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Acquired Carbapenemase-encoding Genes Among Gram-negative Isolates in USA Medical Centers: Report from the MYSTIC Program (2007)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

ABSTRACT

Objective: To evaluate the occurrence of acquired carbapenemase-encoding genes among Gram-negative patient isolates recovered from USA medical centers (MC) in the 2007 MYSTIC Program.

Methods: 1,392 Enterobacteriaceae (ENT), 133 *Acinetobacter* spp. (ASP) and 465 *Pseudomonas* spp. (PSP) clinical isolates from 15 USA MC were susceptibility tested by CLSI reference broth microdilution method. ENT and PSP isolates showing MIC values ≥2 mg/L and ≥16 mg/L, respectively, for imipenem and meropenem were screened for KPC- and metallo-ß-lactamase (MßL)-encoding genes. ASP isolates showing MIC values ≥16 mg/L for imipenem and meropenem were screened for MßL- and OXA-encoding genes. Presence of IS*Aba1* upstream of the bla_{OXA-51} and clonality were also investigated by PCR and PFGE, respectively. Additionally, ASP isolates from previous MYSTIC Program years were tested for OXA genes.

Results: 42 (3.0% of the total) ENT isolates were screened and 35 (2.5%) isolates from MC located in New York (24/35; 68.6%), New Jersey (10/35; 28.6%) and Ohio (1/35; 2.9%) harbored bla_{KPC}. This gene was found in *C. freundii* (2), *E. coli* (1), *E. cloacae* (3), *K. oxytoca* (1) and *K. pneumoniae* (28). Among 133 ASP, 50 (37.6%) isolates were screened and bla_{OXA-23} was detected in 10 isolates from MC in New York (3), Kentucky (3), Arkansas (2) and Colorado (2), while bla_{OXA-24} was detected in 5 isolates from New Jersey. IS*Aba1* was associated with bla_{OXA-51} in 78.8% of the cases and clonal dissemination of bla_{OXA}–carrying ASP within MC was noted. In the retrospective sample of ASP collected from 1999 to 2006, 42 (6.8%) isolates were screened for bla_{OXA}. bla_{OXA-23} was observed in 2 isolates from Tennessee (2004) and one isolate each from Kentucky and Washington (2005). Among the 36 (7.7%) PSP isolates screened, no carbapenemases were detected. MßL-encoding genes were not observed.

Conclusions: Although the results presented here document an alarming presence of bla_{KPC}, the rate (2.5%) was lower when compared with the previous MYSTIC year (4.5%; P = 0.0063; OR = 1.80, CI 95% 1.15-2.82). However, the KPC gene has spread among several different ENT species, while it was detected only in *Klebsiella* spp. in the previous year. bla_{OXA} genes have been detected in several MYSTIC Program MC within the USA; first detected in 2004. bla_{OXA} genes were only observed in consecutive years in Kentucky, suggesting a sporadic appearance in ASP, overall. MßL-encoding genes are still quite rare in the MYSTIC Program.

INTRODUCTION

ß-lactam antimicrobial agents that once were highly active against most Gram-negative pathogens are now less effective due to emerging resistance mechanisms harbored by Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Among ß-lactam resistance mechanisms, ß-lactamases are most worrisome due to their ability to rapidly disseminate, since genes encoding most ß-lactamases reside on mobile plasmids or transposons.

Several ß-lactamase groups are of special concern because of their epidemiologic impact or possible limitations on contemporary antimicrobial therapy. Acquired carbapenemases (i.e. metallo-ß-lactamases [MßLs], oxacillinases and serine-carbapenemases) can have a significant adverse impact on the clinical utility of carbapenems, and carbapenemase-producing isolates are usually non-susceptible to the vast majority of ß-lactam agents.

The main objective of this study was to evaluate the occurrence of acquired carbapenemase-encoding genes among Gram-negative isolates recovered from United States (USA) medical centers participating in the 2007 MYSTIC Program. Additionally, *Acinetobacter* spp. from previous MYSTIC Program years (1999-2006) were screened for acquired oxacillinases.

MATERIALS AND METHODS

Bacterial isolates. A total of 1,392 Enterobacteriaceae, 133 *Acinetobacter* spp. and 465 *Pseudomonas* spp. clinical isolates were consecutively collected from patients in 15 medical centers geographically dispersed across the USA. These strains were recovered from hospitalized patients with each participating institution contributing 200 isolates including Gram-positive and -negative species. Only one isolate per patient from a documented infection was included in the MYSTIC Program 2007 study. Species identification was confirmed by standard biochemical tests and use of the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS; M7-A7, 2006). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for all antimicrobials were those found in M100-S18 (CLSI, 2008). Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. All QC results were within ranges as published in CLSI documents.

Genotypic detection of carbapenemase-encoding genes. Enterobacteriaceae and *Pseudomonas* spp. isolates showing MIC values ≥2 mg/L and ≥16 mg/L, respectively, for imipenem and meropenem were screened for KPC- and MßL-encoding genes. Generic primers were designed to amplify all KPC- (KPC-1 through -5) and MßL- (IMP-like, VIM-like, SPM-1, SIM-1) encoding genes available in the GenBank using a multiplex PCR approach. A similar approach was also used to amplify other serine-carbapenemases (IMI-like, NMC-A and SME-like) among KPC-negative isolates.

