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Increasing prevalence of KPC-producers as an emerging resistance mechanism among carbapenem non-susceptible isolates: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: The dissemination of metallo-ß-lactamase (MßL) and serine-carbapenemase (S-Carb)-producing Enterobacteriaceae jeopardize the clinical utility of carbapenems. The prevalence of MßL and S-Carb is still low, but rapidly increasing. We evaluated the occurrence of MßL (IMPs, VIMs) and S-Carb (KPC, IMI/NMC-A, SME, and OXA-48) in a collection of 102 carbapenem-non-susceptible *E. coli* (EC) and *K. pneumoniae* (KPN) from the SENTRY Program.

Methods: 5,068 EC and KPN isolates were collected during 2007 from Europe, North and Latin America. Isolates were tested by the CLSI broth microdilution method. Strains showing elevated carbapenem MIC values (≥2 µg/ml for imipenem, meropenem or ertapenem) were tested with multiplex PCR approaches for MBL and S-Carb. Amplicons were sequenced and analyzed.

Results: A total of 102 (2% of isolates overall, 7 EC, 95 KPN) isolates showing increased carbapenem MIC values were evaluated. Fifty (49%) isolates were found to harbor bla_{KPC} (49 KPN, 1 EC). These isolates were from the USA (40, mainly east coast), Israel (9) along with one KPC-2-producer from Argentina. KPC-3 was more prevalent than KPC-2. VIM-1 was detected in 6 (6%) KPN; 5 from Italy and 1 from Turkey. IMP-1 was found in 1 isolate from Turkey. OXA-48 was discovered in 2 Turkish KPN. KPC-producing isolates were highly resistant (MIC, ≥8 µg/ml) to all carbapenems, while MIC values for these agents varied considerably among other enzyme-producers.

Conclusions: MßL are still a problem in certain geographic regions, mainly as causes of local outbreaks; however, KPC-producing isolates are becoming of greatest concern in the USA and are, according to recent studies (including ours), rapidly disseminating worldwide. This is the first report of KPC-2 in Argentina, and of multiple carbapenemases being detected in Turkey.

INTRODUCTION

Resistance to carbapenems in Enterobacteriaceae can be caused by over-production of Amp-C ß-lactamases associated with loss of outer membrane porins and/or over-expression of efflux pumps or by production of ß-lactamases with significant hydrolysis activity against these compounds. These latter carbapenemases can be divided in two groups according to the functional requirements and structure of their active site, metallo-ß-lactamases (MßL; Ambler class B) and serine carbapenemases (class A or Bush class 2f).

The genes encoding most of these carbapenemases reside on plasmids or transposons carrying additional resistance genes to other antimicrobial classes. These transferable structures can readily be acquired by Gram-negative pathogens, facilitating the dissemination of these potent resistance mechanisms and also, in many cases, conferring a multidrug resistant (MDR) profile.

In this study, we evaluated the presence of MßL and serine-carbapenemase encoding genes among 102 carbapenem-non-susceptible *Escherichia coli* and *Klebsiella pneumoniae* collected during the SENTRY Antimicrobial Surveillance Program in 2007.

MATERIALS AND METHODS

Bacterial isolates: During 2007, 5,068 *E. coli* and *K. pneumoniae* isolates were collected from medical centers located in Europe, North and Latin America. These isolates were collected from bloodstream, respiratory tract and skin and soft tissue infections 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 use of Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2006) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by Clinical Laboratory Standard Institute (CLSI, 2008) criteria. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were within the published ranges.

Screening for carbapenemase encoding genes: All isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/ml) were tested with the Modified Hodge test (MHT) using imipenem and meropenem disks. Isolates were screened for the presence of several carbapenemase-encoding genes by multiplex PCR reactions. Generic primers designed for bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SME}, bla_{GES} variants and for bla_{IMI}, bla_{NMC-A}, bla_{OXA-48} were combined in two amplification reactions described in Table 1.

Gene sequencing: Amplicons were sequenced on both strands. Results were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin) and compared to available sequences through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- Among 5,068 isolates collected during the SENTRY Program in 2007, 95 *K. pneumoniae* and 7 *E. coli* (102 total; 2% of isolates overall) showed increased carbapenem MIC values (MIC, ≥2 µg/ml).

Table 1. Oligonucleotides used in two multiplex reactions for the detection of carbapenemase encoding genes.

Primer name	Sequence (5' to 3')	Product size
Reaction 1		
KPC-GenF	GTC ACT GTA TCG CCG TCT AG	859
KPC-GenR	CAA TCC CTC GAG CGC GAG TC	
VIM-GenF	GTT TGG TCG CAT ATC GCA AC	382
VIM-GenR	AAT GCG CAG CAC CAG GAT AG	
IMP-GenF	GAA TAG RRT GGC TTA AYT CTC	188
IMP-GenR	CCA AAC YAC TAS GTT ATC	
Reaction 2		
GESGenF	GCG CTT CAT TCA CGC ACT ATT ACT G	860
GESGenR	TTC TAC GGC CGA TAG TTT CG	
SME-1-F	GTG TTT GTT TAG CTT TGT CGG C	804
SME-1-R	GCA ATA CGT GAT GCT TCC GC	
OXA-48F	TGG TGG CAT CGA TTA TCG GA	693
OXA-48R	GCA TAT CCA TAT TCA TCG CA	
IMI/NMC-A-F	GAA CGA TTT CCA TTA TGT AG	533/517
IMI/NMC-A-R	GCA CCG CAA CTA CCA G	

