574

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Emergence and Dissemination of Metallo-ß-Lactamases Producing Strains in Europe: Report from the SENTRY Antimicrobial Surveillance Program (2000-2006)

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AMENDED ABSTRACT

Objective: To evaluate the emergence and dissemination of metallo-beta-lactamase (MßL) producing strains in European medical centers participating in the SENTRY Program.
Methods: Beginning in 2000 all Gram-negative bacilli submitted to SENTRY Program with decreased susceptibility to imipenem (IPM), meropenem and ceftazidime were routinely screened for production of MßL by disk approximation tests and/or MßL Etest (AB BIODISK, Solna, Sweden) strips. Isolates with screen-positive results were evaluated by PCR using generic primers for IMP, VIM, SPM and GIM enzyme types. MßL gene sequencing and molecular typing (ribotyping, PFGE) were additionally performed to characterize MßL and to evaluate clonality.
Results: Since 2000, 4,935 *P. aeruginosa* (PSA), 1,460 *Acinetobacter* spp. (ASP) and 22,950 Enterobacteriaceae (ENT) have been collected from European centers and tested for susceptibility (S) by reference broth microdilution methods (CLSI, 2006). S rates to IMP remained stable among PSA (75.5% in 2000 and 76.1% in 2006), but varied from 78.5% in 2000 to 51.9% in 2006 among ASP. IMP remained very active against ENT (99.8% S in 2006), but the occurrence of strains with MIC >=2 mg/L increased from 3.6% in 2000 to 5.8% in 2006. A total of 105 MßL-producing strains were identified and characterized since 2000. The most common MßL-producing species were PSA with 44 strains from 6 countries and producing 4 different MßLs; followed by *K. pneumoniae* (KPN; 33 VIM-1-producing strains from 5 countries) and *E. cloacae* (ECL; 20 strains, VIM-1 and IMP-1-producers from 4 countries). MßL-producing strains were usually resistant to most antimicrobials tested. Polymyxin B was very active against PSA (100% S) and KPN (94% S); while tigecycline was highly active (100% S) against ASP, KPN and ECL. Molecular typing results indicated clonal dissemination of PSA producing VIM-1 in Germany, Greece and Italy and GIM-1 in Germany; KPN producing VIM-1 in Greece, Italy and Spain; and IMP-1-producing ECL in Turkey. In addition, clonal diversity was observed among IMP-13-producing PSA from Rome, Italy; VIM-producing KPN from Athens, Greece and ECL from Leipzig, Germany and Madrid, Spain; and IMP-1 producing ECL from Istanbul, Turkey.

Organism (no.)	MßL (no. of strains)	Countries (no. of strains)	Detection year
P. aeruginosa (44)	VIM-1 (31)	Germany (4)/Greece (5)/Italy (21)/Turkey(1)	2001-2004
	VIM-2 (2)	France (1)/Poland (1)	2001 and 2003
	IMP-13 (6)	Italy (6)	2002-2003
	GIM-1 (6)	Germany (6)	2002
K. pneumoniae (33)	VIM-1 (33)	Germany (1)/Greece (16)/Italy (12)/Spain (3)/Turkey (1)	2005-2006
E. cloacae (20)	VIM-1 (7)	Germany (3)/Italy (2)/Spain (2)/Turkey(1)	2004-2006
	IMP-1 (12)	Turkey (12)	2003-2004
E. aerogenes (2)	VIM-1 (2)	Greece (2)	2005
Acinetobacter spp. (2)	IMP-2 (1)	Italy (1)	2003
	VIM-1 (1)	Greece (1)	2003
C. koseri (1)	VIM-1 (1)	Italy (1)	2005
K. ozaenae (1)	VIM-1 (1)	Italy (1)	2005
P. mirabilis (1)	VIM-1 (1)	Greece (1)	2005

Conclusions: The emergence and dissemination of MßL-producing strains has been documented in several European countries, and it is of great concern since these enzymes were usually encoded by genes located on integrons with enhanced mobility.

INTRODUCTION

Carbapenems are commonly used as antimicrobials of last resort for treatment of infections caused by Gram-negative bacteria because of their broad-spectrum of activity and stability to hydrolysis by most ß-lactamases, including ESBLs. They are also used as empiric agents for treating serious infections in critical care patients. In recent years, the emergence of various resistance mechanisms that affect carbapenem activity, including reduced outer membrane permeability, hyperproduction of stably-derepressed AmpC ß-lactamases, acquired carbapenemases and overexpression of efflux pumps, has reduced the efficacy of these antimicrobials in some geographic regions including Latin America, Europe and parts of Asia and North America.

Production of acquired metallo-ß-lactamases ([MßLs]; IMP-, VIM-types, SPM-1, GIM-1 and SIM-1) constitutes one of the most important resistance mechanisms since these enzymes can hydrolyze the vast majority of clinically available ß-lactam agents. Furthermore, these enzymes are not inhibited by the ß-lactamase inhibitors available or currently in development.

