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VIM-1 Producing *Enterobacter cloacae* Strains from Madrid, Spain: Report from the SENTRY Antimicrobial Surveillance Program 2005

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Objective: To characterize Enterobacteriaceae (ENT) strains with reduced susceptibility (S) to carbapenems (CARB) isolated in Madrid, Spain.

Methods: As part of the SENTRY Program, bacterial isolates are S tested by reference CLSI methods against >25 antimicrobial agents. ENT isolates (except *Proteus mirabilis* and indole+ Proteae) exhibiting MIC results at ≥ 2 mg/L to imipenem (IMI) and meropenem (MER) were screened for metallo-ß-lactamases (MßL) and Bush-Jacoby-Medeiros group 2f carbapenemases by disk approximation (DA) and PCR. PCR amplicons were sequenced for epidemiological purposes as well as to reveal genetic context of resistance genes. Isolates were also typed by PFGE.

Results: Two *E. cloacae* (ECL) strains (2700A and 726C) showed elevated CARB MIC values as well as resistance to all ß-lactams, except aztreonam (AZT) in strain 726C (Table). The strains were isolated in March 2005 (12 days apart) from bloodstream and respiratory tract infections of patients hospitalized in two distinct hospital units. The strains showed distinct antibiograms and PFGE patterns (Table). Both strains showed positive DA test results and PCR screens positive for bla_{VIM} with a Class 1 integron of approximately 2.5 Kb. Sequencing of integron from the index strain (726C) revealed bla_{VIM-1} along with *aac*A4 and *aad*A1 genes.

	MIC (mg/L)								PFGE
Strain #	IMI	MER	Ertapenem	AZT	Ciprofloxacin	Tetracycline	Tobramycin	TMP/SMX	
726C	8	4	>8	8	>4	>8	4	1	A
2700A	4	4	8	>16	0.12	4	2	≤0.5	B

Conclusions: This is the first report of VIM-1-producing ECL from Spain. VIM-1 has been recently reported in ENT (*K. pneumoniae* and *E. coli*) from Barcelona, Spain. The finding of bla_{VIM-1} in two clonally unrelated strains emphasizes the mobility of these genes. Thus, MßL may be emerging as a significant, geographically diverse resistance mechanism among ENT in Spain. It is imperative to screen ENT isolates with modestly elevated (not resistant) MIC values of either IMI or MER for carbapenemase production and to control the spread of this resistance mechanism in ENT isolates causing nosocomial infections.

INTRODUCTION

Metallo-ß-lactamases (MßL) are increasingly documented among the Enterobacteriaceae from some geographic regions of the world, most notably from Europe and Asia. Enterobacteriaceae isolates carrying carbapenemases generally exhibit lower MIC values for the carbapenems, often in the susceptible range (CLSI definitions), posing difficulties in correctly identifying MßL carrying strains.

An MßL-producing strain was first reported from Spain in 1996, which was a VIM-2 in *Pseudomonas aeruginosa*. After this initial report, a few cases have been found each year and, until recently, these reports were restricted to VIM-producing *P. aeruginosa* strains. The first report of Enterobacteriaceae producing MßL in Spain occurred in 2005, when Tortola et al (2005) reported one *Escherichia coli* strain from a urinary tract infection and one *Klebsiella pneumoniae* strain isolated from feces carrying bla_{VIM-1}. In both strains, bla_{VIM-1} was demonstrated to be carried on a gene cassette inserted into a class 1 integron. The bla_{VIM-1} containing integron was located on a 40 Kb transferable plasmid. We report here two *Enterobacter cloacae* isolates carrying bla_{VIM-1} isolated from a medical center in Madrid.

MATERIALS AND METHODS

Bacterial isolates. A total of 6,399 Enterobacteriaceae isolates were collected by the SENTRY Antimicrobial Surveillance Program in 2005, including 2,682 from Europe. Among these, 358 isolates were from Spain and 211 from the Hospital Universitario Ramón y Cajal located in Madrid. The isolates were consecutively collected from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients according to a common protocol. Species identification was confirmed by standard biochemical tests and the Vitek system, where necessary.

Susceptibility testing. The Enterobacteriaceae isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution methods as described by the CLSI (formerly NCCLS, 2006) using validated dry-form panels manufactured by TREK Diagnostics (Cleveland, OH, USA). Interpretations of susceptibility to all antimicrobials tested were by CLSI (2006) criteria. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853 were routinely included in the testing of quality assurance.

