

# Characteristics and management of Enterobacteriaceae harboring IMP-4 or IMP-8 carbapenemase in a tertiary hospital.

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## Abstract

**Background:** The emergence of *Enterobacteriaceae* harboring IMP-4 or IMP-8 carbapenemases is rare. We report an occurrence of *Enterobacteriaceae* harboring IMP-4 or IMP-8 carbapenemases in a Chinese tertiary care hospital from November 2010 to December 2012.

**Methods:** The clinical characteristics of 30 patients were described. The genetic relationship of isolates was determined by pulsed-field gel electrophoresis (PFGE). Carbapenemases were detected by modified Hodge test (MHT) and polymerase chain reactions (PCRs). Amplicons were sequenced and blasted to determine the genotype.

**Results:** Most infected patients were from intensive care unit and had complex and serious underlying illnesses requiring mechanical ventilation. PFGE revealed that *Klebsiella pneumoniae* showed two major PFGE types. Two *Klebsiella oxytoca* had an indistinguishable PFGE pattern, while four *Enterobacter cloacae* were different strains. The sequencing studies showed *Enterobacteriaceae* harboring IMP-4 or IMP-8 carbapenemase in the 23 infected patients. The majority of patients had infections with the carbapenemase-producing *Enterobacteriaceae* (CPE) strain, most were successfully treated with a range of antibiotics and discharged.

**Conclusion:** It is important to maintain a high index of suspicion to screen for carbapenemase-producing *Enterobacteriaceae* strains. Rapid identification of these strains and implementation of stringent procedures are the key to prevent major outbreaks in a hospital setting.

**Keywords:** Carbapenemase, *Enterobacteriaceae*, IMP-4, IMP-8, clinical outcome.

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## Introduction

Since 2000, spread of community-acquired enterobacterial isolates (*Escherichia coli*) that produce extended-spectrum  $\beta$ -lactamases (ESBLs) capable of hydrolyzing almost all cephalosporins except carbapenems has been reported worldwide<sup>1</sup>. Carbapenems have become antimicrobial drugs of last resort therefore it is important

to maintain the clinical efficacy of these antibiotics. The frequent use of carbapenems, however, combined with the transmissibility of resistance determinants mediated by plasmids, transposons and gene cassettes, has contributed to the increase of carbapenem resistance by *Enterobacteriaceae*<sup>2-3</sup>. In countries such as the United States, Israel, Germany and Greece, carbapenemase-producing *Enterobacteriaceae* (CPE) had emerged and the dissemination of these multidrug-resistant pathogens had become a problem in clinical care of patients and in public health<sup>4-7</sup>. Invasive infections with these organisms have been associated with high rates of morbidity and mortality, due to their resistance to most available antimicrobial agents<sup>8-10</sup>. Patients who are hospitalized for prolonged periods and those with severe underlying disease are at high risk of acquiring these types of pathogens<sup>11</sup>.

The Ambler classification describes various classes of carbapenemases<sup>12</sup>. *Klebsiella pneumoniae* carbapenemases (KPC) belong to class A; they are increasingly prevalent in parts of the United States, Israel and Greece and are also an emerging concern in Western Europe<sup>13</sup>. Class B metallo-beta-lactamases of the types IMP (imipenemase) and VIM (Verona integron-encoded metallo-beta-lactamase)

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mase) are common in non-fermentative bacteria and have been recently recognized in *Enterobacteriaceae* worldwide<sup>13</sup>. Strains carrying the recently reported New Delhi metallo-beta-lactamase1 (NDM-1), found throughout India, Pakistan and Bangladesh, have also been rapidly disseminated to Europe and across the globe<sup>14</sup>. OXA-type (oxacillin-hydrolysing) carbapenemases (Class D) are widespread and have been detected primarily in *Enterobacteriaceae*<sup>15</sup>.

CPE have been sporadically reported in China in recent years. In 2009, two cases of pulmonary infection due to multidrug-resistant *K. pneumoniae* strains producing *bla*<sub>MP-4</sub> and *armA*, which contained intervening sequences highly related to those of a *Vibrio cholerae* plasmid found in Hangzhou, China<sup>16</sup>. In 2010, a study to investigate the resistance mechanism of 49 *Enterobacteriaceae* isolates with decreased susceptibility to carbapenems collected at 16 teaching hospitals in China, confirmed the production of KPC-2, IMP-4 and IMP-8 carbapenemase<sup>17</sup>. In 2011, the first NDM-1 producing *E. coli* isolate was detected in Hong Kong<sup>18</sup>. Here we report the emergence of *Enterobacteriaceae* harboring IMP-4 or IMP-8 carbapenemases in a Chinese tertiary care hospital. This report describes the emergence, the infection control measures implemented, and the results of molecular investigations.

