strains) and was detected in all 7 hospitals from 5 countries. Isolates producing VIM-4 (10 strains; Greece and Poland), VIM-1 (one strain; Poland) and two new VIM variants (Belgium and Poland) were also noted. All tested strains from Belgium, Poland and Smolensk carried VIM MBLs. One strain from Yekaterinburg carried bla_{GES-4} encoding a serine-carbapenemase. The other 36 (25.9%) strains carried genes encoding ESBLs and/or oxacillinases (OXA). Five OXA-types were detected: OXA-2 (16 strains); OXA-10 (16), OXA-14 (5), OXA-4 (2), and OXA-35/-101 (2). bla_{GES-1} (ESBL) was found among 27 strains: 12 from Russia (2 hospitals) and 15 from the Ukraine. A PER-1-encoding gene was observed in Yekaterinburg (2 strains) and the Ukraine (2), whereas bla_{PSE-1} was noted in Greece (2 strains) and Poland (10). A total of 14 enzymes/combinations were detected and 12 enzymes and/or combinations were noted in Poland alone. 27 VIM-producing strains from Greece (4), Poland (22) and Smolensk (1) carried 1-3 additional BLs.

Conclusions: VIM-producing isolates remain very prevalent in the five European countries analyzed and VIM-2-producing strains were observed in all countries. Additionally, Ambler class D enzymes with broad and extended spectrum were detected alone or in combination with other enzymes. Only two strains tested were negative for the presence of BLs. These results highlight a scenario of multiple BLs that is not commonly observed in PSA strains and surveillance of BL resistance mechanisms seems prudent in these and nearly all countries.

INTRODUCTION

Pseudomonas aeruginosa is widespread in the nosocomial environment and an increasing cause of serious infections in hospital practice, primarily affecting the expanding population of patients immunocompromised by disease or by medical and surgical treatments. *P. aeruginosa* is inherently resistant to many antimicrobial agents and has the ability to obtain additional resistance mechanisms, affecting drugs that ordinarily would be active. This could occur by mutations that lead to hyperexpression of efflux systems and chromosomal cephalosporinase or reduced expression of OprD porin, or by acquisition of genes including those encoding ß-lactamases.

The emergence and dissemination of acquired metallo-ß-lactamases (MßLs) among *P. aeruginosa* further limit the treatment options for infections caused by these organisms, since organisms carrying MßL genes are resistant to virtually all ß-lactam agents. Currently, the most prevalent and widespread acquired MßLs are the VIM enzymes, of which numerous variants have been described.

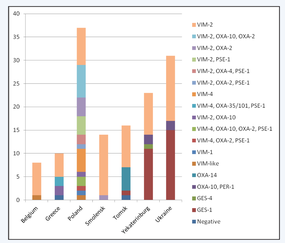
We recently found a high prevalence of ceftazidime- and carbapenem-resistant isolates from several European countries and in this study we describe the epidemiology and ß-lactamase prevalence among these isolates.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility testing. 139 ceftazidime- and/ or carbapenem-resistant *P. aeruginosa* isolates were consecutively collected from seven hospitals located in Belgium, Greece, Poland, Russia (3 hospitals: Smolensk, Tomsk and Yekaterinburg) and Ukraine during 2011 as part of the SENTRY Antimicrobial Surveillance Program. Only one isolate per patient from documented bacteremias was included in the study. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, Missouri, USA), when necessary. All isolates were susceptibility tested using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI). Categorical interpretations for all antimicrobials were those found in M100-S23 and quality control (QC) was performed using *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (CLSI M100-S23).

Genotypic detection of ß-lactamases. All isolates were screened for presence of bla_{IMP}, bla_{VIM}, bla_{GES}, bla_{VEB}, bla_{PER}, bla_{PSE} and oxacillinases with ESBL spectrum (bla_{OXA-2}-, bla_{OXA-10}- and bla_{OXA-30}-group, bla_{OXA-18} and bla_{OXA-45}) by PCR. Amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/). Isolates were additionally tested using the Check-MDR CT101 kit (Check-points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M Groups 1, 2, 8+25 and 9, TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM.

Figure 1. Variety of enzymes detected among *P. aeruginosa* collected in eight European hospitals.



