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Five Year Evaluation of Clonal Dissemination and Expansion of VIM-1 Producing Enterobacteriaceae in Greece: A Report from the SENTRY Antimicrobial Surveillance Program (2001-2005)

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Objective: VIM-1 producing Enterobacteriaceae (ENT) emerged in 2000 and has become endemic in Greece. The objective of this study was to characterize VIM-1 genotypes and to evaluate clonal relationships among VIM-1 producing ENT strains isolated in Greece during the course of the SENTRY Program.

Methods: All isolates received as part of the SENTRY Program were susceptibility tested by reference CLSI methods against >25 antimicrobials. ENT isolates (except *Proteus mirabilis* and indole-positive Proteae) with MIC ≥ 2 mg/L for imipenem (IMI) and meropenem (MER) were screened for metallo-beta-lactamase (MBL) by disk approximation test followed by PCR. ENT isolates from Greece with positive MBL screen test results were further evaluated. MBL gene and its genetic context were revealed by PCR and sequencing techniques. The isolates were also epidemiologically typed by PFGE.

Results: In the 2001-2005 period, 16 *K. pneumoniae* (KPN) and 2 *E. aerogenes* (EAE) isolates from various medical centers in the metropolitan area of Athens, Greece were found to produce VIM-1. Multiple distinct clonal outbreaks of VIM-1 producing ENT were identified during this period. Susceptibility and molecular typing results are summarized in the table.

Organism	PFGE (no. of isolates)	Integron size (bp)	MIC range (mg/L)[a]					
			IMI	MEM	CAZ	P/T	ATM	CIPRO
KPN	C (1)	>2700	>8	>8	>16	>64	>16	>4
	D (9)	2000, 1500, 1300, 800	4- >8	1->16	>16	>64	≤0.12->16	4 ->4
	E (2)	800	8	2-4	>16	>64	>16	1
	F (1)	>2700, 1000	>8	4	>16	>64	>16	2
	G (1)	2000	4	4	>16	>64	≤0.12	≤0.03
	H (1)	>2700	>8	>8	>16	>64	>16	4
	I (1)	2000	4	2	>16	>64	≤0.12	>4
EAE	A (2)	1500, 1300	>8	>8	>16	>64	>16	>4

a. CAZ = ceftazidime, P/T = piperacillin/tazobactam, ATM = aztreonam, and CIPRO = ciprofloxacin

Conclusions: *bla*$_{VIM-1}$ emerged and rapidly became endemic among ENT in Greece. The high mobility of *bla*$_{VIM-1}$ was demonstrated by the finding of: i) different PFGE patterns among VIM-1 producing KPN; ii) variable sizes of the integron amplicons; and iii) the emergence of VIM-1 in EAE. Our results also demonstrated that *bla*$_{VIM-1}$ is disseminating horizontally as well as vertically. These findings represent a critical problem for the healthcare facility as carbapenems remain the last antimicrobial resort for treatment of infections caused by multi-drug resistant Gram-negative bacilli. In addition, this is the first report of EAE producing VIM-1.

INTRODUCTION

The acquired metallo-beta-lactamases (MßLs) comprise a group of enzymes with a broad hydrolytic spectrum that includes carbapenems. Five groups of MßLs have been described, namely IMP, VIM, SPM, GIM and SIM, with the IMP and the VIM types being most prevalent and reported from various regions worldwide. MßLs are more common among non-fermenting Gram-negative bacteria, mainly in the Far East, Southern Europe and South America. However, during the last few years, studies have reported the dissemination of VIM-type MßLs in members of the family Enterobacteriaceae, suggesting the ongoing spread of these resistance determinants among pathogens with higher infectivity.

There are numerous reports of strains producing of the VIM-type MßL from Greece. In this report, we summarize the microbiological characteristics and clonal relationships among VIM-1-producing enteric bacilli collected in Athens, Greece through the SENTRY Antimicrobial Surveillance Program (2001-2005).