## Patients and methods

### Setting

Liaocheng People's Hospital is a 3000-bed tertiary care hospital serving about 139,000 inpatients per year. It has a 43-bed general Intensive Care Unit (ICU) including medical ICU and surgical ICU, a 35-bed Neonatal Intensive Care Unit (NICU), a 30-bed Pediatric Intensive Care Unit (PICU) and a single 30-bed ICU in the Brain Hospital. All ICUs are mainly dealing with critically ill patients. Patients who no longer need mechanical ventilation or are in a stable condition will be transferred to general wards from ICU. Before the occurrence described in this report, no screening for carbapenem-non-susceptible *Enterobacteriaceae* (CNSE) was performed in the ICUs.

### Microbiological methods

The non-duplicate *Enterobacteriaceae* strains were isolated from Liaocheng People's Hospital between November 2010 and December 2012. The strains were sorted and numbered in accordance with the isolates of the time. All isolates were identified using standard conventional microbial methods. The strain identification and antimicrobial susceptibility testing were performed using a

Vitek 2 automated system (bioMérieux, France). Susceptibilities were interpreted using the Clinical and Laboratory Standards Institute (CLSI) M100–S20 breakpoints<sup>19</sup>. If the minimum inhibitory concentration (MIC) to erapenem was elevated (MIC ≥ 2 µg/mL), further testing by the modified Hodge test (MHT) was performed as phenotypic confirmatory method in order to investigate the mechanism of resistance.

### Detection of carbapenemases

The MHT was performed as previously described<sup>19</sup>. PCRs were performed for *bla*<sub>KPC</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>NDM-1</sub> genes as previously described<sup>20–23</sup>. The amplicons were sequenced in Shanghai Sangon company, sequencing instruments and reagents were ABI-PRISM3730 and BigDyeterminator v3.1 respectively. Then the sequencing results blasted to NCBI databases ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) to identify genotype and registered to GenBank sequence database.

### Molecular typing

The genotypes of isolates harboring carbapenemase genes were determined by using PFGE analysis. *Salmonella* Braenderup H9812 strain DNA was used as standard size markers. The genetic relationship of the test strains was generated by the unweighted pair-group method (UPGMA). Isolates that exhibited a PFGE profile with more than 80% similarity (pulsotype, PT) were considered as closely related strains.

## Results

### Epidemiological investigations

Thirty CNSE strains were mainly isolated from patients hospitalized in ICUs of Liaocheng People's Hospital, which includes twenty cases in NICU, three cases in Brain Hospital ICU, one case in PICU and one case in surgical ICU. Other five cases were from other clinical departments. Twenty-two patients were neonatal (73.3%) and five patients were over 60 years old (16.7%). All patients suffered from severe underlying diseases and most patients were treated with invasive procedures. Almost all patients had pulmonary infection (n=28), only two patients had orthopedic wound infections. The patient's characteristics are shown in Table 1.

### Infection control measures

Before April 2011, there had been 10 patients infected with CNSE isolates (patients 1-10, Table 1). Due to the long time interval and lack of experiences, they did not

draw the attention of clinical departments and hospital infection administrators. Follow-up medical records showed that these isolates were mainly isolated from the Brain Hospital ICU (patients 1,6,7). In addition, two or-

thopedic patients (patients 2, 8) were transferred to our hospital after post-operative infection in other hospitals and their information in other hospitals before transferred to our hospital was not retrieved.

