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Occurrence of Carbapenemase-producing Enterobacteriaceae Clinical Isolates in the Asia-Pacific Nations

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objectives: The aim of this study was to determine the occurrence of carbapenemase-producing Enterobacteriaceae isolates in the Asia Pacific (APAC) region during the 2006 SENTRY Program.

Methods: Enterobacteriaceae recovered from hospitalized patients in 37 medical centers (MC) located in 9 countries in the APAC region were tested by CLSI broth microdilution. Isolates showing imipenem and meropenem MIC ≥2 mg/L were screened for MBL- (IMP-like, VIM-like, SIM-1, GIM-1 and SPM-1) and group 2f serine carbapenemase- (KPC, IMI, SME, etc.) encoding genes by PCR and sequencing. Spectrophotometric measurement of meropenem hydrolysis was performed to access carbapenemase activity among PCR-negative isolates. Presence of fluoroquinolone resistance genes (*qnr*-like and *aac[6']-Ib-cr*) was also accessed by PCR and sequencing. The genetic environment of carbapenemase-encoding genes was investigated by plasmid analysis, conjugation and transformation experiments.

Results: Among 844 isolates, 9 (1.1%; 1 *E. coli,* 5 *K. pneumoniae* and 3 *P. mirabilis*) met the screening criteria. The majority of the isolates (7/9; 77.8%) were PCR-negative and did not show meropenem hydrolysis activity. One *K. pneumoniae* isolate (231-21D) harboring bla_{KPC-2} was detected in Zhejiang, China. This isolate was recovered from a venous catheter infection in a 42 y/o male trauma victim on the 77th hospital day. A second *K. pneumoniae* (234-49C) carrying bla_{IMP-4} was recovered in Wuhan (China) from the sputum (2nd hospital day) of a 50 d/o female infant with acute bronchopneumonia. Both cases had favourable clinical outcomes. Plasmid content of the isolate 231-21D revealed three plasmids (60-, 5- and 3.5-kb), while the transconjugant (p231-21D) strain showed only the 60-kb plasmid. The p231-21D strain showed resistance to ß-lactams, aminoglycosides and decreased susceptibility to fluoroquinolones. Further investigations detected *qnrB4* in the isolate 231-21D and recipient strain p231-21D, suggesting both bla_{KPC-2} and *qnrB4* were located in the 60-kb conjugative plasmid. Experiments failed to identify a plasmid-borne location for the bla_{IMP-4}.

		Antimicrobial MIC (mg/L)							
Enzyme	Site	IMI	MER	AZT	CRO	CAZ	CEP	CIP	AMK	TIG
KPC-2	Zhejiang	8	8	>16	16	>16	8	>4	>32	0.5
IMP-4	Wuhan	4	8	8	>32	>16	8	0.5	≤4	0.5

IMI = imipenem; MER = meropenem; AZT = aztreonam; CRO = ceftriaxone; CAZ = ceftazidime; CEP = cefepime; CIP = ciprofloxacin; AMK = amikacin; TIG = tigecycline; COL = colistin.

Conclusion: Occurrence of carbapenemase-encoding genes was low in the APAC region. This is the first report of coproduction of KPC-2 and QnrB4 encoded by resistance genes located in the same conjugative plasmid. This plasmid may further spread and escalate the resistance rates for their respective antimicrobial classes. IMP-4 has been detected in different countries in the APAC region, suggesting a continued dissemination of this gene in this region.

INTRODUCTION

Acquired carbapenemases (i.e. metallo-ß-lactamases [MßLs], oxacillinases and serine-carbapenemases) can have a significant impact on the clinical utility of carbapenems. Isolates producing such enzymes are usually non-susceptible to the vast majority of clinically available ß-lactam agents.

KPC-producing isolates, which were initially restricted to hospitals located in the New York City area, have recently been described in other areas of the United States (USA) and in several nations, including China, Colombia, and Israel. This enzyme, considered a problem for clonal spread, has also been detected among non *Klebsiella pneumoniae* Enterobacteriaceae, emphasizing the global risk of interspecies dissemination of resistance genes. Furthermore, several reports of MßL enzymes have been described among the Asia-Pacific (APAC) nations. The main objective of this study was to determine the occurrence of carbapenemase-producing Enterobacteriaceae isolates in the APAC region during the 2006 SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates. Enterobacteriaceae clinical isolates were consecutively collected from hospitalized patients in 37 medical centers geographically dispersed in nine countries in the APAC region. Only one isolate per patient from a documented infection was included in the 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 (M7-A7, 2006) as described by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS). Cation-adjusted Mueller-Hinton (MH) broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for antimicrobials were those found in M100-S18 document (CLSI, 2008). Tigecycline MIC results were interpreted according to the breakpoints approved by the United States Federal Drug Administration (USA-FDA; ≤2 / ≥8 mg/L for susceptible/resistant). Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within ranges as published in the most recent CLSI document.