- KPC-encoding genes were detected on 50 isolates (49% of the carbapenem non-susceptible strains). These isolates were dominantly distributed in four USA states (40 strains; NY, NJ, WA and WI) and Israel (9; Table 2).
- One *K. pneumoniae* strain carrying bla_{KPC-2} was found in Buenos Aires, Argentina. This isolate showed high levels of carbapenem resistance, but was susceptible to aminoglycosides, fluoroquinolones, tigecycline, tetracycline and trimethoprim/sulfamethoxazole.
- bla_{VIM-1} was detected in six *K. pneumoniae* isolates: five from Genoa, Italy and one from Ankara, Turkey (Table 2).
- Three additional carbapenemase-producing *K. pneumoniae* isolates were detected in Ankara, Turkey: one carrying bla_{IMP-1} and two bla_{OXA-48} (Table 2).
- MHT results showed good correlation with the presence of carbapenem-hydrolyzing ß-lactamases among *K. pneumoniae* and *E. coli* strains. However, two isolates carrying bla_{KPC-3} and two harboring bla_{VIM-1} had repeatedly negative MHT results (Table 2).
- Carbapenemase-producing strains accounted for the majority of carbapenem-non-susceptible isolates detected in the USA and Europe (40/42 and 18/21 respectively). In contrast, only one carbapenemase-producing strain was detected among 21 carbapenem-non-susceptible isolates from Latin America (Figure 1).

Table 2. Occurrence of carbapenemase enzymes among isolates collected in medical centers in the USA, Europe and Latin America during the SENTRY Antimicrobial Surveillance Program (2007).

Carbapenemase encoding gene	Bacterial species	Countries	MHT[a] pos	MHT[a] neg
KPC-2/-3 (23/27)[b]	*K. pneumoniae* (50)	USA (40), Israel (9), Argentina (1)	48	2
	E. coli (1)	USA (1)	1	0
OXA-48	*K. pneumoniae* (2)	Turkey (2)	2	0
IMP-1	*K. pneumoniae* (1)	Turkey (1)	1	0
VIM-1	*K. pneumoniae* (6)	Italy (5), Turkey (1)	4	2

a. MHT: Modified Hodge test.
b. Number of each KPC variant.

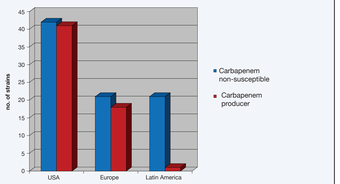
- Overall, KPC-producing isolates showed higher resistance levels against the carbapenems when compared to isolates carrying other resistance mechanisms (Table 3).
- Three isolates carrying bla_{GES} were detected in Mexico. These strains had negative MHT and sequencing results showed that these isolates harbored bla_{GES-1} that has poor activity against carbapenems.

Table 3. Occurrence of carbapenem MIC values for *K. pneumoniae* and *E. coli* isolates collected in the SENTRY Antimicrobial Surveillance Program (2007).

Organism group (no. of isolates)/ Antimicrobial agents	Occurrence of isolates at MIC (µg/ml) ≤0.012	0.25	0.5	1	2	4	8	≥16
KPC-producers (50)								
Doripenem	0	1	1	1	0	8	19	20
Ertapenem	1	0	0	0	1	2	0	46
Imipenem	0	0	1	1	0	0	5	43
Meropenem	1	0	1	2	0	0	3	43
Other mechanisms[a] (52)								
Doripenem	9	3	8	7	8	6	5	6
Ertapenem	6	1	4	2	5	10	7	17
Imipenem	2	5	6	5	17	10	6	2
Meropenem	10	3	5	4	8	9	9	4
Wild-type population[b] (4,966)								
Doripenem	4,943	22	0	1	0	0	0	0
Ertapenem	4,833	68	47	18	0	0	0	0
Imipenem	2,321	2,341	245	59	0	0	0	0
Meropenem	4,945	15	6	0	0	0	0	0

a. Carbapenem non-susceptible strains harboring other resistance mechanisms.
b. Isolates with carbapenem MIC values of ≤1 µg/ml.

Figure 1. Frequency of occurrence of carbapenem non-susceptible and carbapenemase-producing *K. pneumoniae* and *E. coli* collected in the USA, Europe and Latin America as part of the SENTRY Antimicrobial Surveillance Program (2007).



CONCLUSIONS

- Carbapenemase production still remains rare among Enterobacteriaceae in Latin America. Nonetheless, MßL-producing (SPM-1 and others) non-fermentative strains have been reported in most medical sites participating in the SENTRY Program located in this geographic region.
- Four distinct carbapenemases were detected among *K. pneumoniae* strains collected in one Turkish medical center. This high diversity of carbapenemase enzymes seems to be unique to this country.
- According to this study, KPC ß-lactamases are emerging as the most prevalent carbapenemase among *K. pneumoniae* isolates. KPC-producing strains have become endemic on the East Coast USA, and its further dissemination could jeopardize the clinical use of carbapenems in this area.

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