Screening for carbapenemases. All Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC ≥ 2 mg/L) were tested for production of carbapenemases. Indole-positive Proteae and *Proteus mirabilis* were excluded since these species are inherently less susceptible to carbapenems.

Disk approximation. Potential carbapenemase producers were screened using disk approximation techniques. MßL screens were performed using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid (2-MPA) as MßL inhibitors. Screening for serine carbapenemases was achieved by a method described by Pottumarthy et al. (2003) in which imipenem and meropenem were used as substrates and clavulanic acid as the ß-lactamase inhibitor.

PCR. Isolates with positive disk approximation test for MßL were screened for bla_{IMP}, bla_{VIM} and bla_{SPM} as well class 1 integrons using PCR primers described elsewhere. Isolates with elevated carbapenem MIC values and negative PCR results for MßL genes were screened for IMI, KPC, NMC-A and SME genes.

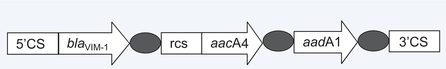
Gene sequencing. PCR amplicons for the carbapenemase genes and the integron were sequenced using a Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to the available sequences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

Epidemiological studies. Multiple isolates from the same medical center harboring carbapenemases belonging to the same family were routinely typed by automated ribotyping and/or pulsed-field gel electrophoresis (PFGE) as part of the SENTRY Program.

RESULTS

- Two *E. cloacae* isolates with elevated imipenem and meropenem MIC were observed among SENTRY isolates submitted by a participating medical center in Madrid, Spain (Table 1). The strains were isolated 12 days apart in March 2005, one from a blood stream infection (66-2700A) and the other from a patient with hospital acquired pneumonia (66-726C).
- Differences on the susceptibility pattern between these two strains were observed with aztreonam, fluoroquinolones, tetracycline and tobramycin (Table 1), suggesting that the strains were not clonally related. Although tetracycline activity varied (MICs, 4 and >8 mg/L), tigecycline was very active against both strains (MICs, 0.25 and 0.5 mg/L; Table 1).
- Both strains demonstrated a positive disk approximation test for MßL using either imipenem or meropenem as substrates in combination with EDTA or 2-MPA. The ceftazidime/EDTA test was negative for both strains, as was the disk approximation test for Bush group 2F enzymes.
- PCR products were obtained from both strains using VIM-1 primers. Class 1 integron primers also yielded PCR products of the same size (2.5 Kb).
- Sequencing of the PCR products of the index strain (66-726C) revealed a bla_{VIM-1} cassette in the first position followed by *aac*A4 and *aad*A1 genes in the class 1 integron. Schematic diagram of the arrangement of these genes in the integron is presented in Figure 1.
- Although the two *E. cloacae* strains were recovered from different units of the same hospital and carried the integron of the same size and characteristics, they showed different PFGE patterns (A and B, Table 1), ruling out clonal dissemination.

Figure 1. Schematic representation of the Class 1 integron carrying bla_{VIM-1} in *E. cloacae*.



Abbreviations: 5'CS - 5' conserved sequence, solid ovals represent 59 base pair elements, rcs - recombination conserved site, 3'CS - 3' conserved sequence. Direction of arrows on the resistance gene markers indicates the direction of transcription.

Table 1. Antimicrobial susceptibility and epidemiologic typing results of the VIM-1 producing *E. cloacae* isolates.

	MIC (mg/L)											PFGE
Strain #	IMP	MER	ERT	AZT	CIP	TET	TIGE	AMK	GENT	TOB	TMP/SMX	Pattern
726C	8	4	>8	8	>4	>8	0.5	2	≤2	4	1	A
2700A	4	4	8	>16	0.12	4	0.25	2	≤2	2	≤0.5	B

Abbreviations: IMP- imipenem, MER- meropenem, ERT- ertapenem, AZT- aztreonam, CIP- ciprofloxacin, TET- tetracycline, TIGE- tigecycline, AMK- amikacin, TOB- tobramycin, TMP/SMX- trimethoprim/sulfamethoxazole.

CONCLUSIONS

- Metallo-ß-lactamase may be emerging as an important resistance mechanism among Enterobacteriaceae in Spain.
- The finding of bla_{VIM-1} in two clonally unrelated *E. cloacae* strains in our study emphasizes the mobility of these genes.
- It is imperative to screen Enterobacteriaceae isolates with modestly elevated (not resistant) MIC values to imipenem or meropenem for carbapenemase production in order to control the spread of this important resistance mechanism in Enterobacteriaceae isolates that cause nosocomial infections.

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