Emergence of Metallo-ß-Lactamases Among Enterobacteriaceae Causing Bloodstream Infections: Report from the SENTRY Antimicrobial Surveillance Program

C2-1353

ICAAC 2004
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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: Carbapenem resistance has been increasing rapidly among *P. aeruginosa* and *Acinetobacter*, and it is sporadically reported among Enterobacteriaceae (ENT). We evaluated the occurrence and characterized ENT strains with decreased susceptibility (S; MIC ≥ 4 µg/ml) to imipenem (IMI) or meropenem (MER) isolated from BSI in the SENTRY Program (2003) worldwide.
Methods: Among 5723 unique ENT strains evaluated, 50 (0.9%) had IMI or MER MIC ≥ 4 µg/ml. Indole + Proteus or *P. mirabilis* (22 strains with IMI MIC of 4 and MER ≤ 0.5 µg/ml) were excluded and the remaining 28 isolates (1 *Citrobacter*, 2 *E. coli*, 16 *Enterobacter*, 9 *Klebsiella*) were screened for metallo-ß-lactamase (MßL) by disk approximation tests and PCR using generic primers for bla_{IMP}, bla_{VIM} and bla_{SPM}. Isolates were also evaluated for hyperproduction of AmpC by testing IMI with and without BRL42715. Multiple possible clonal isolates from the same medical center were ribotyped/PFGE.
Results: Ten isolates (6 *E. cloacae* [Turkey, sites 68 and 69] and 4 *K. pneumoniae* [Greece, site 62]) had MßL screen test positive results. Among those strains, 6 *E. cloacae* (sites 68 and 69 in Turkey) had positive PCR results for bla_{IMP} and 4 isolates showed a unique ribotype/PFGE pattern (187.2/A). Six other *Enterobacter* isolates, each from a unique medical center, were AmpC hyperproducers showing > 4-fold decrease in IMI MIC with 4 or 8 µg/ml BRL42715. Four *K. pneumoniae* (site 62) had positive PCR results for bla_{VIM}. Four small clusters involving 12 isolates (2-4 strains/cluster) were detected in Brazil, Greece, Israel and Turkey.
Conclusions: Isolates with decreased S to IMI (0.8%) or MER (0.4%) remain extremely rare among ENT, but when they occur, intra-hospital dissemination of these pathogens appears frequent. The emergence of MßL-producing ENT is of grave concern since these enzymes are usually codified by genes located on integrons with great mobility.

INTRODUCTION

Carbapenems are the most active agents for the treatment of infections caused by Gram-negative bacteria due to 1.) the stability of these agents against the majority of ß-lactamases, including extended-spectrum ß-lactamases (ESBLs) and AmpC enzymes, and 2.) their high rate of permeation through the bacterial outer membrane. Resistance to carbapenems has been increasingly reported among *Pseudomonas aeruginosa* and *Acinetobacter* spp. strains in some geographic regions; however, it remains extremely rare among Enterobacteriaceae worldwide.

Carbapenem resistance in *P. aeruginosa* is mainly due to hyperproduction of AmpC enzymes associated with decreased bacterial outer membrane permeability. However, there have been increasing reports, especially from Japan, Brazil, and some European countries of *P. aeruginosa* strains that are resistant to carbapenems due to the production of an acquired metallo-ß-lactamase (MßL).

In the present study, we evaluated the occurrence and characterized Enterobacteriaceae strains with decreased susceptibility (MIC, > 2 µg/ml) to imipenem (IMI) or meropenem (MER) isolated from bloodstream infections in the SENTRY Antimicrobial Surveillance Program worldwide in 2003.

MATERIALS AND METHODS

Bacterial isolates. A total of 6,050 unique Enterobacteriaceae strains were collected from bloodstream infections in 2003 from 68 medical centers located in North America, Europe and Latin America (SENTRY Antimicrobial Surveillance Program). Among those, 5,723 strains were evaluated in the present study. Indole-positive Proteae and *Proteus mirabilis* strains were excluded due to intrinsically high MIC values for imipenem.