Screening for carbapenemase-encoding genes. Enterobacteriaceae isolates showing MIC values ≥2 mg/L 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 selected MßL- (IMP-like, VIM-like, SPM-1, SIM-1) encoding genes available in the GenBank using a multiplex PCR approach. A similar screening designed was used to amplify other serine-carbapenemases (IMI-like, NMC-A and SME-like) among KPC PCR-negative isolates. In addition, spectrophotometric measurement of meropenem hydrolysis was performed to assess carbapenemase activity among PCR-negative isolates.

Screening for fluoroquinolones resistance determinants. Isolates were also screened for plasmid encoded quinolone resistance genes (*qnrA, qnrB, qnrS*, *qepA* and *aac(6')-Ib-cr*).

Analysis of plasmid content and conjugation experiments. Plasmid DNA was extracted using the Plasmid DNA Midi Kit (Qiagen GmbH, Hilden, Germany), separated on 1% agarose gel in TAE buffer on a Criterion Sub-cell GT system (Bio-Rad, Hercules, CA). Plasmid sizes were determined by comparison with standard plasmid DNAs extracted from *E. coli* V517 (NCTC 50193). Conjugal transfer of ß-lactam resistance determinants was assayed by mating experiments in MH agar plates and Luria-Bertani broth by mixing equal volume of donor and recipient bacteria (*E. coli* K12) in the exponential phase of growth. Mating plates and broth were incubated for 24 hours at 35°C. Transconjugants were selected in MH agar plates containing streptomycin (150 mg/L) and meropenem (0.25 mg/L). The presence of ß-lactam resistance marker and species identification of the transconjugant strain was confirmed by PCR and the Vitek System, respectively.

Sequencing Analysis. 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

- High resistance rates (15.6-88.5%) were observed among Enterobacteriaceae isolates from the APAC region when tested against 13 agents (Table 1), except for the carbapenems and glycylcyclines (tigecycline; 0.0-1.7% resistant).

Table 1. Antimicrobial susceptibility profile of 844 Enterobacteriaceae isolates recovered from the APAC region.

	MIC (mg/L)		
Antimicrobial Agents	MIC_{50}	MIC_{90}	% susceptible/resistant[a]
Piperacillin/tazobactam	8	>64	69.7/15.6
Aztreonam	>16	>16	21.8/68.6
Cefoxitin	8	32	64.1/21.9
Ceftazidime	>16	>16	35.9/45.6
Ceftriaxone	>32	>32	6.2/88.5
Cefepime	>16	>16	28.8/54.9
Imipenem	0.25	0.5	99.4/0.4
Meropenem	≤0.12	≤0.12	99.3/0.0
Ertapenem	≤0.06	0.5	97.8/1.7
Gentamicin	>8	>8	28.9/71.1
Ciprofloxacin	>4	>4	18.6/73.7
Levofloxacin	>4	>4	28.6/65.6
Tigecycline[b]	0.25	1	99.4/0.0

a. Criteria as published by the CLSI M100-S18.
b. Tigecycline MIC results were interpreted according to the breakpoints approved by the USA-FDA (≤2 / ≥8 mg/L for susceptible/resistant).