**Table 1.Characteristics of patients with carbapenem-non-susceptible Enterobacteriaceae**

Patient number/ Isolate <sup>a</sup>	Sex <sup>b</sup> / age	Ward <sup>c</sup>	Underlying disease <sup>d</sup>	Invasive procedure	Type of specimen tested	Infection due to CPE	Antibiotic therapy	Outcome
1/KPN1	M/65 years	Brain Hospital ICU	TBI	Mechanical ventilation	Tracheal secretion	Pneumonia	Levofloxacin	Alive
2/ECL1	M/24 years	Orthopedics	Tibia and fibula fracture	Plate fixation	Wound secretion	Wound infection	Lomefloxacin	Alive
3/ECL2	M/78 years	Department of cardiovascular	MI, hypertension,diabetes, GCA	None	Tracheal secretion	Pneumonia	Ceftizoxime	Alive
4/KPN2	M/ neonatal	NICU	Premature infant, CHD,NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Imipenem	Alive
5/KPN3	M/ neonatal	NICU	Premature infant, MODS, NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Piperacillin/tazobactam	Death unrelated to CPE
6/ECL3	F/62 years	Brain Hospital ICU	ICH	Mechanical ventilation	Tracheal secretion	Pneumonia	Ciprofloxacin	Alive
7/KPN4	F/83 years	Brain Hospital ICU	TBI	Mechanical ventilation	Tracheal secretion	Pneumonia	Levofloxacin	Alive
8/KOX1	F/54 years	Orthopedics	Right knee purulent arthritis	Arthroscopic Surgery	Wound secretion	Wound infection	Lomefloxacin	Alive
9/KOX2	F/76 years	Surgery ICU	NRF, CHD, RA	Mechanical ventilation	Tracheal secretion	Pneumonia	Moxifloxacin	Death unrelated to CPE
10/KPN5	M/ neonatal	NICU	Premature infant, NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Meropenem	Alive
11/KPN6	M/ neonatal	NICU	Premature infant, NRF	Mechanical ventilation	Tracheal secretion	Pneumonia	Imipenem, cefepime	Alive
12/KPN7	M/ neonatal	NICU	Premature infant, MODS, asphyxia, NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Cefoperazone/sulbactam	Alive
13/KPN8	F/ neonatal	NICU	Premature infant,MODS, NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Cefoperazone/sulbactam, cefepime	Alive
14/KPN9	M/ neonatal	NICU	Premature infant,MODS, CHD, NRF,RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Imipenem, cefepime, cefoperazone/sulbactam	Alive
15/KPN10	M/ neonatal	NICU	Asphyxia, NRF, RDS, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	None	Alive
16/KPN11	M/ neonatal	NICU	Premature infant, MODS, RDS, HIE, IVH	None	Tracheal secretion	Pneumonia	Piperacillin/tazobactam, ceftriaxone	Alive
17/KPN12	M/ neonatal	NICU	Premature infant, NRF, RDS	Mechanical ventilation	Tracheal secretion	Pneumonia	Amoxicillin/sulbactam, ceftriaxone	Alive
18/KPN13	M/ neonatal	NICU	Premature infant, RDS,HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Amoxicillin/sulbactam	Death unrelated to CPE
19/KPN14	M/ neonatal	NICU	Premature infant,MODS, RDS	Mechanical ventilation	Tracheal secretion	Pneumonia	Amoxicillin/sulbactam	Alive
20/ECL4	F/2 years	PICU	CPR, NRF, convulsion	Mechanical ventilation	Tracheal secretion	Pneumonia	Amikacin, ceftazidime, cotrimoxazole	Transferred to other hospitals
21/KPN15	M/ neonatal	Neonatology department(1)	NRF, MODS	None	Tracheal secretion	Pneumonia	Meropenem, piperacillin/tazobactam	Alive
22/KPN16	F/ neonatal	NICU	Fetal macrosomia, HIE, MODS	Mechanical ventilation	Tracheal secretion	Pneumonia	None	Alive
23/KPN17	F/ neonatal	NICU	Premature infant, HIE, NRF, MODS, CHD	None	Tracheal secretion	Pneumonia	Piperacillin/tazobactam	Alive
24/KPN18	M/ neonatal	NICU	Premature infant, HIE	None	Tracheal secretion	Pneumonia	Meropenem, piperacillin/tazobactam	Alive
25/KPN19	M/ neonatal	NICU	HIE, asphyxia, NRF	Mechanical ventilation	Tracheal secretion	Pneumonia	Meropenem	Alive
26/KPN20	M/ neonatal	NICU	Premature infant, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	Ceftriaxone	Alive
27/KPN21	M/ neonatal	NICU	Premature infant, HIE	None	Tracheal secretion	Pneumonia	Meropenem, Ceftriaxone	Alive
28/KPN22	F/ neonatal	NICU	Premature infant, HIE	Mechanical ventilation	Tracheal secretion	Pneumonia	None	Alive
29/KPN23	M/ neonatal	NICU	Premature infant, asphyxia, RDS	Mechanical ventilation	Tracheal secretion	Pneumonia	Imipenem	Alive
<b>30/KPN24</b>	F/ neonatal	Neonatology department(2)	Premature infant, HIE	None	Tracheal secretion	Pneumonia	Meropenem	Alive