Susceptibility testing. The isolates were susceptibility tested by NCCLS reference broth microdilution methods against more than 30 antimicrobial agents, including the carbapenems, imipenem and meropenem. Data was analyzed using NCCLS (2004) categorical interpretive criteria. Quality control tests and colony counts were routinely performed with *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and 35218, and *P. aeruginosa* ATCC 27853.

Screen for hyperproduction of AmpC ß-lactamase. Imipenem MIC results were assessed by agar dilution with three concentrations of the AmpC ß-lactamase inhibitor BRL42715 (2, 4 and 8 µg/ml), as well as without the inhibitor. An isolate was considered an AmpC hyperproducer when imipenem MIC values decreased by ≥ three log_2 dilutions in the presence of any concentration of BRL42715 when compared to the MIC result without the inhibitor.

Metallo-ß-lactamase (MßL) screening test. Enterobacteriaceae isolates with elevated MIC results when tested against imipenem and/or meropenem (> 2 µg/ml) were screened by a double-disk synergy test using EDTA and 2-mercaptopropionic acid (2-MPA) as MßL inhibitors and imipenem, meropenem and ceftazidime as substrate ß-lactams.

Molecular typing. Multiple isolates from the same medical center were molecularly typed by ribotyping and pulsed-field gel electrophoresis (PFGE). Ribotyping was performed by using automated Riboprinter Microbial characterization system (Dupont Qualicon, Inc.). In short, genomic DNA was isolated and digested using EcoRI. DNA was separated by agarose gel electrophoresis. Southern hybridization using probes derived from *E. coli* rRNA operon created characteristic band patterns. These patterns were matched to pre-existing patterns by computer analysis and those with ≥ 93% identity were assigned to the same ribogroup. Isolates with matching riboprint patterns were further evaluated for clonality by PFGE.

Detection of MßL Genes by PCR Amplifications. Isolates were screened for presence of metallo-ß-lactamase by attempts to amplify IMP-, VIM- and SPM-1 determinants using following primers:
bla_{IMP} (5'CTA CCG CAG CAG AGT CTT TG3'; 5' AAC CAG TTT TGC CTT ACC AT3');
bla_{VIM} (5' AGT GGT GAG TAT CCG ACA G3'; 5' ATG AAA GTG CGT GGA GAC GAC3');
bla_{SPM} (5' CCT ACA ATC TAA CGG CGA CC3'; 5' TCG CCG TGT CCA GGTA ATA AC3').
Reactions were set up in 25 µl volumes using Platinum® PCR Supermix (Invitrogen, Inc.), and boiled cell suspensions as template. The cycling parameters for each primer pair were those described previously. PCR products were visualized by electrophoresis on 1.0% agarose gels stained with ethidium bromide.

