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Dissemination of *bla*KPC-2/3 among Clinical Strains of Enterobacteriaceae Isolated in New York City Medical Centers: Report from the MYSTIC Program (2005)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

ABSTRACT

Background: Carbapenem (CARB) resistance (R) remains uncommon among Enterobacteriaceae (ENT) strains with only a few reports worldwide. However, there has been an increasing occurrence of CARB-R ENT in some USA hospitals, especially in the New York City (NYC) area.

Methods: As part of the Meropenem (MEM) Yearly Susceptibility (S) Test Information Collection (MYSTIC) Program, ENT strains are tested for S by CLSI broth microdilution methods against MEM and numerous other antimicrobials. In 2005, 13 of the initial 28 *K. pneumoniae* (KPN) and a *C. freundii* (CF) received from medical center (MC) "02" exhibited R to MEM and R or decreased S to all ß-lactams tested. These strains were screened for the presence of bla_{KPC} using generic primers. In addition, all *Klebsiella* spp. (KSP) and CF strains with elevated MIC values (≥ 2 µg/ml) for MEM and imipenem collected through the MYSTIC Program (1999-2004) were screened for bla_{KPC}. Sequencing of PCR products was performed to characterize the bla_{KPC} in selected clonally unrelated strains. KPC-producing strains were epidemiologically typed by automated ribotyping and PFGE.

Results: 12 of 13 MEM-R KPN strains collected in 2005 from MC "02" had a positive PCR result for bla_{KPC} and an identical ribotype (497.1). Sequencing results for this cluster revealed bla_{KPC-3}. Another MEM-R KPN strain from 2005 with ribotype 497.1 and $bla_{KPC-3/3}$ was observed in a different MC in NYC. Among 1,442 KSP and 515 CF tested in the 1999-2004 period, 13 KSP and 4 CF strains with increased CARB MIC values were identified and PCR results were positive for bla_{KPC} on 10 KPN from 3 MC, all in NYC (5 ribotypes), 2 *K. oxytoca* in Arkansas (ARK; identical ribotypes) and 4 CF (3 from NYC [2 ribotypes] and 1 from Delaware [DEL]). Gene sequence results revealed $bla_{KPC-2/3}$ in a CF strain from center 02 and both bla_{KPC-2} and bla_{KPC-3} in KPN strains isolated in the other 3 sampled NYC MCs.

Conclusions: $bla_{KPC-2/3}$ has emerged widely (NYC,ARK, DEL) among ENT isolated in the MYSTIC Program (1999 - 2005).

INTRODUCTION

Enteric bacilli are responsible for serious infections such as sepsis and pneumonia in hospitalized and critical care patients. Increased use of cephalosporins for treating these infections led to the emergence of extended-spectrum ß-lactamases (ESBL) and other resistance mechanisms. Nevertheless, the carbapenems remain an excellent therapeutic option for treatment of serious infections caused by multidrug-resistant Enterobacteriaceae.

Resistance to carbapenems was initially limited to non-fermentative Gram-negative pathogens and is generally due to hyper-production of AmpC ß-lactamases associated with loss of outer membrane protein and/or the presence efflux pumps. Reports on carbapenem resistance due to production of metallo-ß-lactamases (MßL) as well as serine carbapenemases are still relatively uncommon. However, in the recent years, there has been a significant increase of reports on carbapenemase-producing Gram-negative bacilli, mainly *Pseudomonas aeruginosa* and *Acinetobacter* spp.

MYSTIC Program monitors antimicrobial susceptibility of select pathogens from medical centers in the United States of America (USA) that use meropenem. As part of this program, 13 of the initial 28 *Klebsiella pneumoniae* and *Citrobacter freundii* isolates received by the MYSTIC Program in 2005 from medical centers located in the New York City area showed reduced susceptibility to imipenem and meropenem. The objective of this study was to evaluate the resistance mechanism to carbapenems on these isolates. We also screened all MYSTIC Program Enterobacteriaceae isolates (1999 - 2004) with imipenem and meropenem MIC values of ≥ 2 µg/ml for production of carbapenemases.

