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Characterization of VIM-1-Producing *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Klebsiella pneumoniae* Strains from Germany: Report from SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Objective: To characterize carbapenem (CARB)-resistant *P. aeruginosa* (PSA) and Enterobacteriaceae strains isolated in German medical centers participating in the SENTRY Program. The GIM class of metallo-beta-lactamases (MßL) was originally described by our program in clinical isolates from Germany and has not been observed again or in other nations. In Germany, MßL-producing strains are still rare in contrast to other European countries.

Methods: From 2000 to 2006, a total of 8,846 isolates were submitted to the SENTRY Program monitor from six German centers, including 596 PSA, 256 *E. cloacae* (ECL) and 348 *K. pneumoniae* (KPN). The isolates were tested for susceptibility (S) by reference (CLSI) broth microdilution methods and those with decreased S to imipenem (IPM), meropenem and ceftazidime were routinely screened for MßL production by disk approximation tests and/or MßL Etest (AB BIODISK) strips. Isolates with screen-positive results were confirmed by PCR using generic primers for IMP, VIM, SPM and GIM enzyme types. MßL gene sequencing and molecular typing (automated ribotyping, PFGE) were additionally performed to characterize MßL and to evaluate clonality.

Results: Decreased S to CARB (IMP MIC, >=2 mg/L) was observed in 3 ECL (1.2%) and 1 KPN (0.3%) strains, while 77 PSA (13.1%) were R to IPM (MIC, >=16 mg/L). Among IPM-R PSA, 10 strains had a positive MßL screen test and were PCR-positive for bla_{GIM-1} (6 strains from Düsseldorf described in 2002) or bla_{VIM-1} (4 strains from Frankfurt isolated in 2005-2006). The ECL and KPN strains were from Leipzig (2005 and 2006) and PCR-positive for bla_{VIM-1}. The bla_{VIM-1} was located within a class 1 integron for all VIM-1-producing strains. VIM-1-producing PSA showed identical/similar PFGE patterns, while the ECL strains each had a distinct molecular typing pattern.

MßL-type	Location (no.)	Species (no.)	No. of PFGE patterns
GIM-1	Düsseldorf (6)	*P. aeruginosa* (6)	1
VIM-1	Frankfurt (4)	*P. aeruginosa* (4)	1
	Leipzig (4)	*E. cloacae* (3)	3
		K. pneumoniae (1)	ND

Conclusions: bla_{VIM-1} has emerged and is rapidly disseminating as clones and also among clonally unrelated strains in diverse areas of Germany. Long-term surveillance and continued screening for MßL genes among isolates with decreased S to CARBs will be essential to control dissemination of this important R mechanism.

INTRODUCTION

Carbapenems are the most active ß-lactam agents for the treatment of infections caused by Gram-negative organisms. However, resistance to carbapenems has been increasingly reported among *Pseudomonas aeruginosa* and *Acinetobacter* spp. worldwide. Furthermore, Enterobacteriaceae strains with reduced susceptibility to these compounds have been detected in several geographic regions, especially in European countries.

Carbapenem resistance in *P. aeruginosa* is mainly due to hyperproduction of AmpC ß-lactamases associated with hyperexpression of efflux pumps and/or decreased permeability of bacterial outer membrane. However, there have been increasing reports, especially from Japan, Brazil, and some European countries, of *P. aeruginosa* strains that are resistant to carbapenems mediated by the of acquired metallo-ß-lactamase (MßL).

Five major classes of MßLs have been described, including IMP, VIM, SPM (only SPM-1, Brazil), GIM (only GIM-1, Germany) and SIM (only SIM-1, Korea). While IMP- and VIM-type MßLs have been reported in many countries, reports of SPM-1, GIM-1 and SIM-1 are restricted to their place of origin. Acquired MßL genes possess great mobility because they are usually located in integrons or plasmids.

In the present study, we characterized MßL-producing strains isolated in Germany through the SENTRY Antimicrobial Surveillance Program in 2000-2006.

MATERIALS AND METHODS

Bacterial Isolates: A total of 8,846 isolates were submitted to the SENTRY Program from six German medical centers in 2000-2006, including 596 *P. aeruginosa*, 256 *E. cloacae* and 348 *K. pneumoniae*. Isolates were consecutively collected from bloodstream, respiratory tract, skin and soft tissue, and urinary tract infections according to defined protocols. Only clinically significant isolates were included in the study; one per patient episode. Species identification was confirmed by standard biochemical tests and use of Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested against more than 25 antimicrobials by the broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2006) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by CLSI (2007) criteria. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were concurrently tested for quality assurance.