Table 1. In vitro activity of select agents tested against 5,723 Enterobacteriaceae collected in 2003 from bloodstream infections in North America, Latin America and Europe.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% susceptible	% resistant
Citrobacter spp. (120)					
Imipenem	≤0.5	1	≤0.5-4	100.0	0.0
Meropenem	≤0.06	≤0.06	≤0.06-4	100.0	0.0
Ceftriaxone	≤0.25	32	≤0.25->32	85.0	6.7
Ceftazidime	≤1	>16	≤1->16	83.3	13.3
Piperacillin/Tazobactam	2	64	0.5->64	84.2	7.5
Aztreonam	≤0.12	>16	≤0.12->16	84.2	13.3
Ciprofloxacin	≤0.03	2	≤0.03->4	87.5	10.0
Gentamicin	≤2	≤2	≤2->8	93.3	5.0
E. coli (3,125)					
Imipenem	≤0.5	≤0.5	≤0.5-8	99.9	0.0
Meropenem	≤0.06	≤0.06	≤0.06-8	99.9	0.0
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	96.2	3.2
Ceftazidime	≤1	≤1	≤1->16	96.7	1.7
Piperacillin/Tazobactam	2	4	≤0.5->64	96.6	1.7
Aztreonam	≤0.12	0.25	≤0.12->16	96.5	2.8
Ciprofloxacin	≤0.03	>4	≤0.03->4	83.5	16.4
Gentamicin	≤2	≤2	≤2->8	92.7	6.6
Enterobacter spp. (716)					
Imipenem	≤0.5	1	≤0.5->8	99.6	0.1
Meropenem	≤0.06	0.12	≤0.06->8	99.3	0.1
Ceftriaxone	≤0.25	>32	≤0.25->32	77.0	15.2
Ceftazidime	≤1	>16	≤1->16	75.3	21.2
Piperacillin/Tazobactam	2	64	≤0.5->64	80.2	8.8
Aztreonam	≤0.12	>16	≤0.12->16	76.1	18.2
Ciprofloxacin	≤0.03	4	≤0.03->4	86.6	11.6
Gentamicin	≤2	>8	≤2->8	88.5	10.1
Indole positive Proteae (143)					
Imipenem	2	4	≤0.5-4	100.0	0.0
Meropenem	≤0.06	0.12	≤0.06-0.5	100.0	0.0
Ceftriaxone	≤0.25	4	≤0.25->32	94.4	2.8
Ceftazidime	≤1	8	≤1->16	90.2	4.2
Piperacillin/Tazobactam	≤0.5	4	≤0.5-64	97.9	0.0
Aztreonam	≤0.12	2	≤0.12->16	97.9	1.4
Ciprofloxacin	≤0.03	>4	≤0.03->4	81.8	14.7
Gentamicin	≤2	>8	≤2->8	86.0	10.5
Klebsiella spp. (1,273)					
Imipenem	≤0.5	≤0.5	≤0.5->8	99.7	0.3
Meropenem	≤0.06	≤0.06	≤0.06->16	99.4	0.3
Ceftriaxone	≤0.25	32	≤0.25->32	85.6	9.2
Ceftazidime	≤1	>16	≤1->16	87.4	10.2
Piperacillin/Tazobactam	2	32	≤0.5->64	88.9	9.0
Aztreonam	≤0.12	>16	≤0.12->16	85.3	12.9
Ciprofloxacin	≤0.03	2	≤0.03->4	89.2	9.3
Gentamicin	≤2	>8	≤2->8	86.6	12.0
Salmonella spp. (89)					
Imipenem	≤0.5	≤0.5	≤0.5-1	100.0	0.0
Meropenem	≤0.06	≤0.06	≤0.06	100.0	0.0
Ceftriaxone	≤0.25	≤0.25	≤0.25-16	98.9	0.0
Ceftazidime	≤1	≤1	≤1-8	100.0	0.0
Piperacillin/Tazobactam	4	8	≤0.5-8	100.0	0.0
Aztreonam	≤0.12	≤0.12	≤0.12->16	98.9	1.1
Ciprofloxacin	≤0.03	0.12	≤0.03-1	100.0	0.0
Gentamicin	≤2	≤2	≤2->8	97.8	2.2
Serratia spp. (257)					
Imipenem	≤0.5	1	≤0.5-2	100.0	0.0
Meropenem	≤0.06	≤0.06	≤0.06-1	100.0	0.0
Ceftriaxone	≤0.25	8	≤0.25->32	90.3	4.3
Ceftazidime	≤1	≤1	≤1->16	96.1	2.7
Piperacillin/Tazobactam	2	32	0.5->64	88.3	1.6
Aztreonam	≤0.12	4	≤0.12->16	94.9	4.7
Ciprofloxacin	0.06	1	≤0.03->4	91.1	5.1
Gentamicin	≤2	4	≤2->8	90.3	8.6
All Enterics (5,723)					
Imipenem	≤0.5	≤0.5	≤0.5->8	99.8	0.1
Meropenem	≤0.06	≤0.06	≤0.06->8	99.8	0.1
Ceftriaxone	≤0.25	8	≤0.25->32	90.9	6.1
Ceftazidime	≤1	4	≤1->16	91.5	6.4
Piperacillin/Tazobactam	2	16	≤0.5->64	92.3	4.3
Aztreonam	≤0.12	4	≤0.12->16	91.2	7.2
Ciprofloxacin	≤0.03	>4	≤0.03->4	85.8	13.3
Gentamicin	≤2	4	≤2->8	90.6	8.3