MATERIALS AND METHODS

Bacterial isolates. All Enterobacteriaceae isolates collected from medical centers located in the metropolitan area of Athens, Greece through the SENTRY Program were evaluated. The isolates were consecutively collected from bloodstream infections, skin and soft tissue infections, urinary tract infections and pneumonia in hospitalized patients according to SENTRY Program protocols. Species identification was confirmed by standard biochemical tests and Vitek cards (bioMérieux, Hazelwood, MO, USA), where necessary.

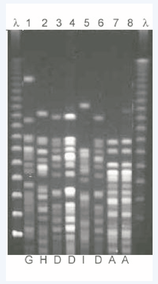
Susceptibility testing. The Enterobacteriaceae isolates were susceptibility tested against more than 25 antimicrobials by reference broth microdilution methods according to the CLSI documents using validated dry-form panels manufactured by TREK Diagnostics (Cleveland, OH, USA). *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 were routinely included in the testing for quality assurance.

Screening for carbapenemases. 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 screened only when highly resistant (MIC ≥ 16 mg/L) to carbapenems since these species are inherently less susceptible to these antimicrobials. MßL screenings were performed by disk approximation test using imipenem, meropenem and ceftazidime as substrates and EDTA, as well as, 2-mercaptopropionic acid (2-MPA) as MßL inhibitors. Isolates with positive disk approximation test were screened for *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{SPM}$ using PCR primers described elsewhere. PCR screening for Class 1 integron were performed to reveal the genetic context of the MßL genes.

Gene sequencing. PCR amplicons 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 the available sequences via NCBI BLAST search.

Epidemiological studies. Isolates harboring MßLs belonging to the same family were routinely typed using Riboprinter™ Microbial Characterization system (Dupont Qualicon, Wilmington, DE, USA) and/or pulsed-field gel electrophoresis (PFGE) as part of the SENTRY Program epidemiology protocols.

Figure 1. PFGE patterns of VIM-1 producing *K. pneumoniae* and *E. aerogenes* isolates. Genomic DNA was digested with SpeI and separated on CHEF DRII at 6 V/cm^2 with pulse times ramping from 5-60 sec over 23 hours. λ represents 48.5 kb Lambda molecular weight ladder. Lanes 1-6 represent *K. pneumoniae* isolates and lanes 7-8 represent *E. aerogenes* strains (PFGE patterns as indicated at the bottom of lanes).



RESULTS

- Among 558 Enterobacteriaceae strains collected in the 2001-2005 period, 16 *K. pneumoniae* and two *E. aerogenes* isolates showed elevated imipenem and meropenem MIC values (2 - ≥ 16 mg/L) and a positive disk approximation screening test for MßL. PCR followed by gene sequencing revealed *bla*$_{VIM-1}$ in all 18 strains.

- All VIM-1 producing isolates were resistant to the tested cephalosporins and ß-lactam/ß-lactamase inhibitor combinations. Susceptibility to aztreonam, ciprofloxacin and aminoglycosides varied and all except two isolates were susceptible to polymyxin B (Table 1).

- PFGE revealed seven distinct banding patterns among VIM-1 producing *K. pneumoniae* (Figure 1). Two patterns were observed to be clonal, one of which (PFGE type D/ribogroup 258.211.6) was the most prevalent and seems to have become endemic at the medical center (isolated from 2003 through 2005). The other probable clonal dissemination episode consisted of two strains (PFGE type E/ribogroup 258.258.4) isolated in 2004.

- PCR with primers targeting conserved sequences of Class 1 integrons yielded products of various sizes (800- >2700 bp), indicating the diversity of genetic backgrounds in which the VIM-1 may have disseminated in this geographic area. Isolates belonging to the same PFGE pattern also showed different sized integron amplicons.

