Brief Original Article

Detection of the novel IMP-38 among carbapenemase-producing Enterobacteriaceae in a university hospital, China

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Abstract

Introduction: This study set out to investigate the molecular epidemiology of carbapenemase-producing *Enterobacteriaceae* isolates collected from Xiang Ya Hospital, Hunan, China.

Methodology: The carbapenemase genes from *Enterobacteriaceae* isolates were determined by PCR and sequencing. Relatedness of *Klebsiella pneumoniae* isolates was evaluated by pulsed-field gel electrophoresis.

Results: Twenty-four out of 738 non-repetitive *Enterobacteriaceae* isolates harbored carbapenemase genes including IMP-38, a novel IMP-type metallo-enzyme. Nine IMP-38-producing isolates were shown to originate from the same clone and caused a small outbreak in the neonatal ward.

Conclusions: IMP-38, a novel IMP-type metallo-enzyme, was one of the predominant types of carbapenemase in the clinical carbapenem-resistant *Enterobacteriaceae* isolates in our hospital.

Key words: carbapenemase; *Enterobacteriaceae*; IMP; KPC.

J Infect Dev Ctries 2014; 8(8):1044-1048. doi:10.3855/jidc.4179

(Received 27 August 2013 – Accepted 23 November 2013)

Introduction

Carbapenems, such as imipenem and meropenem, have a broad activity of spectrum and stability against β-lactamases [1,2]. Carbapenem-resistant *Enterobacteriaceae* are being increasingly reported worldwide, representing a significant threat to public health; great efforts toward detection and infection control strategies are required. A variety of carbapenemases has been reported in *Enterobacteriaceae*, including the Ambler class A β-lactamases of KPC-type, the metallo-β-lactamases (MBL) (Ambler class B) of VIM-, IMP-, and NDM-types, and the carbapenem-hydrolysing class D β-lactamases of the OXA-48-type [3].

In our previous study, we showed that *Enterobacteriaceae* clinical isolates collected from three affiliated hospitals of Central South University carried a high incidence (33.4%) of ESBLs, predominately the CTX-M-type [4]. In this study, we investigated the molecular epidemiology and the antimicrobial-resistant mechanisms of carbapenemases in *Enterobacteriaceae* clinical isolates.

Methodology

Bacterial strains and plasmid

A total of 738 non-repetitive clinical *Enterobacteriaceae* isolates were collected from Xiang Ya Hospital, Hunan, China, between March 2010 and February 2011 (Table 1). The isolates were confirmed by Vitek2 GN card (bioMérieux, Marcy l'Etoile, France), an automatic microorganism identification system. PUC19 (Thermo Fisher Scientific, Waltham, MA, USA) was used as a vector to clone the amplified $bla_{\text{IMP-38}}$ gene.

Antimicrobial susceptibility testing

Carbapenem-resistant *Enterobacteriaceae* isolates were screened using an agar diffusion method with imipenem, meropenem, and ertapenem disks (10 μg for each antibiotic) first. The minimal inhibitory concentrations (MICs) of the following antibiotics in carbapenemase-producing isolates were then determined by agar dilution test: imipenem, meropenem, ertapenem, cefotaxime, cefepime, amikacin, ciprofloxacin, aztreonam, ceftriaxone, gentamicin, piperacillin/tazobactam, tetracycline, and

polymyxin B (Oxoid Basingstoke, UK). *Escherichia coli* ATCC 25922 was used as the internal quality control strain. The results of both tests were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [5].

Carbapenemase phenotypic screening

The modified Hodge test was used to determine phenotypes of KPC-type carbapenemase-producing isolates. Class B carbapenemase-producing strains were detected by imipenem and the EDTA synergy test [6].

PCR amplification and sequencing for carbapenemase genes

Extracted whole-cell lysates or plasmids isolated from ertapenem-resistant isolates were used as the templates for PCR amplification to determine various carbapenemase genes, including bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA-48} [7-10]. PCR products were then subjected to direct sequencing with an automatic sequencer (model 3730; Applied Biosystems, Foster City, CA, USA).

Pulsed-field gel electrophoresis (PFGE) typing

PFGE was performed to evaluate clonal relationships between some carbapenemase-producing *K. pneumoniae* strains. The procedures included digestion with XbaI restriction enzyme at 37°C for 12 hours and electrophoresis at 120°C, 6 V/cm, 0.5–70 seconds for 24 hours. The XbaI restriction patterns of genomic DNA of the isolates were analyzed and interpreted according to the criteria described by Tenover *et al*. [11].