MATERIALS AND METHODS

Bacterial isolates. In the January 1999 - May 2005 period of the MYSTIC Program, 7,261 Enterobacteriaceae isolates were collected in the USA. All isolates were collected from clinical infections from patients hospitalized in medical centers that use meropenem. Species level identification on these isolates was confirmed by standard biochemical tests and Vitek cards where necessary.

Susceptibility testing. The Enterobacteriaceae isolates were susceptibility tested against multiple classes of antimicrobials by broth microdilution procedure as described by the CLSI/NCCLS (2003) using validated dry-form panels manufactured by Trek Diagnostics (Cleveland, OH, USA). Interpretations on susceptibility to all antimicrobials tested were by CLSI (2005) criteria where available. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *P. 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 µg/ml) were tested for production of carbapenemases. Indole-positive proteae and *Proteus mirabilis* were excluded because of their inherent decreased susceptibility to carbapenems.

a. Disk approximation: Potential carbapenemase producers were screened using disk approximation techniques. MßL screens were set up using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid (2-MPA) as ß-lactamase inhibitors. Screening for serine carbapenemases was performed as described by Pottumarthy et al (2002), in which imipenem and meropenem were used as substrates and clavulanic acid as ß-lactamase inhibitor.

b. PCR: Isolates with positive disk approximation test for MßL were screened for IMP and VIM family of enzymes using PCR primers described elsewhere. In addition, all isolates with elevated carbapenem MIC values and negative MßL screening tests were screened for IMI-, KPC-, NmcA and SME- type serine carbapenemases using specific primers.

Sequencing. PCR amplicons for the carbapenemase genes were sequenced using Sanger based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to the pre-existing sequences via NCBI BLAST search.

Molecular typing. Multiple isolates from the same medical center harboring carbapenemases belonging to the same family were typed using Riboprinter™ Microbial Characterization system. Isolates with identical ribotypes were further characterized by pulsed-field gel electrophoresis (PFGE).

RESULTS

- The susceptibility patterns of 7,261 Enterobacteriaceae isolates collected by the MYSTIC Program are presented in Table 1. The three medical centers located in the New York City area (two in New York City and one in Mineola) contributed with 17.5% of isolates (1,270 isolates). Isolates received from these three New York sites showed resistance rates significantly higher to all antimicrobial agents when compared to the entire collection.
- Imipenem and meropenem were the most active agents tested against Enterobacteriaceae with susceptibility rates ranging from 98.8 to 100% (Table 1).
- Third generation cephalosporins and aztreonam were very active against *E. coli*, *Klebsiella* spp., and *Serratia* spp. (93.3 - 98.3% susceptible), while among *Enterobacter* spp. and *Citrobacter* spp. resistance rates to these agents varied from 5.7 to 16.3%. *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. strains from the New York City area showed high resistance rates (19.6 - 21.5%) to ceftazidime (Table 1).
- Cefepime showed excellent in vitro activity with 98.1 - 99.5% overall susceptibility rate. Resistance rates ranged from 0.2% in *E. coli* to 1.3% in *Klebsiella* spp., and rates were generally higher among strains from the New York City area.
- Gentamicin was highly active against enteric bacilli, with resistance rates ranging from 3.1% (*Serratia* spp.) to 7.1% (*Citrobacter* spp.; Table 1).
- Resistance rates to ciprofloxacin were highest among *E. coli* (12.6%), while among other species ranged from 3.7% in *Serratia* spp. to 6.3% in *Enterobacter* spp.
- A total of 42 isolates (0.58% of the Enterobacteriaceae isolates collected in the MYSTIC Program) showed elevated carbapenem MIC values. Thirty-four of these isolates (81.0%) harbored a $bla_{KPC-2/3}$. The vast majority of KPC-2/3-producing strains (88.2%) were detected in the New York City area (Table 2).