Screening for carbapenemases: Beginning in 2000, *Pseudomonas* spp. and *Acinetobacter* spp. isolates resistant to imipenem, meropenem (MIC, ≥16 mg/L) and ceftazidime (MIC, ≥32 mg/L), and Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC, ≥2 mg/L) are routinely screened for production of carbapenemases.

Potential carbapenemase producers were screened using disk approximation techniques and/or MßL Etest strips (AB BIODISK, Solna, Sweden). Isolates exhibiting positive MßL screening test were evaluated for presence of bla_{IMP}, bla_{VIM}, bla_{SPM-1} and bla_{GIM-1} using PCR primers as previously described.

Gene sequencing: PCR products for the MßL genes were sequenced using a Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to available sequences via NCBI BLAST search to determine the enzyme type.

Molecular typing: Multiple isolates of the same species from a medical center harboring MßLs belonging to the same enzyme family were typed using the Riboprinter™ Microbial Characterization System (Qualicon Inc., Wilmington, Delaware, USA) and/or pulsed-field gel electrophoresis (PFGE) using a CHEF DRII apparatus (Bio-Rad, Hercules, California, USA) to evaluate clonality.

RESULTS

- Polymyxin B (99.8% susceptible) and amikacin (94.5% susceptible) were the most active compounds tested against *P. aeruginosa* isolates and only 75.8% of strains were susceptible to imipenem, less than cephalosporins (81.4-84.3%) and piperacillin/tazobactam (86.7%).
- Among *K. pneumoniae*, 13.1% of strains exhibited an ESBL phenotype while 26.8% of *E. cloacae* showed decreased susceptibility to ceftazidime (probably AmpC mechanism). Meropenem (99.6-99.7% susceptibility), imipenem (99.2-99.7%), cefepime (96.3-98.4%) and amikacin (96.8-99.2%) were highly active against these Enterobacteriaceae (Table 1).
- Ten carbapenem-resistant *P. aeruginosa* strains exhibited positive MßL screen tests and PCR positive results for bla_{GIM-1} (six strains from Düsseldorf reported in 2002) or bla_{VIM-1} (four strains from Frankfurt isolated in 2005-2006).
- MßL-producing strains generally showed multidrug-resistant phenotypes (Table 2).
- *E. cloacae* and *K. pneumoniae* strains with elevated carbapenem MIC values and positive MßL screen tests exhibited PCR positive results for bla_{VIM-1}. These strains were isolated in 2005 and 2006 in a medical center located in Leipzig (Table 3).
- All four VIM-1-producing *P. aeruginosa* were isolated in a single medical center (Frankfurt) and exhibited identical molecular typing patterns, indicating clonal dissemination (Table 3).
- Integrons of the same size were amplified from all *P. aeruginosa* strains, only one was sequenced (strain #88-6759A; Figure 1). The integron also harbored *aac*A4 and *aph*15.
- Although all three VIM-1-producing *E. cloacae* had distinct molecular typing patterns (PFGE patterns A, B and C; Table 3), the bla_{VIM-1} carrying integron of all strains showed identical gene sequence in their variable region, indicating that it was transferred among these strains (Figure 1).
  - All had bla_{VIM-1} located in the first position of a class 1 integron and *aad*A1 near the *qac*/*sul*1 at the 3' end.
  - The variable region of integron in *E. cloacae* also carried *aac*A4 and *aad*A1 genes along with bla_{VIM-1}.
- The integron amplified from *K. pneumoniae* strain harbored *aac*A7 and *dhfr* (trimethoprim resistance gene) downstream from bla_{VIM-1}.

Table 1. Antimicrobial susceptibility profiles of Gram-negative pathogens in which metallo-ß-lactamases were isolated in Germany (SENTRY Program, 2000-2006).