<sup>a</sup> KPN, *Klebsiella pneumoniae*; ECL, *Enterobacter cloacae*; KOX, *Klebsiella oxytoca*.

<sup>b</sup> M, male; F, female.

<sup>c</sup> TBI, traumatic brain injury; MI, myocardial infarction; GCA, gastric cardiac adenocarcinoma; CHD, congenital heart disease; NRF, neonatal respiratory failure; RDS, respiratory distress syndrome; HIE, hypoxic-ischemic encephalopathy; ICH, intracerebral hemorrhage; RA, rheumatoid arthritis; IVH, intraventricular hemorrhage; CPR, cardiopulmonary resuscitation.

<sup>d</sup> NICU, neonatal intensive care units; PICU, pediatric intensive care unit.

Until late April 2011, consecutive cases with invasive infections for CNSE in the NICU (Patients 12-14) were detected, and the strains were all isolated from bronchial aspirate specimen. By PCRs and subsequent sequencing, blaIMP-4 genes were detected from most strains, and the isolated *K. pneumoniae* and *Klebsiella oxytoca* were clonally related. Positive control measures were implemented and

health care workers were informed about CNSE by leaflets and informative meetings. The outbreak control team was organized and extended infection control measures were implemented in our hospital with the support of hospital administrators. In our hospital, Positive control 1 measures were immediately implemented at the time of admission of CPE-positive patients, to ensure that no

patients came into contact with a case. All CPE-positive patients were isolated immediately after detection by contact isolation, and the objective of these measures was to prevent future patient-to-patient transmission and included:

- (a) Isolating patients in single rooms or isolating cohorts with the same species and same resistance profile, flagging the presence of cases by displaying a specific poster or logo on the CPE-positive patients beds and in the part of the ward where these patients were cohorted;
- (b) All ICU personnel, including cleaning staff and physical therapists, were instructed about standard hygiene procedures such as appropriate hand hygiene, use of gloves and gowns by medical personnel during physical contact, and use of masks by medical personnel when exposure to respiratory secretions was expected;
- (c) environmental sites were investigated for CPE contamination. The environmental samples were taken from medical devices, personal computers, telephones, door handles, floors and various other surfaces. Environmental sampling demonstrated no CPE were detected.

Despite the introduction of these measures, during the following three months, four more patients (patients 15-18) were identified in the NICU, who were infected with pneumonia with CNSE. After mid-July, no other cases were detected during the following two months.

However, on September 25, 2011 and October 19, 2011, two cases (patient 19, 20) infected with CNSE were detected, and the resistance was similar to the previous strains. But these strains were confirmed producing IMP-8 carbapenemase, then the same control measures were implemented. From December 5, 2011 to December 29, 2011, nine cases (patient 21-29) infected CNSE led to another larger outbreak. All strains were isolated from tracheal secretions in the NICU, including a case detected in the neonatology department which was transferred from NICU. The outbreak control team drew up more strict control measures, more strict control measures – Positive control 2 was implemented for the key departments, including stopping the transfer of CPE-positive patients to other units or hospitals until the case was discharged and obtaining targeted surveillance samples from all inpatients and contact patients in kinds of ICU for additional measures.

Since then, such strains have been under effective control and have not been detected in the NICU. Only one patient (patient 30) was detected in another neonatology

department, and had been transferred from the NICU. The spatio-temporal description of patients infected with CNSE is shown in Figure 1.

### Clinical outcomes

Although there was no specific antibiotic treatment program, most patients were alive and discharged (n=28). Only two patients died of causes unrelated to CNSE. The clinical outcomes of patients are shown in Table 1.