RESULTS

- Reduced susceptibility to the carbapenems, imipenem and meropenem, was extremely rare among the Enterobacteriaceae isolates collected from bloodstream infections by the SENTRY Program in 2003 (Table 1). Among 5,723 isolates evaluated, only 28 isolates (0.5%) showed MIC results of > 2 µg/ml for imipenem or meropenem (Table 2).
- Both carbapenems (imipenem and meropenem) were active against 99.8% of the Enterobacteriaceae isolates at the NCCLS susceptible breakpoint (≤4 µg/ml). Isolates resistant to these carbapenems (MIC, ≥ 16 µg/ml) were detected only among *Enterobacter* spp. (0.1%) and *Klebsiella* spp. (0.3%; Table 1).
- The third-generation cephalosporins, ceftriaxone and ceftazidime, were active against 90.9 and 91.5% of the Enterobacteriaceae strains, respectively. Susceptibility rates to these ß-lactams varied from 98.9 to 100.0% for *Salmonella* spp. and 77.0 to 75.3% for *Enterobacter* spp., respectively.
- The highest rates of resistance were observed for ciprofloxacin (13.3%) and gentamicin (8.3%).
- Four clusters of Enterobacteriaceae strains with reduced susceptibility to carbapenems were identified in Turkey (four *E. cloacae* isolates), Greece (four *K. pneumoniae*), Israel (two *K. pneumoniae*) and Brazil (two *K. pneumoniae*).

Table 2. Summary of tests performed to evaluate resistance mechanisms and clonality of Enterobacteriaceae isolates with reduced susceptibility (MIC, > 2 µg/ml) to imipenem or meropenem (SENTRY Program, 2003).

Continent	Medical center	Bank #	Organism[a]	IMP/MER[b] MIC	MßL screen	MßL PCR[c]	Ribotype	PFGE[d]
North America	15	9679	CF	4/4	Negative	ND[e]	ND	ND
	15	11262[f]	ECL	2/4	Negative	ND	ND	ND
	15	13717	KPN	>8/>8	Negative	ND	520.4	ND
	30	7133	ECL	4/2	Negative	ND	ND	ND
Latin America	46	9374	KPN	2/8	Negative	ND	520.4	KPN46A
	46	10688	KPN	2/8	Negative	ND	520.4	KPN46A
Europe	58	14474[f]	EAE	8/-	Negative	ND	ND	ND
	61	1259[f]	EBS	4/2	Negative	ND	ND	ND
	61	5563[f]	EAE	8/2	Negative	ND	ND	ND
	62	1795	KPN	>8/>8	Positive	VIM-1	211.6	KPN62D
	62	2634	KPN	>8/>8	Positive	VIM-1	211.6	KPN62D
	62	2638	KPN	4/8	Positive	VIM-1	211.5	KPN62D
	62	2639	KPN	>8/>8	Positive	VIM-1	211.6	KPN62D
	63	3124	KPN	2/4	Positive	Negative	520.4	KPN63B
	63	3130	KPN	2/8	Negative	ND	520.4	KPN63B
	63	5787	EC	8/8	Negative	ND	185.5	ND
	63	3123	EC	8/>8	Negative	ND	215.2	ND
	68	1467	ECL	4/4	Negative	ND	213.2	ND
	68	10526	ECL	4/4	Positive	IMP-1	213.5	ND
	69	7329	ECL	4/8	Positive	IMP-1	187.2	ECL69A
	69	7338	ECL	2/8	Positive	IMP-1	187.2	ECL69A
	69	7374	ECL	4/8	Positive	IMP-1	187.2	ECL69A
	69	7592	ECL	4/>8	Positive	IMP-1	187.2	ECL69A
	69	15213	ECL	4/4	Positive	IMP-1	213.6	ND
	86	6303[f]	ECL	4/4	Negative	ND	ND	ND
	90	9627	ECL	4/-	Negative	ND	107.1	ND
	90	9626	ECL	-/8	Negative	ND	213.3	ND
	113	16274[f]	EAE	>8/8	Positive	Negative	ND	ND

