The Emergence of Metallo-ß-Lactamases and Increasing Carbapenem Resistance Among *P. aeruginosa* and *Acinetobacter* spp. from Bloodstream Infections: Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: Carbapenem-resistance (R) among *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) is becoming a growing problem worldwide.

Methods: R rates to imipenem (IMP) and selected ß-lactams among PSA and ASP were analyzed for bloodstream infections (BSI; 1997 and 2002) in Europe (EU), North America (NA) and Latin America (LA); and correlated with the emergence of metallo-ß-lactamase (MßL) in the SENTRY Program participants centers (PC). Isolates were tested by NCCLS broth microdilution methods and isolates R to IMP, meropenem (MER) and ceftazidime (CAZ) were screened for MßL production by phenotypic tests (disk approximation or MßL Etest strip) and hydrolyses assays. Characterization of MßL was assessed by PCR for bla_{IMP}, bla_{VIM}, bla_{SPM} and gene sequencing.

Results:

		% R by year				
		1997		2002		
Organism	Region (no. tested; 1997/2002)	IMP	CAZ	IMP	CAZ	Characterized MßL group
PSA	EU (257/408)	4.7	10.5	12.3	17.7	VIM/GIM/IMP
	LA (92/155)	10.9	19.6	16.8	23.9	SPM/VIM/IMP
	NA (359/300)	8.6	7.0	4.7	10.0	
ASP	EU (111/111)	15.3	27.0	16.2	53.2	VIM
	LA (87/91)	6.9	44.8	9.9	49.5	IMP
	NA (117/87)	2.6	11.1	8.0	25.3	

IMP- and CAZ-R rates increased for both pathogens in all 3 geographic regions analyzed, except for IMP-R among PSA in NA. A significant number of MßLs, some described for the first time (SPM-1, GIM-1, IMP-13 and -16) were detected in LA and EU, where IMP-R increased significantly overall among PSA from 1997 - 2002. Some MßLs have been detected in genotypically distinct strains from > 1 site within a nation (SPM-1) or in different countries (VIM-2). The largest number of MßLs was detected in Brazil, where IMP-R PSA increased 3-fold to 26.8% in 2002. Although IMP-R increased among ASP in all 3 regions, MßL was detected only in Brazil and Greece.

Conclusions: The emergence and dissemination of mobile MßLs represent an alarming factor for increasing R to carbapenems in several regions evaluated by the SENTRY Program and especially LA.

BACKGROUND

The carbapenems (meropenem and imipenem) are usually active against multi-drug resistant *Pseudomonas* spp. and *Acinetobacter* spp.; however, resistance to these compounds has been increasing rapidly. Resistance to carbapenems in *P. aeruginosa* is usually secondary to reduced uptake as a result of OprD porin loss. Resistance by this mechanism depends on continued expression of the chromosomal AmpC ß-lactamase. Low level carbapenem resistance can also arise via overexpression of the MexA-MexB-OprM efflux system. On the other hand, some high-level resistances (MIC >32 μg/ml) are due to the production of metallo-ß-lactamases (MßL).

MßLs (Ambler class B) are usually zinc dependent. These metal ions co-ordinate water molecules that serve as nucleophiles, which attack and break the cyclic amide bond of the ß-lactam ring, rendering the ß-lactam compound biologically inactive. The recognized enzyme types are IMP, VIM and the recently described SPM ß-lactamase. In the present study we evaluated the variation of resistance rates to several ß-lactam compounds among *Pseudomonas aeruginosa* and *Acinetobacter* spp. between 1997 and 2002; and the production of metallo-ß-lactamases among isolates found to be resistant to both imipenem and meropenem.

MATERIALS AND METHODS

Study Design. The SENTRY Antimicrobial Surveillance Program monitors pathogen frequency and antimicrobial resistance patterns of nosocomial and community-acquired infections through >90 sentinel hospitals worldwide. Among other selected pathogens, *Pseudomonas* spp. and *Acinetobacter* spp. strains resistant to imipenem (MIC, ≥ 16 μg/ml), meropenem (MIC, ≥ 16 μg/ml), and ceftazidime (MIC, ≥ 32 μg/ml) have been routinely examined for antimicrobial resistance genes through the amplification and sequencing of the variable region of class 1 integrons. Also multiple phenotypic and enzymatic analyses are routinely applied.

Susceptibility testing. All isolates are susceptibility tested by the reference broth microdilution method as described by the NCCLS. Antimicrobial agents were obtained from the respective manufacturers and quality control was performed by concurrent testing of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212.

Phenotypic detection of ß-lactamases. Production of MßLs was screened by the disk approximation test. Briefly, a 100mm Mueller-Hinton agar plate was inoculated using a 0.5 McFarland suspension from a fresh culture.