### Antimicrobial susceptibility testing

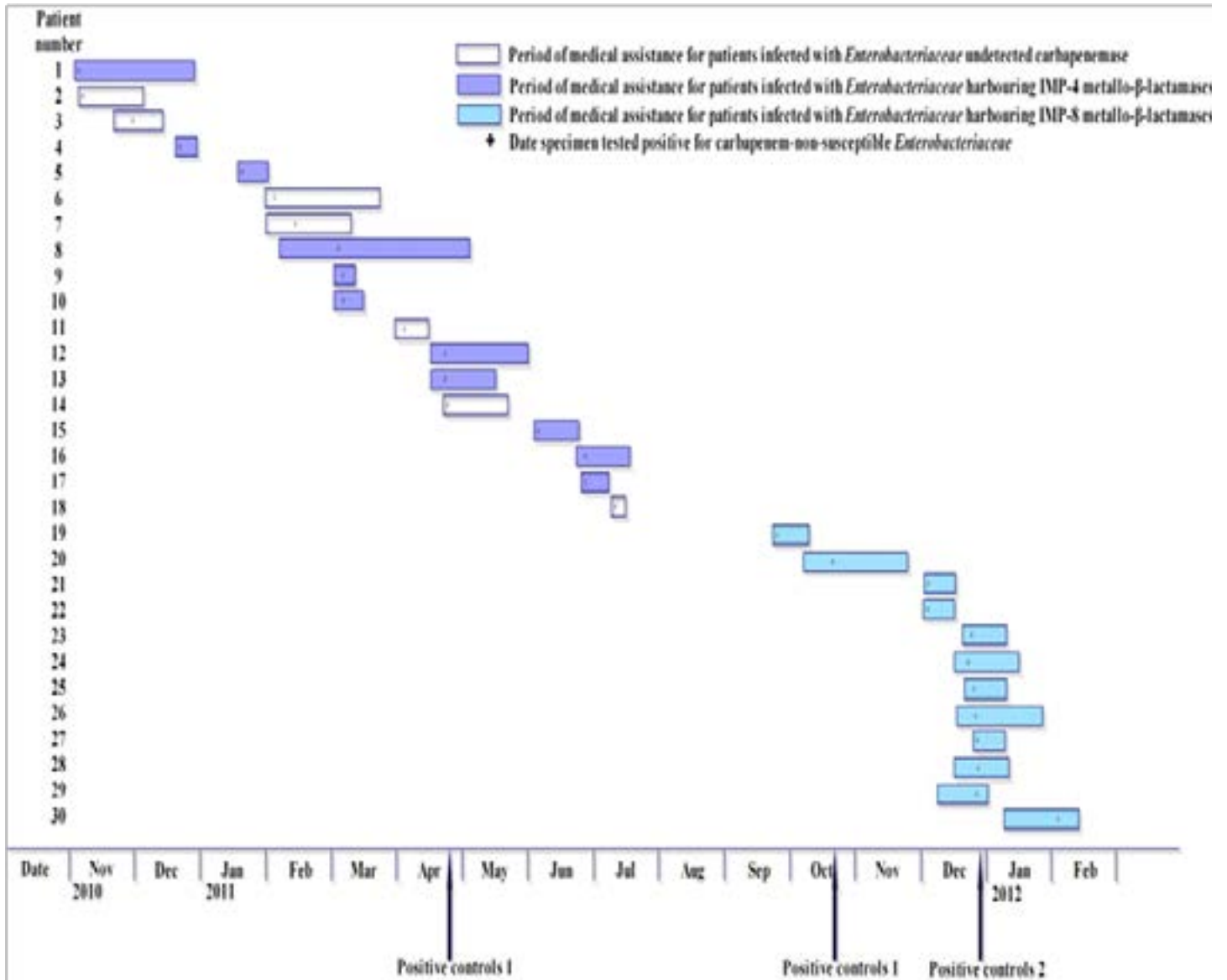
According to 2010 CLSI criteria<sup>19</sup>, these recommendations include performing a MHT on isolates resistant to at least one extended-spectrum cephalosporin and with carbapenem MICs at the upper end of the susceptible range (MICs of 2 to 4 µg/ml for imipenem or meropenem or 2 µg/ml for ertapenem), as they may produce carbapenemases. If the MHT is negative, carbapenem MICs are to be reported as indicating susceptibility; if the MHT is positive, carbapenem MICs are to be reported without an interpretation and with the following comment. “This isolate demonstrates carbapenemase production. The clinical efficacy of the carbapenems has not been established for treating infections caused by *Enterobacteriaceae* that test carbapenem susceptible but demonstrate carbapenemase production *in vitro*.” The strains exhibited the same multidrug-resistant profile, all strains demonstrated insensitivity to ertapenem and 18 strains showed elevated MIC to imipenem (accounted for 60%).