- Both VIM-1 producing *E. aerogenes* strains shared unique molecular typing results (PFGE type A) indicating possible clonal dissemination of this pathogen (Table 1). In our recent survey of SENTRY Program isolates, a total of 21 *E. aerogenes* with elevated carbapenem MIC values were encountered from 2000-2004, but none harbored an MßL or a group 2F carbapenemase. This is the first report of an MßL from this species.

Table 1. Characterization of VIM-1 producing Enterobacteriaceae isolates from Greece.

Organism	PFGE pattern (no. of isolates)	Year of Isolation	Integron size (bp)[a]	MIC range (mg/L)[b]							
				IMI	MEM	ERT	ATM	CIP	PolyB	AMK	GEN
K. pneumoniae	C (1)	2002	>2700	>8	>8	>8	>16	>4	≤1	16	>8
	D (9)	2003, 2004, 2005	2000, 1500, 1300, 800	4- >8	1->16	2- >8	≤0.12->16	4 ->4	≤1- 8	8- 32	≤2->8
	E (2)	2004	800	8	2-4	4	>16	1	0.5	16- 32	>8
	F (1)	2001	>2700, 1000	>8	4	ND[c]	>16	2	2	4	4
	G (1)	2005	2000	4	4	2	≤0.12	≤0.03	≤0.5	16	>8
	H (1)	2005	>2700	>8	>8	>8	>16	4	≤0.5	16	8
	I (1)	2005	2000	4	2	2	≤0.12	>4	≤0.5	16	8
E. aerogenes	A (2)	2005	1500, 1300	>8	>8	>8	>16	>4	≤0.5	8	≤2

a. Integron sizes are approximate based on mobility in the gel.
b. All the VIM-1 producing isolates were resistant to ceftazidime (MIC, >16 mg/L) and piperacillin/tazobactam (MIC, > 64 mg/L). IMI = imipenem, MEM = meropenem, ERT = ertapenem, ATM = aztreonam, CIP = ciprofloxacin, PolyB = Polymyxin B, AMK = amikacin and GEN = gentamicin.
c. ND = not determined.

CONCLUSIONS

- *bla*$_{VIM-1}$ emerged and became endemic among Enterobacteriaceae in the Athens metropolitan area (first report of *E. aerogenes* producing VIM-1).

- The high mobility of *bla*$_{VIM-1}$ was demonstrated by the findings of: i) various PFGE patterns among VIM-1 producing *K. pneumoniae*; ii) variable sizes of the integron amplicons; and iii) the emergence of VIM-1 in *E. aerogenes*.

- Results demonstrate that *bla*$_{VIM-1}$ has disseminated horizontally as well as vertically.

- These findings are of great concern since carbapenems generally remain the antimicrobial of last resort for treatment of infections caused by multi-drug resistant Gram-negative bacilli.

SELECTED REFERENCES

Clinical and Laboratory Standards Institute. (2006) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A7*. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute. (2006) *Performance standards for antimicrobial susceptibility testing. Document M100-S16*. Wayne, PA: CLSI.

Giakkoupi P, Petrikkos G, Tzouvelekis LS, Tsonas S, Legakis NJ, Vatopoulos AC, WHONET Greece Study Group (2003) Spread of integron-associated VIM-type MßL genes among imipenem-nonsusceptible *Pseudomonas aeruginosa* in Greek hospitals. *J Clin Microbiol* 41, 822-825.

Ikonomidis A, Tokatlidou D, Kristo I, Sofianou D, Tsakaris A, Mantzana P, Pournaras S, Maniatis A (2005) Outbreaks in distinct regions due to a single *Klebsiella pneumoniae* clone carrying a *bla*$_{VIM-1}$ metallo-ß-lactamase gene. *J Clin Microbiol* 43, 5344-5347.

Jones RN, Biedenbach DJ, Sader HS, Fritsche TR, Toleman MA, Walsh TR (2005) Emerging epidemic of metallo-ß-lactamase-mediated resistances. *Diag Microbiol Infect Dis* 51, 77-84.