RESULTS

- Among 139 selected *P. aeruginosa* strains, 100 (71.9%) produced metallo-ß-lactamases (MßLs). VIM-2 was the most prevalent (87 strains; 62.6%) and was detected in all hospitals analyzed (Table 1).
- The prevalence of VIM-2 among all detected enzymes in the five countries was: 45.2% in Ukraine, 60.4% (range, 39.1 - 100.0%) in Russia, 70.0% in Greece, 73.0% in Poland and 87.5% in Belgium.
- VIM-4 was observed among 10 (7.2%; Table 1) strains from Poland (8 strains) and Greece (2 strains) and a single VIM-1-producing *P. aeruginosa* isolate was detected in Poland.
- Two strains produced new VIM variants: one was distinct from VIM-4 by one amino acid substitution (A58S) detected in Belgium and another from Poland that was most similar to VIM-2 and VIM-6, and displayed one amino acid substitution when compared to these enzymes (Q60R and S148N, respectively); see poster P1283 for the characterization of the new enzymes.
- One strain from Russia carried a GES-4 serine-carbapenemase encoding gene (Table 1).
- Among 36 (25.9%) carbapenemase-negative strains, five OXA-types were detected: OXA-2 (16 strains), OXA-10 (16), OXA-14 (5), OXA-4 (2), and OXA-35/-101 (2).
- bla_{GES-1} (ESBL) was found among 27 strains: 12 from Russia (2 hospitals) and 15 from the Ukraine. A PER-1-encoding gene was observed in Yekaterinburg (2 strains) and the Ukraine (2), whereas bla_{PSE-1} was noted in Greece (2 strains) and Poland (10).
- Poland was the country with the widest variety of enzymes combinations (Figure 1). VIM-2- and VIM-4-producing isolates from Poland often harboured oxacillinase-encoding genes and/or PSE-1 and 12 different combinations were observed among 22 strains (59.5% among isolates from this country).
- Only two strains (1.4%), one from Greece and one from Tomsk, Russia did not yield positive amplification with any of the genes tested.

Table 1. Distribution of different ß-lactamases detected among *P. aeruginosa* collected in five European countries.

	Country/city (no. of isolates [percentage per country/city])							
				Russia				
ß-lactamases/combinations results	Belgium (8)	Greece (10)	Poland (37)	Smolensk (14)	Tomsk (16)	Yekaterinburg (23)	Ukraine (31)	Total no. of isolates (no. of isolates [percentage overall])
GES-1					1 (6.2)	11 (47.9)	15 (48.4)	27 (19.4)
GES-4						1 (4.3)		1 (0.7)
OXA-10, PER-1						2 (8.7)	2 (6.5)	4 (2.9)
OXA-14					5 (31.3)			5 (3.6)
VIM-1			1 (2.7)					1 (0.7)
VIM-2	7 (87.5)	5 (50.0)	8 (21.6)	13 (92.9)	9 (56.3)	9 (39.1)	14 (45.2)	65 (46.8)
VIM-2, OXA-10		2 (20.0)	1 (2.7)					3 (2.2)
VIM-2, OXA-10, OXA-2			7 (18.9)					7 (5.0)
VIM-2, OXA-2			4 (10.8)	1 (7.1)				5 (3.6)
VIM-2, OXA-2, PSE-1			1 (2.7)					1 (0.7)
VIM-2, OXA-4, PSE-1			2 (5.4)					2 (1.4)
VIM-2, PSE-1			4 (10.8)					4 (2.9)
VIM-4			5 (13.5)					5 (3.6)
VIM-4, OXA-10, OXA-2, PSE-1			2 (5.4)					2 (1.4)
VIM-4, OXA-2, PSE-1			1 (2.7)					1 (0.7)
VIM-4, OXA-35/101, PSE-1		2 (20.0)						2 (1.4)
VIM-like	1 (12.5)		1 (2.7)					2 (1.4)
Negative		1 (10.0)			1 (6.2)			2 (1.4)

CONCLUSIONS

- VIM-2 was highly prevalent among ceftazidime- and/or carbapenem-resistant *P. aeruginosa* isolates collected from five European countries. Other VIM enzymes, serine-carbapenemases, and Ambler class A and D enzymes were also observed among these isolates.
- Combinations of ß-lactamases often observed among Enterobacteriaceae were also noted in several *P. aeruginosa* isolates surveyed from Poland. Further investigation on the traits of these *P. aeruginosa* isolates might elucidate how these isolates were able to accumulate several ß-lactamase encoding genes.
- Enforcement of infection control practices appears needed in these medical centers/nations to decrease the dissemination of these resistance genes and strains.

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