Cloning of the bla_{IMP-38} gene

The entire bla_{IMP-38} gene was amplified using two pairs of primers flanked with specific restriction sites. The PCR products were cloned into pUC19 vector for sequencing.

Results

Phenotypic testing for carbapenemase

Among the 738 strains, 27 strains were positive by the modified Hodge test, 20 were positive by the EDTA-disk synergy test (Table 1).

Antimicrobial susceptibility

The phenotypes of the 738 strains were shown in Table 1, among which 24 isolates were identified as carbapenemase-producing strains. These isolates were resistant to multiple antibiotics. Resistant rates to various antimicrobial agents, such as meropenem, ertapenem cefotaxime, cefepime, ciprofloxacin, aztreonam, ceftriaxone, gentamicin, piperacillin/tazobactam, and tetracycline, ranged from 58.3% to 100%. However, the resistant rates to imipenem, amikacin, and polymycin were much lower (25%, 33.3%, and 0%, respectively) (Table 2).

Determination of carbapenemase genes

Twenty-four out of forty-four ertapenem-resistant isolates were found to carry carbapenemase genes, among which four were KPC type-positive and twenty were IMP-type positive strains. The plasmids isolated from these carbapenemase-producing strains were found to carry carbapenemase genes. Sequencing analysis showed that eleven strains contained IMP-4, among which five were *K. pneumoniae*, three were *E. cloacae*, one was *E. coli*, one was *C. freundii*, and one was *K. oxytoca*. In addition, KPC-2 was found in four *K. pneumonia* isolates. IMP-38, a novel IMP-type metallo-enzyme, was found in nine *K. pneumoniae* isolates. The gene showed a contig of 874 bp with an open reading frame of 741 nucleotides encoding a putative protein of 246 amino acids. It differed from bla_{IMP-4} [12] by one point mutation (A640G), which led to one amino acid substitution (Ser214Gly). Since this one amino acid substitution has not been described yet in IMP-type enzymes produced by clinical strains, it was designated as IMP-38.

PFGE typing

The PFGE patterns of the nine IMP-38-positive *K. pneumoniae* isolates showed homologous appearance, with differences of only one to three bands; they can, therefore, be considered to be five subclones of one pulsed-field-type clone. Among them, KP8, KP9, KP12, KP13, and KP15 belonged to the same subclone (Figure 1A). The PFGE patterns of the four KPC-2-positive *K. pneumoniae* isolates were identical (Figure 1B).

Table 1. Carbapenemase phenotypes and genotypes of *Enterobacteriaceae* isolates

Species	No. of isolates	ETP-R	MEM-R	IMP-R	MHT-positive	EDTA synergy positive	KPC-2positive	IMP-4positive	IMP-38 positive
E. coli	324	4	1	1	1	1	-	1	-
K. pneumoniae	204	24	12	6	18	14	4	5	9
E. cloacae	103	14	3	3	6	3	-	3	-
P. mirablis	27	-	-	-	-	-	-	-	-
K. ozaenae	18	-	-	-	-	-	-	-	-
C. freundii	14	1	-	-	1	1	-	1	-
K. oxytoca	12	1	-	-	1	1	-	1	-
S. marcescens	12	-	-	-	-	-	-	-	-
E. aerogenes	9	-	-	-	-	-	-	-	-
P. vulgaris	8	-	-	-	-	-	-	-	-
M. morganii	7	-	-	-	-	-	-	-	-
Total	738	44	16	10	27	20	4	11	9

ETP-R: ertapenem resistant; MEM-R: meropenem-resistant;
IMP-R: imipenem-resistant; MHT-positive: modified Hodge test positive

Table 2. Antibiotic susceptibility for carbapenemase-producing isolates

Antibiotic	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range (μg/mL)	Resistant %
Imipenem	1	8	0.5–16	25
Meropenem	4	8	0.5–8	62.5
Ertapenem	16	32	2–32	100
Cefotaxime	256	512	32≥512	100
Cefepime	32	64	4–256	58.3
Amikacin	4	1,024	1–1024	33.3
Ciprofloxacin	4	32	0.06–128	58.3
Aztreonam	128	>512	16≥512	100
Ceftriaxone	512	512	32–512	100
Gentamicin	128	>512	16≥512	100
Piperacillin/tazobactam	256/4	1,024/4	8/4–1024/4	83.3
Tetracycline	32	256	8–256	91.7
Polymyxin	0.5	1	<0.12–1	-

