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Carbapenemase Production in United States (USA) Hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2009)

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: Recent worldwide reports of NDM-1 metallo-beta-lactamase (MBL)-producing strains increased the awareness for the spread of this resistance mechanism that has also been detected in the USA. We evaluated the presence of MBLs, including NDM-1 and serine-carbapenemases (CARBase) among Enterobacteriaceae from USA hospitals submitted via the SENTRY Antimicrobial Surveillance Program in 2009.

Methods: 3,414 Enterobacteriaceae clinical strains collected in 42 USA medical sites were susceptibility (S) tested by CLSI methods (M07-A8 and M100-S21). CARBase production was assessed in all strains meeting the CLSI screening criteria for CARBase (imipenem and/or meropenem MIC, ≥2 µg/mL) and current CLSI breakpoints for non-S were evaluated. Strains were subjected to Modified Hodge Test (MHT) and PCR was performed in multiplex reactions for genes encoding IMP, VIM, NDM-1, KPC, SME, IMI, NMC-A, GES, OXA-48. Selected amplicons were sequenced on both strands.

Results: 69 (2.0%) of the clinical Enterobacteriaceae showed elevated CARB MIC values (50 *K. pneumoniae* [KPN], 8 *E. cloacae* [ECL], 5 *S. marcescens* [SM], 4 *E. coli* [EC], and 2 *K. oxytoca* [KOX]). 59 (85.5% of CARB-R and 1.7% overall) of 69 strains were positive for CARBase encoding genes: 58 KPC-2 and -3 and 1 SME-2 in SM (Indianapolis, IN). KPC harboring species were: 45 KPN, 8 ECL, 2 SM, 2 KOX and 1 EC. CARB MIC values for CARBase-producers ranged from 2 to >8 µg/mL for ertapenem, imipenem and meropenem. Only one KPC-producing KOX had a doripenem MIC value at 1 µg/mL. 42 (72.4%) of KPC-producing strains were detected in (states): NY and NJ; but were also noted in CT, KY, IN, MA, OH and VA. Only one CARBase-producer (1.7%; SME-2) was negative for MHT. No MBLs were observed.

Conclusions: KPC production (mainly in NY and adjacent states) are currently responsible for the CARBase rates. Although bla_{NDM-1} was not detected in this collection, it merits monitoring since this resistance gene has quickly spreading throughout the world. CLSI-revised breakpoints for carbapenem compounds facilitated the accurate detection of CARBase-producing strains in the USA.

INTRODUCTION

Detection and surveillance of carbapenemase-producing organisms have become matters of major importance for the selection of appropriate therapeutic schemes and the implementation of infection control interventions. Acquired carbapenemases belonging to either molecular class B (metallo-ß-lactamases, MßL) or molecular classes A and D (serine carbapenemases and oxacillinases) have emerged globally and represent serious public health challenges.

The MßL NDM-1 was recently described and this enzyme appears to be spreading rapidly. Isolates producing NDM-1 have been detected in several countries, including in the United States (USA), and among various Enterobacteriaceae species and *Acinetobacter* spp. NDM-1 demonstrates a broad-spectrum of hydrolytic activity against ß-lactam agents that includes the carbapenems; and as with other MßLs, is not inhibited by ß-lactamase inhibitors currently marketed or in clinical development for clinical use. Furthermore, the detection of NDM-1-producing strains appears to be troublesome and cases of false-negative Modified Hodge Test (MHT) results and MIC values at or below the current carbapenem breakpoints have been described.

In this study, we report the prevalence of carbapenemases, among Enterobacteriaceae strains collected in USA medical sites as part of the SENTRY Antimicrobial Surveillance Program (2009).