Most strains exhibited resistance to cephalosporins and sulfonamides, the resistance rates to ceftriaxone, ceftazidime, cefepime and trimethoprim/sulfamethoxazole were 100%, 100%, 80% and 93.3% respectively. However, the isolates showed sensitivity to aminoglycosides, fluoroquinolones and beta-lactamase inhibitor combination, the resistance rates to gentamicin, tobramycin, ciprofloxacin and piperacillin/tazobactam were 56.7%, 16.7%, 13.3% and 20% respectively. No full resistance to tigecycline and polymyxin B was observed among the tested strains (Table 2).

### Carbapenemase characterisation

All isolates were screened for carbapenemase production using phenotypic and molecular methods. The MHT yielded positive results in 25 of the 30 isolates, including 23K. pneumoniae (KPN1-KPN3, KPN5-KPN24), one strain of *Enterobacter cloacae* (ECL4) and one strain of *K. oxytoca* (KOX2), indicating the presence of a carbapenemase.

**Fig 1. Spatio-temporal description of patients infected with carbapenem-non-susceptible Enterobacteriaceae (n=30)**



By PCRs and subsequent sequencing,  $bla_{IMP-4}$  genes were detected in 11 strains isolated from the patients before May 2011, while isolates from twelve patients had  $bla_{IMP-8}$  genes detected. No  $bla_{KPC}$ ,  $bla_{VIM}$ ,  $bla_{OXA}^{-48}$ , and  $bla_{NDM}^{-1}$  genes were amplified positive in all strains (Table 2).

#### Nucleotide sequence accession number

The sequence of the  $bla_{IMP-4}$ -containing plasmid in *K. pneumoniae* was submitted to GenBank under accession no. JQ808503, the  $bla_{IMP-4}$ -containing plasmid in *K. oxytoca* under accession no. JQ820404. And the sequence of the  $bla_{IMP-8}$ -containing plasmid in *K. pneumoniae* was sub-

mitted to GenBank under accession no. JQ820405, the  $bla_{IMP-8}$ -containing plasmid in *E. cloacae* under accession no. JQ820406.

#### Phylogenetic analysis

PFGE of 24 *K. pneumoniae* isolates identified 6 pulsotypes, pulsotype (PT) PT1 to PT6 using 80% similarity as the cut-off. PT1 and PT2 were the major cluster, included 8 isolates (KPN2, KPN3, KPN5, KPN7, KPN8, KPN10-KPN12) and 12 isolates (KPN13-KPN24) respectively. PT3-6 included the other 4 isolates (KPN1, KPN4, KPN6, KPN9). PFGE revealed that two *K. oxytoca* had an indistinguishable PFGE pattern, while four *E. cloacae* were different strains.