Table 1. In vitro activities of selected antimicrobial agents against 7,261 Enterobacteriaceae isolates collected through MYSTIC Program (1999 - 2005) compared with susceptibilities of isolates from three New York medical centers.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml) 50%	90%	Range	% Category (all US/New York City area): Susceptible	Resistant
E. coli (2,705)					
Meropenem	≤0.016	0.03	≤0.016-2	100.0/100.0	0.0/0.0
Imipenem	0.12	0.25	0.03-2	100.0/100.0	0.0/0.0
Ceftazidime	≤0.12	0.5	≤0.12->16	98.0/97.1	1.4/1.8
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	98.3/98.0	0.8/1.1
Cefepime	≤0.12	≤0.12	≤0.12->16	99.5/99.1	0.2/0.7
Piperacillin/Tazobactam	1	4	≤1->128	98.0/97.8	1.3/1.8
Aztreonam	≤1	≤1	≤1->16	98.2/97.5	1.1/1.6
Gentamicin	≤2	≤2	≤2->8	93.7/93.3	5.6/5.8
Ciprofloxacin	≤0.25	>2	≤0.25->2	87.2/83.9	12.6/16.1
Levofloxacin	≤0.06	>8	≤0.06->8	83.2/75.0	16.8/23.6
Citrobacter spp. (666)					
Meropenem	0.03	0.06	≤0.016-4	100.0/100.0	0.0/0.0
Imipenem	0.25	1	0.03-4	100.0/100.0	0.0/0.0
Ceftazidime	0.25	>16	≤0.12->16	84.8/76.9	13.8/21.5
Ceftriaxone	0.25	32	≤0.016->32	85.6/81.8	5.7/8.3
Cefepime	≤0.12	0.5	≤0.12->16	99.4/98.3	0.3/0.0
Piperacillin/Tazobactam	2	16	≤1->128	90.0/89.3	4.6/9.1
Aztreonam	≤1	>16	≤1->16	84.6/77.7	10.8/15.7
Gentamicin	≤2	≤2	≤2->8	92.3/90.1	7.1/7.4
Ciprofloxacin	≤0.25	0.5	≤0.25->2	93.1/90.9	5.5/5.8
Levofloxacin	≤0.06	1	≤0.06->8	92.5/87.9	4.5/10.3
Enterobacter spp. (989)					
Meropenem	0.03	0.12	≤0.016->32	99.6/97.8	0.3/1.6
Imipenem	0.5	1	0.06->32	99.6/97.8	0.3/1.6
Ceftazidime	0.25	>16	≤0.12->16	79.7/73.9	16.3/21.2
Ceftriaxone	≤0.25	32	≤0.25->32	82.5/78.3	9.9/15.8
Cefepime	≤0.12	2	≤0.12->16	98.1/94.0	0.8/2.2
Piperacillin/Tazobactam	2	64	≤1->128	84.4/78.3	7.1/11.4
Aztreonam	≤1	>16	≤1->16	80.7/74.5	14.5/22.3
Gentamicin	≤2	≤2	≤2->8	93.5/84.8	5.5/13.0
Ciprofloxacin	≤0.25	≤0.25	≤0.25->2	92.6/85.3	6.2/13.6
Levofloxacin	≤0.06	1	≤0.06->8	91.3/78.3	6.1/18.8
Klebsiella spp. (1,873)					
Meropenem	0.03	0.03	≤0.016->32	98.8/93.8	1.1/5.9
Imipenem	0.12	0.5	0.03->32	98.9/94.4	0.6/2.9
Ceftazidime	≤0.12	1	≤0.12->16	93.6/79.8	5.9/19.6
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	94.8/81.5	3.1/12.6
Cefepime	≤0.12	0.25	≤0.12->16	98.1/91.5	1.3/5.9
Piperacillin/Tazobactam	2	8	≤1->128	93.6/78.9	4.7/16.6
Aztreonam	≤1	≤1	≤1->16	93.3/78.9	6.1/20.5
Gentamicin	≤2	≤2	≤2->8	94.3/85.0	4.0/12.9
Ciprofloxacin	≤0.25	0.5	≤0.25->2	93.3/80.4	5.7/17.0
Levofloxacin	≤0.06	1	≤0.06->8	92.2/72.6	6.7/27.4
Serratia spp. (660)					
Meropenem	0.06	0.06	≤0.016->32	99.5/99.1	0.3/0.0
Imipenem	0.5	1	0.12->32	99.5/99.1	0.5/0.9
Ceftazidime	0.25	0.5	≤0.12->16	97.4/95.5	2.0/3.6
Ceftriaxone	≤0.25	2	≤0.25->32	96.4/95.5	1.2/1.8
Cefepime	≤0.12	0.25	≤0.12->16	99.4/99.1	0.5/0.9
Piperacillin/Tazobactam	2	4	≤1->128	96.4/92.8	0.3/1.8
Aztreonam	≤1	≤1	≤1->16	97.2/95.5	2.6/3.6
Gentamicin	≤2	≤2	≤2->8	95.2/91.9	3.1/4.5
Ciprofloxacin	≤0.25	1	≤0.25->2	93.5/89.2	3.7/4.5
Levofloxacin	0.12	1	≤0.06->8	97.2/98.2	1.4/1.8