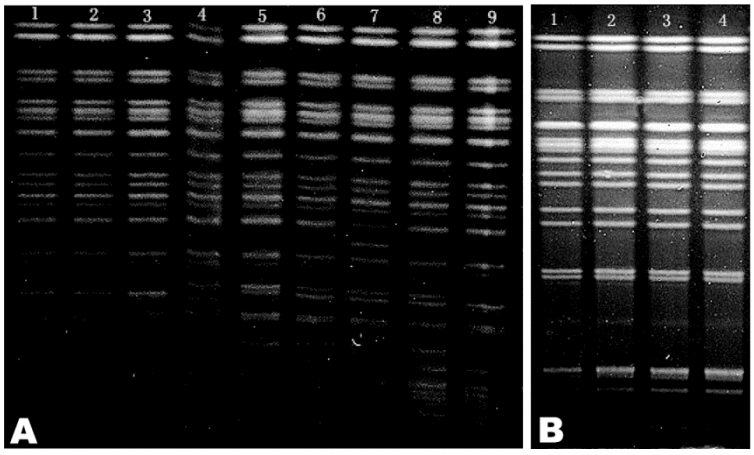

Figure 1. PFGE patterns of carbapenemase-producing *K. pneumoniae* isolates. **A)** PFGE patterns of KP8, KP9, KP10, KP11, KP12, KP13, KP14, KP15, and KP16 IMP-38-positive *K. pneumoniae* isolates from lanes 1 to 9; **B)** PFGE patterns of KP1, KP2, KP3, and KP4 KPC-2-positive *K. pneumoniae* isolates from lanes 1 to 4, respectively.

Discussion

The metallo-β-lactamases are the most molecularly diverse carbapenemases, showing the greatest clinical threat. During a one-year period in our hospital, 738 non-repetitive clinical *Enterobacteriaceae* isolates were collected, among which 24 were identified as carbapenemase-producing isolates, with 6 strains showing resistance to impenem and 15 to meropenem. Therefore, carbapenemase phenotypic screening of *Enterobacteriaceae* isolates with decreasing susceptibility should be enhanced in order to avoid missing the carbapenemase-producing isolates.

IMP-4, the first MBL found in a plasmid from a *C. youngae* isolate in 2001 in China, now appears to be the most dominant variant in China [13]. In the present study, we detected twenty IMP-producing isolates, including eleven IMP-4-positive and nine IMP-38-positive isolates. Our study showed that bla_{IMP} genes were disseminated in different enterobacterial species. The IMP-38 was identified as differing from IMP-4 by a point mutation, suggesting that IMP-38 may originate from IMP-4. The nine IMP-38-positive *K. pneumoniae* isolates belonged to the same clone and were collected from the trachea cannula of nine newborn patients who suffered from respiratory distress syndrome in the neonatal ward in October 2010. Our finding drew great concerns from clinicians of the neonatal ward and the hospital's infection control department. Strengthening disinfection of the ward and medical facilities, as well as quarantining the nine neonates, effectively controlled the spread of the IMP-38 carrying clone.

The worldwide spread of KPC-producing isolates has become a serious problem in the past five years, ever since it was first described in the northeastern United States [2]. A high prevalence rate of KPC-2 *Enterobacteriaceae* isolates has been reported in Zhejiang province, China, and sporadic reports have been made in Shanghai City, Jiangsu, and Jiangxi provinces [14-16]. In this study, four KPC-2-producing *K. pneumoniae* isolates with identical origination were detected. They were isolated from three non-adjacent departments in our hospital – the integrated ward, ICU, and orthopedics department. These data – the first report in Hunan province – suggest that clone dissemination is the way KPC-possessing *K. pneumoniae* isolates are spread in the hospital.

In summary, carbapenem resistance in our hospital has mainly appeared in *K. pneumoniae* clinical isolates and has mostly resulted from production of IMP and KPC types of metallo-beta-lactamases. IMP-38 is a novel IMP-type metallo-enzyme identified as one of the predominant types of carbapenemase in the clinical carbapenem-resistant *Enterobacteriaceae* isolates in our hospital.

Nucleotide sequence

The sequence of bla_{IMP-38} has been deposited to GenBank under the accession number of HQ875573.

Acknowledgements

The authors are grateful to Dr. Fangyou Yu at the First Affiliated Hospital of Wenzhou Medical College and Prof. Minggui Wang at Huashan Hospital, Fudan University, China, for their generosity of providing us the KPC-2 producing *C. freundii* and IMP-1 producing *Pseudomonas aeruginosa*. This work was supported by the Science and Technology Bureau of Hunan Province Grant 08FJ3175 (to W. L.).

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Conflict of interests: No conflict of interests is declared.