Acquired MßL genes are frequently located on integrons, which usually carry other resistance genes and are related to multidrug-resistant phenotypes. Integrons exhibit high mobility, especially in environments with high antimicrobial-use pressure and appear to be widespread among Gram-negative pathogens in European hospitals.

As part of SENTRY Antimicrobial Surveillance Program, we evaluated the emergence and dissemination of MßL-producing strains in participating European medical centers.

MATERIALS AND METHODS

Bacterial isolates. During 2000-2006, the SENTRY Program collected 4,935 *Pseudomonas aeruginosa,*1,460 *Acinetobacter* spp. and 22,950 Enterobacteriaceae from participating medical centers in Europe, Turkey, Israel and Russia. Consecutive, non-duplicate isolates were collected from patients hospitalized with bloodstream infections, pneumonia, skin and soft tissue infections, and urinary tract infections according to defined protocols. Species identification was confirmed by standard biochemical tests and use of the Vitek System (bioMérieux, Hazelwood, Missouri, USA), where necessary.

Susceptibility testing. All isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedures described by the CLSI (2006) using validated dry-form panels manufactured by TREK Diagnostics (Ohio, USA). Interpretations of susceptibility testing results were by CLSI (2007) and EUCAST (2006) criteria. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853 were routinely included during testing 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), as well as Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC, ≥2 mg/L) were screened for production of carbapenemases. Indole-positive Proteae and *Proteus mirabilis* were screened only when frankly resistant (MIC ≥16 mg/L).

MßL screening was performed by a disk approximation technique using imipenem, meropenem and ceftazidime as substrates, and EDTA and 2-mercaptopropionic acid (2-MPA) as MßL inhibitors. All Enterobacteriaceae strains were also screened for serine carbapenemases by a disk potentiation test using clavulanic acid as the ß-lactamase inhibitor.

Gene sequencing. Isolates with positive screen test results were evaluated by PCR using primers for bla_{IMP}, bla_{VIM}, bla_{SPM-1} and bla_{GIM-1}. 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.

Epidemiological Typing. Multiple isolates from the same 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 by pulsed-field gel electrophoresis (PFGE) using a CHEF DRII apparatus (Bio-Rad, Hercules, California, USA).

RESULTS

- *P. aeruginosa* showed high rates of antimicrobial resistance with most active antimicrobials being polymyxin B (99.5% susceptible), amikacin (82.5-87.9%), piperacillin/tazobactam (81.5%), meropenem (73.1-80.3%) and imipenem (77.2%; Table 1).
- Polymyxin B (MIC_{90}, ≤0.5 mg/L; 97.9% susceptible) and tigecycline (MIC_{90}, 2 mg/L; 96.5% susceptible) showed excellent potency and spectrum against *Acinetobacter* spp., while imipenem and meropenem (62.6-72.6% susceptible) showed limited activity against this pathogen.
- Enterobacteriaceae isolates remained highly susceptible to carbapenems (>99% susceptible), tigecycline (96.9% susceptible), amikacin (96.2-97.9% susceptible) and cefepime (91.7-96.4% susceptible). In contrast, susceptibility to ciprofloxacin was relatively low (85.2-86.7%; Table 1).
- ESBL rates based on CLSI screening criteria ranged from 24.6% to 25.6% among *K. pneumoniae*.
- Susceptibility rates to imipenem remained stable among *P. aeruginosa* (75.5% in 2000 and 76.1% in 2006), but varied from 78.5% in 2000 to a low of 51.9% in 2006 among *Acinetobacter* spp.
- Although imipenem remained very active against Enterobacteriaceae (99.8% susceptible in 2006), the occurrence of strains with MIC values at ≥2 mg/L increased from 3.6% in 2000 to 5.8% in 2006.
- MßL-producing strains were resistant to most antimicrobials tested. Only polymyxin B showed activity against *P. aeruginosa*. Tigecycline (94.8-100% susceptible) and amikacin (65.5-98.3% susceptible) maintained activity against MßL-producing Enterobacteriaceae strains.
- MßL producers were most frequently isolated in Italy (VIM-1[no. of isolates, 37], IMP-2[1] and IMP-13[6]) followed by Greece (VIM-1[25]) and Turkey (VIM-1[3] and IMP-1[12]; Table 2).
- VIM-1 was the most predominant and widely distributed MßL identified in Europe (Table 2). This MßL was also detected among species that are usually susceptible to most antimicrobial agents such as *E. aerogenes* (Greece), *Proteus mirabilis* (Greece) and *Citrobacter koseri* (Italy; Table 2).
- The most common MßL-producing species was *P. aeruginosa* with 44 strains isolated from six countries and producing four different MßLs (VIM-1, VIM-2, IMP-13 and GIM-1; Table 2).
- Thirty-three VIM-1-producing strains of *K. pneumoniae* isolated from five countries and 20 *E. cloacae* strains from four countries producing VIM-1 and IMP-1 were detected among Enterobacteriaceae.
- Although *Acinetobacter* spp. showed high resistance rates to carbapenems, MßLs were rarely detected in this pathogen; only one IMP-1 and one VIM-1 producers were encountered among 240 carbapenem-resistant strains screened.
- Molecular typing results indicated clonal dissemination of *P. aeruginosa* producing VIM-1 in Germany, Greece and Italy and GIM-1 in Germany; *K. pneumoniae* producing VIM-1 in Greece (two clusters); *E. aerogenes* producing VIM-1 in Greece and three clonal outbreaks of IMP-1-producing *E. cloacae* in Turkey (Table 3).
- Clonal diversity was observed among IMP-13-producing *P. aeruginosa* from Rome; VIM-1-producing *K. pneumoniae* from Athens and Rome; *E. cloacae* from Leipzig and Madrid; and IMP-1 producing *E. cloacae* from Istanbul.