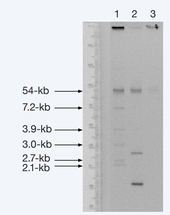
- Among 844 Enterobacteriaceae isolates collected, only 9 (1.1%; 1 *E. coli,* 5 *K. pneumoniae* and 3 *Proteus mirabilis*) met the carbapenamase screening criteria. The majority of them (7 of 9; 77.8%) were PCR-negative for carbapenemases and did not show meropenem hydrolysis activity.
- One *K. pneumoniae* isolate (231-21D) harboring bla_{KPC-2} was detected in Zhejiang (China). This isolate was recovered from a venous catheter infection in a 42 year-old male trauma victim on the 77th hospital day.
- A second *K. pneumoniae* (234-49C) carrying bla_{IMP-4} was recovered in Wuhan (China) from the sputum of a 50 day-old female infant with acute bronchopneumonia on the 2nd hospital day.
- Isolate 231-21D showed resistance or decreased susceptibility to several antimicrobials including carbapenems but remained susceptible to tigecycline and the polymyxins. Isolate 234-49C also exhibited resistances to several antimicrobials, remaining susceptible to aminoglycosides, fluoroquinolones, tigecycline and polymyxins (Table 2).

Table 2. Antimicrobial susceptibility profile of *K. pneumoniae* clinical isolates 231-21D and 234-49C, the *E. coli* K12 strain carrying the conjugative plasmid p231-21D and the recipient strain *E. coli* K12.

	MIC (mg/L)			
Antimicrobial Agents	*K. pneumoniae* 234-49C	*K. pneumoniae* 231-21D	*E. coli* K12 (p231-21D)	*E. coli* K12
Ampicillin	>16	>16	>16	4
Piperacillin/tazobactam	>64	>64	32	2
Aztreonam	8	>16	>16	0.06
Cefoxitin	>16	>16	>16	4
Cefotaxime	>16	>16	4	≤0.25
Ceftazidime	>32	>32	32	0.25
Ceftriaxone	16	>32	32	0.06
Cefepime	8	8	4	0.06
Imipenem	4	8	4	0.03
Meropenem	8	8	2	0.03
Ertapenem	4	>8	>8	0.06
Amikacin	≤4	>32	>32	≤4
Gentamicin	≤2	>8	>8	≤2
Ciprofloxacin	0.5	>4	0.25	0.06
Levofloxacin	0.5	>4	0.25	0.06
Tigecycline	0.5	0.5	0.12	0.06
Colistin	≤0.5	≤0.5	≤0.5	≤0.5
Polymyxin B	1	≤0.5	≤0.5	≤0.5

- Plasmid DNA analysis of isolate 231-21D revealed three plasmids (60-, 5- and 3.5-kb), while the transconjugant (p231-21D) strain showed only the 60-kb plasmid (Figure 1). The p231-21D strain showed resistance to ß-lactams, aminoglycosides and elevated MIC values for the fluoroquinolones. Further investigations detected *qnrB4* in the index isolate and recipient strain (p231-21D), suggesting both bla_{KPC-2} and *qnrB4* were located in the 60-kb conjugative plasmid (Table 2).
- Experiments failed to identify a plasmid-borne location for the bla_{IMP-4} in *K. pneumonia* strain 234-49C.

Figure 1. Lane 1 represents plasmid DNAs from *E. coli* strain V517 (NCTC 50193) used as molecular size marker. Lane 2 represents plasmid DNAs from *K. pneumoniae* clinical isolate 231-21D. Lane 3 represents plasmid DNA from *E. coli* K12 transconjugant strain.



CONCLUSIONS

- Overall, carbapenem resistance rates and occurrence of carbapenemase-encoding genes were very low among clinical isolates from the APAC region. However, cephalosporin resistance rates were high; suggesting the production of ß-lactamases lacking carbapenemase activity (extended-spectrum ß-lactamases and AmpC cephalosporinases) may be of a greater clinical concern.
- This is the first report of co-production of KPC-2 and QnrB4 encoded by resistance genes located in the same conjugative plasmid. The plasmid-mediated aminoglycoside, fluoroquinolone and ß-lactam (carbapenems, cephalosporins and penicillins) resistance determinants may further spread among clinical isolates of enteric bacilli and escalate resistance rates.
- bla_{IMP-4} has been previously detected in *Acinetobacter* spp. and *Citrobacter youngae* isolates from China, followed by the emergence of bla_{IMP-4}-carrying *P. aeruginosa* and the rapid dissemination of this resistance gene among several Gram-negative pathogens in Australia. Although the prevalence of MßL genes in Enterobacteriaceae from our study was very low, the detection of a bla_{IMP-4}-carrying *K. pneumoniae* underscores the continue spread of this gene in this particular geographic region.
- These findings highlight the importance of active hospital surveillance in order to avoid possible outbreaks by such resistant organisms, as previously reported in Australia.

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