MATERIALS AND METHODS (Continued)

Imipenem, meropenem, and ceftazidime disks were strategically aligned around disks contained either EDTA (750 μg) or 2-mercaptopropionic acid (2-MPA, 360 μg). The appearance of either an enhanced or phantom zone between the carbapenems and/or ceftazidime and either one of the disks containing the metallo-ß-lactamase inhibitor (EDTA or 2-MPA) was considered a positive test. *Acinetobacter baumannii* 54/97 was used as a positive control. MßL Etest® strips (AB Biodisk, Solna, Sweden) were used to confirm the disk approximation test results. The strips contain imipenem ± EDTA. In addition, ceftazidime/ceftazidime-clavulanic acid and cefepime/cefepime-clavulanic acid ESBL Etest® strips were also used to evaluate the possible concurrent production of extended-spectrum ß-lactamase (ESBL).

Antimicrobial resistance gene screening. Oligonucleotide primers targeting to conserved regions of bla_{IMP}, bla_{VIM}, bla_{SPM} genes were initially used to determine the genetic basis of the resistance for screen-positive strians. Additional primers designed for the 5' conserved segment (CS) and 3'CS regions of class 1 integrons were used to amplify the integron resident in those strains where MßL resistance genes were initially detected. The cycling parameter were: 95ºC for 5 minutes followed by 30 cycles of 95ºC for 1 minute, annealing at 45ºC for 1 minute and extension 68ºC ranging from 1 to 4 minutes and ending with 5 minutes incubation at 68ºC.

DNA sequencing. Primers for the 5'CS and 3'CS of the class 1 integron, as well as primers for the gene cassette yielded PCR products which were sequenced on both strands using DuPont Automated systems.

Plasmid analyzes and transformation. Transfer of ß-lactam resistance markers into DH5α was performed using a Bio-Rad Gene Pulser apparatus (Bio-Rad, Richmond, CA) set at 2.5kV, 25μF and 400Ω. DH5α harboring the plasmid DNA was selected on nutrient agar plates containing ceftazidime (2μg/ml).

Computer sequence analysis. Nucleotides sequences and their deduced protein products, alignments and phylogenetic relationships were determined using the Lasergene software package (DNASTAR, Madison, WI).

COMMENTS

- In the North American region (USA and Canada), ß-lactam resistance rates did not vary significantly among *P. aeruginosa*, but a notable increase in resistance rates to ß-lactams was detected among *Acinetobacter* spp. in recent years (Table 1).
- Only one MßL was detected among isolates from the North American region. *P. aeruginosa* strains producing the recently described VIM-7 were detected in Texas.
- In the Latin American region, an important trend to increased resistance rates to all ß-lactams evaluated was noted among *P. aeruginosa*, especially for meropenem (from 5.4 to 15.5%, p=0.01) and *Acinetobacter* spp.
- Several MßLs have been detected among *P. aeruginosa* from the Latin American region, including enzymes first described by the SENTRY Program, such as SPM-1 (Sao Paulo and Brasilia, Brazil) and IMP-16 (Brasilia, Brazil). The bla_{SPM} has already been detected in a large number of strains in several Brazilian cities, while the bla_{IMP-16} was only detected in one isolate (Table 2).
- Other ß-lactamases detected in *Pseudomonas* spp. from Latin America include VIM-2 (*P. aeruginosa* in Caracas, Venezuela and *P. fluorescens* in Santiago, Chile), IMP-1 (*P. fluorescens* in Sao Paulo, Brazil); and GES-1, which is a new class A ß-lactamase (*P. aeruginosa* in Sao Paulo, Brazil; Table 3).
- The greatest increases in resistance rates and also the greatest variety of MßLs were detected in Europe. MßL enzymes detected among *P. aeruginosa* included GIM-1 (Dusseldorf, Germany), IMP-13 (Rome, Italy – recently described by the SENTRY Program), VIM-1 (Rome, Genoa and Catania, Italy) and VIM-2 (Warsaw, Poland and Paris, France).
- IMP-2 and VIM-1 were also detected among *Acinetobacter* spp. strains from Europe (Rome, Italy and Athens, Greece, respectively).
- Some MßLs, such as IMP-1, VIM-1, VIM-2, and SPM-1, were detected in different species and/or in several geographic regions. Some enzymes were also detected in several isolates within a geographic region. These results indicate a rapid dissemination of MßL-encoding genes.

RESULTS

Table 1. Comparison of the in vitro activities of selected ß-lactams between the year 1997 and 2002 - SENTRY Program.