**Table 2. Antimicrobial susceptibility profiles of carbapenem-non-susceptible Enterobacteriaceae**

Isolate <sup>a</sup>	MH T <sup>b</sup>	Genotype	Antimicrobial agent and MIC( $\mu\text{g}/\text{mL}$ )								Trimethoprim / sulfamethoxazole	
			Ertapenem	Imipenem	Gentamicin	Tobramycin	Piperacillin/tazobactam	Ceftriaxone	Ceftazidime	Cefepime		Ciprofloxacin
KPN1	+	bla <sub>IMP-4</sub>	4	8	≤1	≤1	8	≥64	≥64	32	≤0.25	≥320
KPN2	+	bla <sub>IMP-4</sub>	≥8	2	≥16	8	64	≥64	≥64	≥64	2	≥320
KPN3	+	bla <sub>IMP-4</sub>	4	≤1	≥16	8	8	≥64	≥64	≥64	1	≥320
KPN4	-	None	4	2	≥16	8	8	≥64	≥64	32	1	≥320
KPN5	+	bla <sub>IMP-4</sub>	4	≤1	≥16	8	8	≥64	≥64	8	0.5	≥320
KPN6	+	None	4	8	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN7	+	bla <sub>IMP-4</sub>	≥8	≤1	≥16	8	64	≥64	≥64	16	2	≥320
KPN8	+	bla <sub>IMP-4</sub>	4	≤1	≥16	8	32	≥64	≥64	≥64	2	≥320
KPN9	+	None	4	≤1	≥16	8	16	≥64	≥64	≥64	2	≥320
KPN10	+	bla <sub>IMP-4</sub>	≥8	≤1	≥16	8	8	≥64	≥64	8	1	≥320
KPN11	+	bla <sub>IMP-4</sub>	4	≤1	≥16	8	8	≥64	≥64	8	2	≥320
KPN12	+	bla <sub>IMP-4</sub>	4	2	≥16	8	8	≥64	≥64	≥64	2	≥320
KPN13	+	None	4	≤1	≥16	8	8	≥64	≥64	8	1	≥320
KPN14	+	bla <sub>IMP-8</sub>	4	≤1	≥16	8	≥128	≥64	≥64	≥64	1	≥320
KPN15	+	bla <sub>IMP-8</sub>	4	4	2	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN16	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN17	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN18	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN19	+	bla <sub>IMP-8</sub>	4	2	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN20	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN21	+	bla <sub>IMP-8</sub>	4	8	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN22	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN23	+	bla <sub>IMP-8</sub>	4	4	4	8	8	≥64	≥64	≥64	≤0.25	≥320
KPN24	+	bla <sub>IMP-8</sub>	4	2	≤1	8	8	≥64	≥64	16	≤0.25	≥320
ECL1	-	None	4	≤1	≥16	≥16	≥128	≥64	≥64	≥64	≥4	≥320
ECL2	-	None	4	≤1	≥16	≥16	≥128	≥64	≥64	≥64	≥4	≤20
ECL3	-	None	4	≤1	≥16	≥16	≥128	≥64	≥64	≥64	0.5	≤20
ECL4	+	bla <sub>IMP-8</sub>	≥8	4	≤1	≤1	8	≥64	≥64	32	≤0.25	≥320
KOX1	-	bla <sub>IMP-4</sub>	≥8	8	≥16	≥16	≥128	≥64	≥64	≥64	≥4	80
KOX2	+	bla <sub>IMP-4</sub>	≥8	≥16	≥16	≥16	≥128	≥64	≥64	≥64	≥4	≥320

**Discussion**

The emergence of *Enterobacteriaceae* harboring IMP-4 or IMP-8 carbapenemases described in this report faced two different outbreaks in a tertiary hospital in China. Although cases from other cities in China have been reported, all of these reports were individually IMP-4 or IMP-8 genotype, and the number of cases were small<sup>16,24-28</sup>. This occurrence included a variety of *Enterobacteriaceae*, which was mainly *K. pneumoniae*. Two kinds of PEGF types that presented by *K. pneumoniae* originated in the NICU, which could have been the source of such strains. Infection control departments were focused on monitoring the depart-

ments during the occurrence, and had achieved significant results. Together with the indistinguishable PFGE pattern, this strongly suggests that this mechanical ventilation represented a persistent source of contamination. The source of the IMP-producing *K. oxytoca* could not be fully elucidated, although the facts suggest that the PFGE pattern was closely related. Because the two patients were transferred to our hospital after post-operative infection in other hospitals. Four *E. cloacae* had a long separation time interval, and PFGE proved to be a different pattern, the source was not easy to determine.

The main characteristics of this occurrence are that patients were mainly from ICU and had suffered from severe underlying diseases which were treated with invasive procedure. Patients suffering from severe underlying disease are routinely and extensively given broad-spectrum anti-infective agents as prophylaxis or targeted therapy. Broad-spectrum antibiotic therapy exerts a selective pressure towards resistant organisms and affects the normal body flora. Furthermore, intensive care medicine, including use of medical devices, allows pathogens to colonize or infect patients.