MATERIALS AND METHODS

Bacterial isolates: A total of 3,414 Enterobacteriaceae isolates were collected from 42 medical centers located in nine USA Census Regions during 2009. These isolates were recovered 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 2 Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2009) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results followed the M100-S21 document (CLSI, 2011). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were within the CLSI 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 also screened for the presence of bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{SME}, bla_{GES}, bla_{IMI}, bla_{NMC-A}, bla_{OXA-48} variants by multiplex PCR reactions. 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 3,414 Enterobacteriaceae strains collected in USA medical sites during 2009, 69 (2.0%) displayed elevated MIC values for imipenem and/or merorpenem (≥2 µg/mL) and were selected for molecular evaluation.
- Carbapenem-resistant strains belonged to five species: *Klebsiella pneumoniae* (50), *Enterobacter cloacae* (8), *Serratia marcescens* (5), *E. coli* (4), and *Klebsiella oxytoca* (2) and were recovered from patients hospitalized in fourteen hospitals, ten USA states.
- Molecular screening showed that 58 strains carried bla_{KPC}. One *S. marcescens* strain harbored bla_{SME-2}. All strains were negative to MßL-encoding genes, including bla_{NDM-1}, or genes encoding other serine-carbapenemases.

Table 1. Distribution of 58 KPC-producing Enterobacteriaceae strains collected during 2009 among eight USA states.

State (no. of isolates)	Organism (no. of isolates)
Connecticut (1)	*S. marcescens* (1)
Indiana (1)	*S. marcescens* (1)
Kentucky (1)	*E. cloacae* (1)
Massachusetts (1)	*K. pneumoniae* (1)
New Jersey (12)	*K. pneumoniae* (10)
	E. cloacae (2)
New York (30)	*K. pneumoniae* (27)
	E. cloacae (2)
	E. coli (1)
Ohio (6)	*K. pneumoniae* (5)
	E. cloacae (1)
Virginia (6)	*E. cloacae* (2)
	K. oxytoca (2)
	K. pneumoniae (2)

- KPC-producers were collected in hospitals located in eight states (Table 1). Isolates belonged to five species tested (*K. pneumoniae* [45], *E. cloacae* [8], *S. marcescens* [2], *K. oxytoca* [2], and *E. coli* [1]).
- All strains carrying KPC-encoding genes were positive by MHT using imipenem and meropenem substrates. The SME-2-producing *S. marcescens* had a negative MHT result with both substrates. No MHT false-positive results were observed.
- KPC-producing strains displayed MIC values for imipenem, meropenem and ertapenem that would be categorized as non-susceptible using current CLSI non-susceptible breakpoints (M100-S21). One (1.7%) KPC-positive *K. oxytoca* had a doripenem MIC of 1 µg/mL that would be considered susceptible according to the current breakpoint criteria.

Table 2. MIC distributions for carbapenem-resistant (R; 69 strains) and KPC-producing (58 strains) Enterobacteriaceae strains from USA medical centers tested against four carbapenems during the SENTRY Antimicrobial Surveillance Program (2010). Carbapenem non-susceptible MIC values according to the CLSI document M100-S21 are highlighted in blue.

	No. of isolates at MIC (µg/mL; cumulative %):								
	≤0.06	0.12	0.25	0.5	1	2	4	8	>8
Imipenem									
Carbapenem-R	-	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.9)	7 (13.0)	6 (21.7)	17 (46.4)	37 (100.0)
KPC positive	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	4 (8.6)	17 (37.9)	36 (100.0)
Meropenem									
Carbapenem-R	-	5 (7.2)	0 (7.2)	0 (7.2)	0 (7.2)	2 (10.1)	9 (23.2)	32 (46.4)	37 (100.0)
KPC positive	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.4)	7 (12.1)	14 (39.7)	35 (100.0)
Ertapenem									
Carbapenem-R	5 (7.3)	0 (7.3)	0 (7.3)	0 (7.3)	0 (7.3)	0 (7.3)	3 (11.6)	10 (26.1)	51 (100.0)
KPC positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.2)	9 (20.7)	46 (100.0)
Doripenem									
Carbapenem-R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.7)	(17.4)	34 (49.3)	52 (75.4)	69 (100.0)
KPC positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	5 (10.3)	20 (44.8)	18 (75.7)	14 (100.0)

CONCLUSIONS

- KPC-producing isolates represented 84.0% of the carbapenem non-susceptible strains collected in USA hospitals during the SENTRY Program (2009). These strains were mainly recovered from hospitals located in New York and adjacent states.
- CLSI breakpoint criteria for carbapenems have been recently revised and these results confirm that all KPC-producing strains collected in USA medical centers would be considered non-susceptible when testing imipenem, meropenem and ertapenem.

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