	1997		2002	
Organisms/antimicrobial agent	% S	% R	% S	% R
North America				
P. aeruginosa	(n=359)		(n=300)	
Imipenem	87.5	8.6	89.0	4.7
Meropenem	95.0	1.7	91.0	4.0
Ceftazidime	86.9	7.0	88.3	10.0
Cefepime	88.3	4.5	87.7	4.7
Aztreonam	76.3	10.6	73.3	12.0
Acinetobacter spp.	(n=117)		(n=87)	
Imipenem	94.0	2.6	89.7	8.0
Meropenem	94.9	5.1	88.5	11.5
Ceftazidime	74.4	11.1	65.5	25.3
Cefepime	74.4	12.0	66.7	18.4
Aztreonam	23.1	47.9	11.5	64.4
Latin America				
P. aeruginosa	(n=92)		(n=155)	
Imipenem	84.8	10.9	79.4	16.8
Meropenem	94.6	5.4	79.4	15.5
Ceftazidime	71.7	19.6	67.7	23.9
Cefepime	72.8	14.1	72.3	15.5
Aztreonam	65.2	14.1	57.4	20.0
Acinetobacter spp.	(n=87)		(n=91)	
Imipenem	92.0	6.9	90.1	9.9
Meropenem	93.1	5.7	84.6	9.9
Ceftazidime	39.1	44.8	39.6	49.5
Cefepime	41.4	32.2	44.0	42.9
Aztreonam	12.6	77.0	12.1	84.6
Europe				
P. aeruginosa	(n=257)		(n=408)	
Imipenem	88.3	4.7	78.4	12.3
Meropenem	90.7	5.8	80.4	13.2
Ceftazidime	87.5	10.5	76.2	17.7
Cefepime	82.9	11.7	76.2	8.3
Aztreonam	83.3	9.3	65.2	18.4
Acinetobacter spp.	(n=111)		(n=111)	
Imipenem	82.0	15.3	80.2	16.2
Meropenem	75.7	15.3	75.7	16.2
Ceftazidime	54.1	27.0	40.5	53.2
Cefepime	62.2	24.3	43.6	41.8
Aztreonam	31.5	43.2	12.6	75.7

Table 2. New metallo-ß-lactamases detected in *P. aeruginosa* and characterized by the SENTRY Program.

ß-lactamase	No. of isolates	No. of ribogroups	Clinical specimens[a]	City, country	Date of isolation
SPM-1	21	6	Blood, Resp, SST	Sao Paulo and Brasilia, Brazil	2001-2003
GIM-1	5	3	Blood, Resp	Dusseldorf, Germany	2002
VIM-7[b]	2	1	Resp	Houston, USA	2001
IMP-13	8	2	Blood	Rome, Italy	2001-2002
IMP-16	1	1	Resp	Brasilia, Brazil	2002

a. Resp = Clinical specimens from the respiratory tract; SST = skin and soft tissue.
b. VIM-7 was not detected by the SENTRY Program, but was characterized by that research team.

Table 3. List of all ß-lactamases identified by the SENTRY Program among *Pseudomonas* spp. and *Acinetobacter* spp.

ß-lactamase	Species[a]	No. of isolates	No. of ribogroups	Clinical specimens[b]	City, country	Date of isolation
IMP-1	ACB	2	2	Blood, Resp	Buenos Aires, Argentina	2001-2002
IMP-1	ACB	1	1[a]	Blood	Rosario, Argentina	2002
IMP-1	ACB	7	5	Blood, Resp SST	Sao Paulo, Brazil	2001-2002
IMP-1	PSFL	1	1	Blood	Sao Paulo, Brazil	2001
IMP-2	ACB	1	1	Blood	Roma, Italy	2003
VIM-1	ACB	2	1	Blood	Athens, Greece	2002-2003
VIM-1	PSA	2	1	Blood	Athens, Greece	2003
VIM-1	PSA	17	1	Blood, Resp SST	Genoa, Italy	2000-2001
VIM-1	PSA	4	3	Blood, Resp Urine	Catania, Italy	2000-2001
VIM-1	PSA	1	1	Blood	Roma, Italy	2002
VIM-2	PSFL	1	1	Blood	Santiago, Chile	2002
VIM-2	PSA	3	1	Resp.	Caracas, Venezuela	2002
VIM-2	PSA	1	1	Blood	Warsaw, Poland	2001
VIM-2	PSA	1	1	Blood	Paris, France	2003
GES-1	PSA	1	1	Blood	Sao Paulo, Brazil	2002

a. ACB = *Acinetobacter baumannii*; PSA = *P. aeruginosa*; and PSFL = *P. fluorescens*.
b. Resp = clinical specimens from the respiratory tract; SST = skin and soft tissue.

CONCLUSIONS

- The SENTRY Program has detected and characterized several new MßL enzymes within a 12 - 18 month period.
- MßL genes are emerging and disseminating very rapidly among and within geographic regions evaluated by the SENTRY Program.
- The emergence and dissemination of mobile MßLs represents an important factor leading to increasing resistance for carbapenems in several regions evaluated by the SENTRY Program including the USA.