a. CF = *Citrobacter freundii*; ECL = *Enterobacter cloacae*; KPN = *Klebsiella pneumoniae*; EAE = *Enterobacter aerogenes*; EBS = *Enterobacter* spp., EC = *E. coli*.
b. IMP/MER = Imipenem/meropenem.
c. Only isolates with positive MßL screen test were evaluated by PCR.
d. PFGE was performed only on isolates with identical ribotypes.
e. ND = Not done.
f. Isolates with positive screen test for hyperproduction of AmpC.

- Only six isolates showed a positive screen test for hyperproduction of AmpC ß-lactamases.
- Twelve of 28 isolates with elevated carbapenem MICs showed a positive MßL screening test. Seven *Enterobacter* spp. (six from Turkey and one from Germany) and five *K. pneumoniae* isolates (four from Greece and one from Israel) showed strong positive reactions to more than one substrate-inhibitor combination.
- Four clonal *E. cloacae* isolates (site 69) as well as two unrelated isolates (sites 68 and 69) from Turkey showed PCR products using IMP-1 primers (Table 2).
- Four clonal *K. pneumoniae* isolates from Greece (site 62) showed PCR products with VIM-1 primers (Table 2).
- Two isolates (*Enterobacter aerogenes* and *K. pneumoniae*) had positive MßL screen test results and negative PCR results for all primer sets tested (Table 2).

CONCLUSIONS

- Enterobacteriaceae isolates generally remain very susceptible to carbapenems (99.8%).
- Carbapenem resistance mediated by metallo-ß-lactamases is emerging and clinical microbiology laboratories should become more vigilant at detecting the isolates with diminished susceptibility to carbapenems.
- Clonality appears to play a major role in dissemination of MßL-producing Enterobacteriaceae and non-fermentative Gram-negative bacilli (not shown).
- Other mechanisms such as hyperproduction of Ambler class C enzymes and serine carbapenemases as well as changes in porins are also responsible for changing carbapenem susceptibility profiles among Enterobacteriaceae.

SELECTED REFERENCES

Giakkoupi P, Xanthaki A, Kanelopoulou M, Vlahaki A, Miriagou V, Kontou S, Papafraggas E, Malamou-Lada H, Tzouvelekis LS, Legakis NJ, Vatopoulos AC. (2003). VIM-1 metallo-ß-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. *Journal of Clinical Microbiology* 41:3892-3896.

Luzzaro F, Docquier J-D, Colinon C, Endimiani A, Lombardi G, Amicosante G, Rossolini GM, Toniolo A. (2004). Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo-ß-lactamase encoded by a conjugative plasmid. *Antimicrobial Agents and Chemotherapy* 48:648-650.

Mushtaq S, Ge Y, Livermore DM. (2004). Comparative activities of doripenem versus isolates, mutants, and transconjugants of Enterobacteriaceae and *Acinetobacter* spp. with characterized ß-lactamases. *Antimicrobial Agents and Chemotherapy* 48:1313-1319.

Nordmann P, Poirel L. (2002). Emerging carbapenemases in Gram-negative aerobes. *Clinical Microbiology and Infection* 8:321-331.

Pfaller MA, Hollis RJ, Sader HS. (1992). PFGE analysis of chromosomal restriction fragments. In: *Isenberg HD. Clinical Microbiology Procedures Handbook (Supplement 1)*. Washington, ASM Press, pp. 10.5.c.1-10.5.c.11.