Table 1. In vitro activity of select antimicrobial agents tested against Gram-negative pathogens isolated in Europe by the SENTRY Program (2000-2006).

Organism (no. tested)/ antimicrobial agent	MIC (mg/L): 50%	MIC (mg/L): 90%	% Susceptible: CLSI[a]	% Susceptible: EUCAST[b]
P. aeruginosa (4,935)				
Ceftazidime	4	>16	74.8	74.8
Cefepime	4	>16	75.4	75.4
Piperacillin/tazobactam	8	>64	81.5	-[c]
Aztreonam	8	>16	61.0	-
Imipenem	1	>8	77.2	77.2
Meropenem	1	>8	80.3	73.1
Gentamicin	≤2	>8	72.3	72.3
Amikacin	≤4	32	87.9	82.5
Ciprofloxacin	≤0.25	>4	69.8	65.9
Tigecycline	>4	>4	-	-
Polymyxin B	≤1	2	99.5	-
Acinetobacter spp. (1,460)				
Ceftazidime	>16	>16	36.0	-
Cefepime	16	>16	41.8	-
Ampicillin/sulbactam	16	>16	47.2	-
Piperacillin/tazobactam	>64	>64	35.4	-
Imipenem	1	>8	72.6	69.1
Meropenem	1	>8	68.3	62.6
Gentamicin	>8	>8	37.0	37.0
Amikacin	32	>32	49.1	45.9
Ciprofloxacin	>2	>2	34.3	34.3
Tigecycline	0.5	2	96.5[d]	-
Polymyxin B	≤1	≤1	97.9	-
Enterobacteriaceae (22,950)				
Ceftazidime	≤1	4	91.4	86.9
Ceftriaxone	≤0.25	16	90.6	86.6
Cefepime	≤0.12	1	96.4	91.7
Aztreonam	≤0.12	8	91.2	-
Cefoxitin	4	>32	78.1	-
Ampicillin/sulbactam	8	>16	52.9	-
Piperacillin/tazobactam	2	16	90.8	-
Imipenem	≤0.5	≤0.5	99.7	99.2
Meropenem	≤0.12	≤0.12	99.8	99.7
Gentamicin	≤2	≤2	91.3	90.2
Amikacin	≤4	≤4	97.9	96.2
Ciprofloxacin	≤0.25	>2	86.7	85.2
Tigecycline	0.5	1	96.9[d]	92.5
Polymyxin B	≤1	>4	84.4	-
K. pneumoniae (2,963)				
Ceftazidime	≤2	>16	81.2 (25.6)[e]	74.4
Ceftriaxone	≤0.25	>32	79.5 (25.3)[e]	74.7
Cefepime	≤0.12	16	88.4	78.7
Aztreonam	≤0.12	>16	79.8 (24.6)[e]	-
Cefoxitin	≤4	16	86.7	-
Ampicillin/sulbactam	8	>16	59.8	-
Piperacillin/tazobactam	4	>64	83.2	-
Imipenem	≤0.5	≤0.5	99.4	98.9
Meropenem	≤0.12	≤0.12	99.6	99.3
Gentamicin	≤2	>8	82.1	80.7
Amikacin	≤4	16	92.8	86.7
Ciprofloxacin	≤0.25	>2	86.9	84.7
Tigecycline	0.5	1	98.8[d]	93.6
Polymyxin B	≤1	≤1	98.2	-
Enterobacter spp. (2,532)				
Ceftazidime	≤2	>16	69.5	53.5
Ceftriaxone	≤0.25	>32	72.3	64.6
Cefepime	≤0.12	4	96.6	81.9
Aztreonam	≤0.12	>16	72.6	-
Cefoxitin	>32	>32	4.7	-
Ampicillin/sulbactam	>16	>16	24.1	-
Piperacillin/tazobactam	4	>64	74.6	-
Imipenem	≤0.5	1	99.0	98.1
Meropenem	≤0.12	≤0.12	99.2	98.6
Gentamicin	≤2	>8	89.3	88.4
Amikacin	≤4	≤4	96.9	94.3
Ciprofloxacin	≤0.25	>2	84.0	82.6
Tigecycline	0.5	1	97.3[d]	91.7
Polymyxin B	≤1	>4	89.0	-