Acinetobacter spp. isolates showing MIC values ≥16 mg/L for imipenem and meropenem were screened for MßL- and OXA- (OXA-23-, OXA-24-, OXA-51- and OXA-58-clusters) encoding genes. Upstream DNA sequences of bla_{OXA-51} were studied by PCR using primers targeting IS*Aba1*, *2* or *3*. Additionally, 42 *Acinetobacter* spp. isolates exhibiting MIC values ≥16 mg/L for imipenem and meropenem from prior MYSTIC Program years (1999-2006) were tested for OXA genes.

Molecular typing and sequencing analysis. *Acinetobacter* spp. chromosomal DNA was subjected to pulsed-field gel electrophoresis (PFGE) after digestion with SmaI. PCR amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

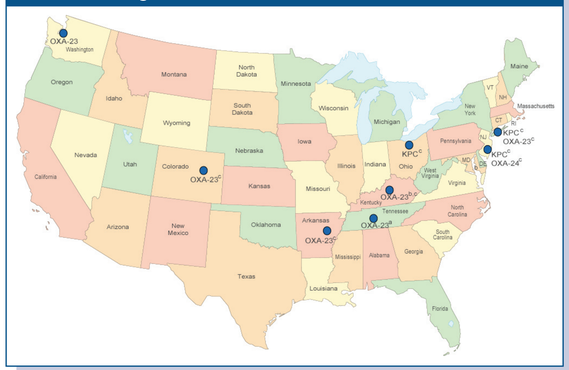
- A total of 42 (3.0% of the total) Enterobacteriaceae isolates were screened and 35 (2.5%) isolates from medical centers located in New York (24/35; 68.6%), New Jersey (10/35; 28.6%) and Ohio (1/35; 2.9%) harbored bla_{KPC-2} (Table and Figure 1).
- bla_{KPC-2} was also detected in *Citrobacter freundii* (2), *E. coli* (1), *Enterobacter cloacae* (3), *Klebsiella oxytoca* (1) and *K. pneumoniae* (28; Table 1). Other serine-carbapenemases, such as IMI-like, NMC-A and SME-like were not identified among KPC-negative isolates.
- Among 133 *Acinetobacter* spp., 50 (37.6%) isolates were screened and bla_{OXA-23} was detected in 10 isolates from medical centers in New York (3), Kentucky (3), Arkansas (2) and Colorado (2), while bla_{OXA-24} was observed in 5 isolates from New Jersey (Table and Figure 1).
- According to PFGE patterns, the three bla_{OXA-23}-carrying isolates from Kentucky were considered epidemiologically unrelated. The remaining OXA-23-producing *Acinetobacter* spp. were genetically related within specific medical institutions. The OXA-24-producing isolates recovered from a single hospital in New Jersey, belonged to a unique PFGE pattern (Table 1).
- Among *Acinetobacter* spp. showing negative results for acquired oxacillinases (OXA-24, -23 and -58), the IS*Aba1* was detected upstream of the bla_{OXA-51} in 78.8%, which could explain the carbapenem-resistance phenotype.
- A total of 42 (6.8%) *Acinetobacter* spp. isolates recovered between 1999 and 2006 were screened for bla_{OXA}. bla_{OXA-23} was observed in 2 isolates from Tennessee (2004) and one isolate each from Kentucky and Washington (2005), showing its recent escalation in the 2007 sample.
- Among the 36 (7.7%) *Pseudomonas* spp. isolates screened, no carbapenemases were detected.

Table 1. Distribution of the characterized ß-lactamases in USA medical centers (MYSTIC Program, 2007).

ß-lactamase (no. of isolates)	Medical center location (no. of isolates)	Bacterial species (no. of isolates)
KPC-2 (35)	New York, New York (15) New Brunswick, New Jersey (10) Mineola, New York (9) Cleveland, Ohio (1)	*K. pneumoniae* (28), *Enterobacter cloacae* (3), *Citrobacter freundii* (2), *E. coli* (1), *K. oxytoca* (1)
OXA-23 (10)	Lexington, Kentucky (3) New York, New York (3) Denver, Colorado (2) Little Rock, Arkansas (2)	*A. baumannii* (10)[a]
OXA-24 (5)	New Brunswick, New Jersey (5)	*A. baumannii* (5)[a]

a. Clonal dissemination within medical centers were identified.

Figure 1. Distribution of bla_{KPC-2}, bla_{OXA-23} and bla_{OXA-24} detected in Enterobacteriaceae and *A. baumannii* isolates within the USA. [a] represents *A. baumannii* isolates recovered during 2004; [b] represents *A. baumannii* isolates recovered during 2005; and [c] represents *A. baumannii* isolates recovered during 2007.



CONCLUSIONS

- Although these 2007 MYSTIC Program results presented here document an alarming presence of bla_{KPC-2}, the rate (2.5%) was significantly lower when compared with the previous MYSTIC Surveillance year (4.5%; *P* = 0.0063; OR = 1.80, CI 95% 1.15-2.82).
- Most KPC-producing isolates in this study were recovered from the New York City area, confirming the continued presence of this serious resistance problem.
- While the rate of bla_{KPC}-carrying Enterobacteriaceae isolates was lower overall, this gene has spread among several different species, whereas it was detected only in *Klebsiella* spp. in earlier sampling.
- While bla_{OXA} genes have now been detected in *Acinetobacter* spp. collected from several MYSTIC Program medical centers within the USA (first detected in 2004), bla_{OXA} genes were only observed in consecutive years in Kentucky, suggesting a rather sporadic appearance in *Acinetobacter* spp.; but recent expansion overall.

ACKNOWLEDGEMENT

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