Emergence of VIM-1 in *Proteus mirabilis* and *Citrobacter koseri* Isolated from European Medical Centers: Report from the SENTRY Antimicrobial Surveillance Program

LM DESHPANDE, HS SADER, TR FRITSCHE, RN JONES

JMI Laboratories, North Liberty, IA, USA

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JMI Laboratories

North Liberty, IA, USA

www.jmilabs.com

319.665.3370

fax 319.665.3371

ronald-jones@jmilabs.com

ABSTRACT

Background: The prevalence of metallo-ß-lactamase (MßL) -producing Enterobacteriaceae (ENT) continues to increase ß-lactam resistance (R) in Europe and parts of Southeast Asia. The SENTRY Program has reported bla_{VIM-1} and bla_{IMP-1} in *K. pneumoniae* and *Enterobacter* spp., in Europe and bla_{IMP-1} from *S. marcescens* in Japan. We report here bla_{VIM-1} detected in *P. mirabilis* (PM) and *C. koseri* (CK) isolates both from separate European medical centers.

Methods: As part of the SENTRY Program, PM observed as R (MIC, >8 µg/ml), and other ENT species with elevated MIC values (≥2 µg/ml) to imipenem or meropenem were routinely screened for MßL production by disk (EDTA, 2-MPA) approximation test (DA) and Etest MßL strips. Isolates with a positive screen test and PCR for bla_{IMP} or bla_{VIM} were gene sequenced for MßL and class 1 integron components.

Results: Among 2,813 PM and 419 CK isolates tested during 2000-2005, one PM from Greece and one CK from Italy showed elevated carbapenem (CARB) MIC results.

		MIC (µg/ml) for[a]:								
Organism	Country	IMP	MEM	AZT	CAZ	P/T	AMK	TOB	PB	TIG
PM 2216D	Greece	>8	4	1	>16	64	16	16	>4	2
CK 8776A	Italy	8	1	≤0.12	>16	>64	0.5	4	≤0.5	0.12

a. Abbreviations: IMP = imipenem; MEM = meropenem; AZT = aztreonam; CAZ = ceftazidime; P/T = piperacillin/tazobactam; AMK = amikacin; TOB = tobramycin; PB = polymyxin B; TIG = tigecycline.

Both isolates exhibited positive screening tests and yielded PCR products with VIM-1 and class 1 integron primers. Integron sequencing revealed bla_{VIM-1}, along with the aminoglycoside R genes *aadA1* and *aac(6')* in PM, and *aac(6'), aadA1* and chloramphenicol-R gene *catB2* in CK. Both strains remained susceptible to AZT, AMK, gentamicin (MIC, ≤2 µg/ml) and TIG.

Conclusions: This is the first report of MßL-producing PM and CK. The SENTRY Program has documented the emergence and dissemination of MßL genes in many ENT species, especially in Europe. It is important to recognize this R mechanism among ENT as the isolates may exhibit elevated CARB MIC results within the CLSI susceptible range (≤4 µg/ml).

INTRODUCTION

Among Gram-negative bacteria, metallo-ß-lactamases (MßL; class B) were first detected in non-fermentative species, including *Stenotrophomonas* spp., *Pseudomonas* spp. and *Acinetobacter* spp., mainly in Asia, Europe and Latin America. VIM-1 was the second integron-borne Class B ß-lactamase identified from *P. aeruginosa* in an Italian hospital in 1999 and has become one of the most prevalent and widespread. It has been reported from multiple countries in Europe and Asia, as well as from Latin America and most recently from the United States (USA) and Canada.

The Enterobacteriaceae comprise a major group of pathogens causing hospital-acquired infections. Until recently Enterobacteriaceae species, feared for their versatile ESBLs and expanded spectrum cephalosporinases, were combated successfully with the use of carbapenems. As a consequence of soaring antimicrobial pressure and high mobility of class 1 integrons carrying MßL genes, carbapenem-resistant enteric bacilli have started to emerge. The degree of resistance to carbapenems varies significantly among carbapenemase-producing Enterobacteriaceae. MßL-producing Enterobacteriaceae may show imipenem and/or meropenem MIC values within the susceptible category when using the Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria, and thus go unidentified.