- bla_{KPC-3} was predominant among *E. cloacae* strains isolated in the New York City medical center #2 while a major clonal outbreak (n=13) of *K. pneumoniae* at the same center consisted of KPC-2 producing isolates. This *K. pneumoniae* clone (molecular type 105.497.1/A) became endemic in this medical center (2002 - 2005). Interestingly, these *K. pneumoniae* isolates generally showed higher MIC values to meropenem compared to imipenem (Table 3).
- Clonal dissemination of KPC-3-producing *K. pneumoniae* was identified in the New York City medical center #6 (four isolates), while two isolates producing KPC-2 from this site showed distinct molecular patterns.
- Three KPC-2-producing *K. pneumoniae* isolates with an identical molecular typing pattern were identified in two distinct medical centers located in the New York City area, indicating inter-hospital dissemination (Table 2).
- $bla_{KPC-2/3}$ positive strains of *K. oxytoca*, *C. freundii* and *E. coli* were identified in participating medical centers located in Arkansas, Delaware and as far west as Ohio.
- No MßL-producing isolates were detected among Enterobacteriaceae isolated in the USA by the MYSTIC Program.

Table 2. Occurrence of bla_{KPC} among Enterobacteriaceae isolates within the medical centers monitored by MYSTIC Program.

Site	Organism (no. isolates)	Year of isolation	KPC-type	Molecular pattern (no. of isolates)
New York, NY (site #2)	*C. freundii* (3)	2002, 2005	KPC-3	258.264.5/B (2)
		2004	KPC-2	258.264.7 (1)
	E. cloacae (3)	2000	KPC-3	105.1755.4 (1)
		2001	KPC-3	258.107.1 (1)
		2004	KPC-3	258.275.7 (1)
	E. gregoviae (1)	2002	KPC-3	258.107.3 (1)
	K. pneumoniae (14)	2004, 2005	KPC-2	105.497.1/A (13)
		2002	KPC-3	258.107.4 (1)
New York, NY (site #6)	*K. pneumoniae* (6)	2000	KPC-3	105.204.2/A (4)
		2001	KPC-2	258.267.7 (1)
		2000	KPC-2	105.1752.5 (1)
Mineola, NY (site #4)	*K. pneumoniae* (3)	2004	KPC-2	105.430.8 (1)
		2004	KPC-2	258.268.7 (1)
		2005	KPC-2	105.497.1/A (1)
Little Rock, AK	*K. oxytoca* (2)	2003	KPC-2	258.269.6/A (2)
Wilmington, DE	*C. freundii* (1)	2001	KPC-3	258.264.4 (1)
Cleveland, OH	*E. coli* (1)	2004	KPC-2	-[a]

a. - = not determined.

Table 3. Distribution of carbapenem MIC values among 42 Enterobacteriaceae isolates with reduced susceptibility to carbapenems.

Antimicrobial agent	No. of isolates at MIC (µg/ml) 2	4	8	16	32	>32
Imipenem	5	10	10	7	4	6
Meropenem	7	6	4	9	9	7

CONCLUSIONS

- KPC-2 and KPC-3 are predominant and endemic carbapenemases in the New York City area. The results of this study showed that these genes have disseminated rapidly across species and geographic regions.
- Although clonal dissemination of KPC-producing strains were observed in some medical centers, horizontal gene transfer seems to be a major factor in the dissemination of this resistance mechanism.
- No serine carbapenemases other than the KPC family (eg. NmcA, IMI- or SME- type) or MßLs were detected among Enterobacteriaceae collected by the MYSTIC Program in the USA.
- The rapid and wide dissemination of bla_{KPC} among Enterobacteriaceae species is of great concern since many KPC-producing strains are resistant to all antimicrobial agents available for clinical use in humans.

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