In an outbreak described by Yan et al.<sup>24</sup>, sixteen patients were detected to carry *bla*<sub>IMP-8</sub> and all infections were nosocomially acquired. The organisms caused wound infections in eight patients and bloodstream infections in three patients. They were not directly associated with the death of nine patients<sup>24</sup>. The outcome of our patients seemed to be better compared to the previously reported data. As the isolates were broadly resistant to most antibiotic classes, various combination therapies were used with little effect according to the *in vitro* susceptibility results. Fortunately, most patients were successfully treated with quinolones guided by the antimicrobial susceptibility, and the other patients were also successfully treated with broad-spectrum cephalosporins and carbapenem in spite of no reliable *in vitro* activity. Maybe it could be explained based on principles of PK/PD and clinical data, appropriate drainage in surgical and prompt removal of ventilator in NICU lifted the source of bacterial infection effectively. The favorable clinical outcomes experienced by the patients treated with beta-lactam antibiotics have been previously reported<sup>29</sup>, but the degree to which the antibiotic treatment contributed to these outcomes is difficult to ascertain. In the United States, three *K. pneumoniae* harboring IMP-4 carbapenemase were recovered from pediatric patients, and these isolates had a similar resistance with our strains<sup>30</sup>. Good clinical response to antibiotics with no *in vitro* activity might have been achieved by the increased antimicrobial concentration.

MHT is CLSI recommended method to detect carbapenem of *Enterobacteriaceae*, and has a good sensitivity and specificity to detect KPC but still uncertain to Ambler B metals enzyme<sup>19</sup>. Doyle et al.<sup>31</sup> reported that MHT had a good effect to detect KPC and OXA-48 carbapenemase, but was insufficient to the metal enzyme IMP, VIM and NDM-1. Our study demonstrated that MHT had a high sensitivity to detect IMP-4- or IMP-8-type carbapen-

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emase, that confirmed MHT can effectively detect *Enterobacteriaceae* producing IMP-type carbapenemases. The false-positive results of MHT of the CPE isolates may be attributed to the high level AmpC or ESBLs enzymes producers combined with loss or decreased expression of major porins<sup>32</sup>. It should be noted that early detection of carbapenem resistant isolates is of utmost importance in allowing adequate antimicrobial therapy to be initiated and in avoiding cross-transmission. Thus, suspicion must be high in multi-resistant enterobacterial isolates with elevated MICs to ertapenem. MICs derived by the Vitek 2 system were usually comparably low for imipenem and meropenem, whereas testing with microbroth dilution revealed higher MICs. This problem has been reported previously<sup>33</sup>. However, the Vitek 2 system is widely used in Chinese laboratories, and if included in the testing panel such as AST-GN13, ertapenem may serve as an indicator for the detection of IMP carbapenemase. A study recommended using molecular tests for the optimal detection of carbapenemase-producing *Enterobacteriaceae*<sup>31</sup>. When obtaining the possibility of CPE strains, they should be promptly examined by PCR to confirm the genotype.

IMP-type metalloenzymes have been reported worldwide, with a higher prevalence in southern Europe and Asia. The first acquired MBL, IMP-1, was reported in *Serratia marcescens* in Japan in 1991<sup>34</sup>. Since then, IMP-type enzymes has been described worldwide, and endemicity of IMP-type enzymes has been reported in many countries<sup>35</sup>. However, Most MBL producers are hospital acquired and multidrug-resistant *K. pneumoniae*<sup>36</sup>. The first metallo- $\beta$ -lactamase reported from China was IMP-4, which was detected on a plasmid in *C. youngae* and reported in a 2001 publication<sup>36</sup>, followed by reports of IMP-1 and IMP-9 in a *P. aeruginosa* isolate<sup>37,38</sup>.

In an outbreak described by Peleg et al.<sup>39</sup>, five different gram-negative genera from patients with close epidemiological links were involved. It showed *bla*<sub>IMP-4</sub> appearing highly mobile in different gram-negative strains, which brought greater challenges to infection control. Additional cross-transmission was then prevented by strict patient isolation, intensified hand disinfection and the routine analysis of targeted surveillance cultures. Preventive contact precautions should be carried out for such patients until the negative results of microbiology studies have been obtained. Although we may have terminated the outbreak in our institution, the high number of contact patients that resulted from long hospitalisation times and frequent transferring of the highly care-dependant patients makes it unlikely that the establishment of IMP in China could be prevented.

## Conclusion

CPE harboring IMP-4 or IMP-8 carbapenemase strains had emerged in our hospital. Rapid dissemination of CPE was a serious concern in clinical patient care and these pathogens were now also present in China. Therefore, using surveillance cultures and initiating strict hygiene procedures is mandatory for the prevention and early detection of carbapenem-resistant *Enterobacteriaceae* in units where high-risk patients receive care.

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## Declaration of interest

None.

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