a. Susceptibility rates were calculated by applying Clinical and Laboratory Standards Institute (CLSI) breakpoints.
b. Susceptibility rates were calculated by applying EUCAST breakpoints.
c. - = no breakpoint has been established.
d. Susceptibility rates were calculated by applying breakpoints approved by the United States Food and Drug Administration (US-FDA) for indicated Enterobacteriaceae species.
e. Percentage of isolates with ESBL phenotypes according to CLSI criteria.

Table 2. Geographic distribution of metallo-ß-lactamase producing strains isolated in Europe (SENTRY Program 2000-2006).

Country	City	Organism (no.)	Metallo-ß-lactamase	Detection year
Italy (44)	Genoa, Catania	*P. aeruginosa* (21)	VIM-1	2000-2001
		E. cloacae (2)	VIM-1	2004
	Rome	*P. aeruginosa* (6)	IMP-13	2000-2003
		A. baumannii (1)	IMP-2	2003
	Genoa	*K. pneumoniae* (12)	VIM-1	2005-2006
		C. koseri (1)	VIM-1	2005
		K. ozaenae (1)	VIM-1	2006
Greece (25)	Athens	*P. aeruginosa* (5)	VIM-1	2003-2004
		K. pneumoniae (16)	VIM-1	2001-2005
		E. aerogenes (2)	VIM-1	2005
		A. baumannii (1)	VIM-1	2003
		P. mirabilis (1)	VIM-1	2005
Turkey (15)	Istanbul	*P. aeruginosa* (1)	VIM-1	2005
	Ankara	*K. pneumoniae* (1)	VIM-1	2005
		E. cloacae (1)	VIM-1	2005
	Istanbul, Ankara	*E. cloacae* (12)	IMP-1	2003-2005
Germany (14)	Frankfurt	*P. aeruginosa* (4)	VIM-1	2005-2006
	Düsseldorf	*P. aeruginosa* (6)	GIM-1	2002
	Leipzig	*K. pneumoniae* (1)	VIM-1	2005
		E. cloacae (3)	VIM-1	2006
Spain (5)	Madrid	*K. pneumoniae* (3)	VIM-1	2005-2006
		E. cloacae (2)	VIM-1	2005
France (1)	Nantes	*P. aeruginosa* (1)	VIM-2	2003
Poland (1)	Warsaw	*P. aeruginosa* (1)	VIM-2	2001

Table 3. Clonality observed among metallo-ß-lactamase producing strains isolated from Europe.

Organism	No. isolates with molecular typing	Metallo-ß-lactamase	City/ Country	Ribotype/PFGE[a] (no. isolates)
P. aeruginosa	6	GIM-1	Düsseldorf/Germany	798.3/PSA-B (3) 134.1/PSA-B (2) 134.2/ ND (1)
	4	VIM-1	Frankfurt/Germany	337.2/PSA-C (4)
	4	VIM-1	Athens/Greece	1034.2/PSA-A (4)
	16	VIM-1	Genoa/Italy	1034.2/PSA-D (16)
	2	VIM-1	Catania/Italy	1034.2/PSA-E (2)
K. pneumoniae	8	VIM-1	Athens/Greece	211.6/KPN-A (6) 258.4/KPN-B(2)
E. cloacae	11	IMP-1	Istanbul/Turkey	250.1/ECL-B (5) 187.2/ECL-A (4) 213.6/ECL-C (2)
E. aerogenes	2	VIM-1	Athens/Greece	ND/EAE-A (2)

a. Capital letter suffix attached to species abbreviation designates a unique PFGE type. Different letter suffixes indicate distinct patterns observed in that species. ND = Not Done.

CONCLUSIONS

- The emergence and dissemination of MßL-producing strains was documented in several European countries and it is of great concern since these enzyme genes are located on mobile integrons.
- VIM-1 is the most widespread MßL among Gram-negative pathogens isolated in Europe.
- MßL-producing Enterobacteriaceae may include carbapenem MIC values within the susceptible category, posing a great risk for inadequate antimicrobial selection and treatment failures.
- Tigecycline and the polymyxins appear to be very promising alternatives for treatment of infections caused by MßL-producing Enterobacteriaceae and *P. aeruginosa*, respectively.

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