Organism (no. tested)/ antimicrobial agent	MIC (mg/L): 50%	90%	% Susceptible[a]	% Resistant[a]
P. aeruginosa (586)				
Ceftazidime	2	>16	81.4	14.3
Cefepime	4	16	84.3	6.7
Piperacillin/tazobactam	8	>64	86.7	13.3
Imipenem	1	>8	75.8	13.1
Meropenem	0.5	8	82.9	9.2
Amikacin	≤4	16	94.5	2.7
Tobramycin	0.5	16	85.8	11.6
Ciprofloxacin	≤0.25	>4	72.0	23.5
Polymyxin B	≤1	≤1	99.8	0.2
K. pneumoniae (348)				
Ceftazidime	≤1	16	89.9	9.2 (13.1)[b]
Cefepime	≤0.12	1	96.3	2.9
Piperacillin/tazobactam	4	32	89.7	7.5
Imipenem	≤0.5	≤0.5	99.7	0.3
Meropenem	≤0.12	≤0.12	99.7	0.0
Amikacin	≤4	≤4	96.8	0.3
Tobramycin	0.5	4	90.2	6.3
Ciprofloxacin	≤0.25	2	89.9	6.3
Tigecycline	0.5	2	98.8	0.0
Polymyxin B	≤1	≤1	97.6[c]	1.8[c]
E. cloacae (256)				
Ceftazidime	≤1	>16	73.2	20.1
Cefepime	≤0.12	2	98.4	1.2
Piperacillin/tazobactam	4	>64	79.9	6.3
Imipenem	≤0.5	1	99.2	0.8
Meropenem	≤0.12	≤0.12	99.6	0.0
Amikacin	≤4	≤4	99.2	0.4
Tobramycin	0.5	1	94.5	5.1
Ciprofloxacin	≤0.25	0.5	92.1	6.3
Tigecycline	0.5	1	99.1	0.0
Polymyxin B	≤1	2	91.1[c]	8.0[c]

a. Susceptibility interpretations per CLSI (M100-S17) criteria where available; US-FDA susceptibility criteria (≤2 mg/L) for tigecycline were used for *K. pneumoniae* and *E. cloacae*.
b. Percentage of isolates with ESBL phenotype.
c. *P. aeruginosa* breakpoints (≤2/≥8 mg/L) established by the CLSI (2007) were used.

Table 2. Antimicrobial susceptibility profiles of metallo-ß-lactamase-producing strains isolated in Germany (14 strains).

Organism (no. strains)	City	MßL	MIC (mg/L) for: IMI	MER	AZT	CAZ	CPM	P/T	AMK	TOB	CIP	PB	TIG
P. aeruginosa (6)	Düsseldorf	GIM-1	>8	>8	8-16	>16	≥16	>64	4-16	≥16	>4	≤1-2	NA
P. aeruginosa (4)	Frankfurt	VIM-1	>8	>8	>16	>16	>16	>64	16-32	>16	>4	1	>4
E. cloacae (3)	Leipzig	VIM-1	4->8	1->8	>16	>16	8->16	>64	≤4-8	4-8	0.25->4	≤0.5->4	0.25-1
K. pneumoniae (1)	Leipzig	VIM-1	>8	8	>16	>16	>16	>64	8	>16	>4	>4	2

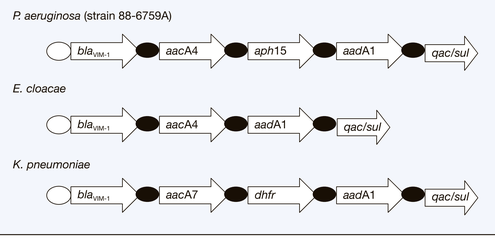
a. Abbreviations: MßL= metallo-ß-lactamase; IMP = imipenem; MEM = meropenem; AZT = aztreonam; CAZ = ceftazidime; CPM = cefepime; P/T = piperacillin/tazobactam; AMK = amikacin; TOB = tobramycin; CIP = ciprofloxacin; PB = polymyxin B; TIG = tigecycline.
b. NA = MIC values not available.

Table 3. Characterization of metallo-ß-lactamase-producing strains isolated in Germany as part of the SENTRY Program (2000-2006).

MßL type	Location (no.)	Organism (no.)	Molecular typing patterns: Ribotype/PFGE (no.)
GIM-1[a]	Düsseldorf (6)	*P. aeruginosa* (6)	105.798.3/A (3) 258.134.1/A (2) 258.134.2/-[b] (1)
VIM-1	Frankfurt (4)	*P. aeruginosa* (4)	105.1034.2/A(4)
	Leipzig (4)	*E. cloacae* (3)	-/A (1) -/B (1) -/C (1)
		K. pneumoniae (1)	-/-

a. Previously reported by Castanheira et al. (2004).
b. - = not determined

Figure 1. Schematic representation of variable regions of integrons carrying bla_{VIM-1} in strains isolated from medical centers in Germany. Genes are represented as open arrows, direction of the arrows indicate the direction of transcription of the resistance markers. Filled ellipses represent 59 base elements. Open ellipses represent attI1 sites.



CONCLUSIONS

- The results of this SENTRY Program report indicate that bla_{VIM-1} has emerged and appears to be rapidly disseminating among multiple Gram-negative species in diverse geographic areas of Germany.
- Long-term surveillance and continued screening for MßL genes in isolates with decreased susceptibility to carbapenems are warranted to adequately control this important, mobile resistance mechanism.

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