In the last few years these MßLs have been described in Enterobacteriaceae species, such as *Serratia marcescens*, *Klebsiella pneumoniae*, and *Enterobacter* spp. The SENTRY Program has reported bla_{VIM-1} and bla_{IMP-1} in *K. pneumoniae* and *Enterobacter* spp. in Europe, and bla_{IMP-1} from *S. marcescens* in Japan. In the present study, we now report bla_{VIM-1} in *P. mirabilis* and *C. koseri* isolates, both from separate European medical centers.

MATERIALS AND METHODS

Bacterial Isolates. During 2000-2005, the SENTRY Program collected 2,813 *P. mirabilis* and 419 *C. koseri* isolates from medical centers located in North America (n=44), Latin America (n=14), Asia-Pacific (n=14) and Europe (n=33). Isolates were consecutively collected from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients 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/or by the Vitek System (bioMérieux, Hazelwood, Missouri, USA), where necessary.

Susceptibility Testing. All isolates were tested for susceptibility against more than 25 antimicrobials by broth microdilution procedures as described by the CLSI (2006a) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility to antimicrobials tested were by CLSI (2006b) criteria, where available. Tigecycline breakpoints were those approved by the USA-FDA for some Enterobacteriaceae species (Susceptible / Intermediate / Resistant at ≤ 2 / 4 / ≥ 8 µg/ml). *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 Metallo-ß-lactamase. As part of the SENTRY Program, *P. mirabilis* and indole-positive Proteae isolates observed as carbapenem-resistant (MIC, >8 µg/ml), and other Enterobacteriaceae species with elevated MIC values (≥2 µg/ml) to imipenem or meropenem were routinely tested for MßL production by disk approximation test (DA) and Etest MßL strips (AB BIODISK, Solna, Sweden). DA screening was performed using imipenem, meropenem and ceftazidime as substrates, and EDTA and 2-mercaptopropionic acid (2-MPA) as MßL inhibitors. Antimicrobial disks were strategically positioned around enzyme inhibitors (see Figure 1). Isolates exhibiting a "key hole" or "phantom zone" effect between one or more substrate/inhibitor combination(s) were regarded as potential MßL producers. Isolates exhibiting a positive DA test were screened for bla_{IMP}, bla_{VIM} and bla_{SPM} using generic PCR primers.

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.

RESULTS

- Antimicrobial susceptibilities of 2,813 *P. mirabilis* and 419 *C. koseri* isolates are listed in Table 1. A significant proportion of the *P. mirabilis* population was resistant to gentamicin (10.2% resistant), ciprofloxacin (14.0%) and trimethoprim/sulfamethoxazole (23.0%). *C. koseri* on the other hand showed >96% susceptibility to all agents tested. Meropenem and imipenem exhibited the best in vitro activity against both species with all isolates susceptible to meropenem and only one isolate resistant to imipenem.
- Only one *P. mirabilis* (2216D) from a participant medical center in Greece and one *C. koseri* (8776A) from Italy showed elevated carbapenem MIC results. Both strains remained susceptible to meropenem, aztreonam, amikacin, gentamicin and tigecycline (Table 2). Both the strains were found to harbor bla_{VIM-1}.
- *P. mirabilis* is intrinsically less susceptible to carbapenems, and in this case (2216D) meropenem MIC value was only slightly elevated (4 µg/ml) and within the susceptible range.
- *C. koseri* 8776A showed a low MIC value (1 µg/ml) to meropenem, eight-fold lower than that of imipenem, despite harbouring a MßL.

Table 1. Antimicrobial susceptibility profiles of *P. mirabilis* and *C. koseri* isolates collected by the SENTRY Antimicrobial Resistance Surveillance Program (2000-2005).

	MIC (µg/ml)			
Antimicrobial tested (no. isolates)	MIC_{50}	MIC_{90}	% susceptible[a]	% resistant[a]
P. mirabilis (2,813)				
Imipenem	1	2	99.8	<0.01[b]
Meropenem	≤0.12	≤0.12	100.0	0.0
Ceftazidime	≤2	≤2	98.0	1.5
Ceftriaxone	≤0.25	≤0.25	94.7	3.5
Aztreonam	≤0.12	≤0.12	98.9	0.6
Piperacillin/tazobactam	≤0.5	1	99.0	0.1
Gentamicin	≤2	>8	87.9	10.2
Amikacin	4	8	98.0	1.6
Ciprofloxacin	≤0.25	4	81.4	14.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	77.0	23.0
Tetracycline	>8	>8	12.3	97.7
Polymyxin B	>8	>8	0.7[a]	–[a]
C. koseri (419)				
Imipenem	≤0.5	≤0.5	99.8	0.0
Meropenem	≤0.12	≤0.12	100.0	0.0
Ceftazidime	≤2	≤2	97.6	2.1
Ceftriaxone	≤0.25	≤0.25	98.6	1.2
Aztreonam	≤0.12	≤0.12	98.3	1.4
Piperacillin/tazobactam	2	8	98.6	0.7
Gentamicin	≤2	≤2	98.8	0.9
Amikacin	1	2	99.5	0.0
Ciprofloxacin	≤0.25	≤0.25	97.9	1.6
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	97.2	2.8
Tetracycline	≤4	≤4	96.5	2.4
Polymyxin B	≤1	≤1	100.0[a]	–[a]

a. Susceptibility criteria per M100-S16 (CLSI, 2006); susceptibility criteria established for *Pseudomonas* spp. and *Acinetobacter* spp. for polymyxin B was utilized for comparison purposes only.

b. Only one isolate showed MIC >8 µg/ml (2216D, VIM-1 producer).

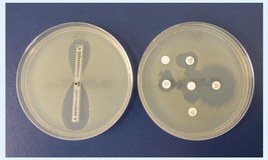
- MßL Etest failed to clearly detect the MßL in *P. mirabilis* (Figure 1). However DA test produced a very clear indication of the presence of enzyme inhibited by EDTA and 2-MPA in both isolates.
- Both isolates yielded PCR products with bla_{VIM-1} and class 1 integron primers spanning the 5' and 3' conserved regions.
- Sequencing of integron amplicons from *P. mirabilis* discovered bla_{VIM-1} along with the aminoglycoside resistance genes *aac(6')Ib, aac3-Ia* and *aadA1*.

Table 2. Antibiotic susceptibility profile of VIM-1 producing *P. mirabilis* and *C. koseri*.

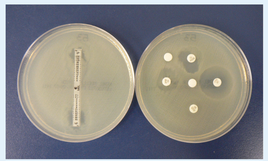
	Broth microdilution MIC (µg/ml) for:	
Antimicrobial tested	*P. mirabilis* 2216D	*C. koseri* 8776A
Imipenem	>8	8
Meropenem	4	1
Aztreonam	1	≤0.12
Ceftazidime	>16	>16
Piperacillin/tazobactam	64	>64
Amikacin	16	0.5
Gentamicin	≤2	≤2
Tobramycin	16	4
PolymyxinB	>4	≤0.5
Tigecycline	2	0.12

Figure 1. Detection of metallo-ß-lactamase from Enterobacteriaceae using Etest and disk approximation test.

8776A *C. koseri*



2216D *P. mirabilis*



Etest consists of imipenem and imipenem plus 320 µg/ml EDTA on each half of the strip. Disk approximation test consists of EDTA (750 µg) disk in the center with imipenem to the left, meropenem at the top, aztreonam to the right and ceftazidime at the bottom. 2-mercaptopropionic acid (3 µl of 1:10 dilution) was added to a blank paper disk situated diagonally opposite between imipenem and meropenem.

- *C. koseri* integron sequence revealed bla_{VIM-1} in the first position at the 5' end along with *aac(6')Ib*, *aadA1* and the chloramphenicol resistance gene, *catB2* at the 3' end.

CONCLUSIONS

- This is the first report of VIM-1 MßL-producing *P. mirabilis* and *C. koseri* from the SENTRY Program. Another report of a clonal outbreak of VIM-1 producing *P. mirabilis* was recently published from a different medical center in Greece. The SENTRY Program continues to document the emergence and dissemination of MßL genes in many species of Enterobacteriaceae, especially in Europe.
- It is important to recognize this resistance mechanism among Enterobacteriaceae as the isolates may exhibit elevated carbapenem MIC results, but within the CLSI susceptible range (≤4 µg/ml).
- The DA test for MßL production is a preferred option for screening Enterobacteriaceae strains since Etest may not detect the enzyme due to lower, off-scale imipenem MIC values among MßL-producing Enterobacteriaceae.
- The threat of horizontally transferring MßL within Enterobacteriaceae appears on the rise and needs to be controlled via stricter infection control practices and surveillance networks to monitor the movement and spread of these important resistance mechanisms.

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