

Molecular Epidemiology of Clonally Related Metallo- β -Lactamase-Producing *Klebsiella pneumoniae* Isolated from Newborns in a Hospital in Shandong, China

Yan Jin,¹ Chunzhong Dong,¹ Chunhong Shao,¹ Yong Wang,¹ and Yun Liu^{1*}

¹Clinical Laboratory of Provincial Hospital affiliated with Shandong University, Jinan, Shandong, China

*Corresponding author: Yun Liu, 324 Jingwu Road, Jinan, Shandong, China. Tel/Fax: +86-53168776574, E-mail: lyunsd@126.com

Received 2016 December 24; Revised 2017 May 25; Accepted 2017 June 24.

Abstract

Background: The infection caused by metallo- β -lactamase (MBL)-producing *Klebsiella pneumoniae* has become a challenge to anti-infection treatments for newborns.

Objectives: The aim of this study is to investigate the molecular epidemiological characteristics of clonally related MBL-producing *K. pneumoniae* isolated from newborns in a hospital in Northern China.

Methods: A total of 16 isolates of carbapenem-resistant *K. pneumoniae* were obtained from newborns between September 2011 and June 2014. Resistance genes were identified by PCR and sequencing. The isolates were classified by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

Results: Our results show that the most prevalent genotypes of carbapenem-resistant *K. pneumoniae* were ST54 and ST705. There were respectively 6 strains and 9 strains of *K. pneumoniae* ST54 harboring the *bla*_{NDM-1} and *bla*_{IMP-4} genes, while the *K. pneumoniae* ST705 harbored the *bla*_{IMP-4} gene. Other resistance genes included extended-spectrum β -lactamases, plasmid-mediated AmpC enzymes and a class 1 gene cassette. The transferability of metallo- β -lactamase was determined by conjugation experiments, which showed that the *bla*_{NDM-1} and *bla*_{IMP-4} genes were transferable and likely through a plasmid-mediated event.

Conclusions: Our results showed that New Delhi metallo-beta-lactamase-1 (NDM-1) and imipenemase-4 (IMP-4) carbapenemases were the major players responsible for the carbapenem-resistance. Co-production of NDM-1 with CTX-M-15 in *K. pneumoniae* isolates was detected for the first time in our neonatal intensive care unit. Early detection of these drug resistance genes will help in prevention and control of the infection of *K. pneumoniae*.

Keywords: Molecular Epidemiology, Beta-Lactamases, Infant, Newborn, *Klebsiella pneumoniae*

1. Background

Neonates have high risk for infection of *Klebsiella pneumoniae* due to the undeveloped body physiology, resulting in high morbidity and mortality (1, 2). Approximately one million neonatal deaths are caused by infection, which is frequently associated with contaminated medical devices and instrumentation. Factors, like high occupation rates and extensive use of antibiotics, are inevitably leading to the prevalence of drug-resistant strains. As one of the most frequently isolated bacteria in a hospital setting, *K. pneumoniae* is resistant to many antimicrobial agents by producing a variety of drug-resistance genes, including extended spectrum β -lactamases (ESBLs), AmpC β -lactamases, 16S rRNA methylases, aminoglycoside modifying enzymes and carbapenemases (3).

The increasing number of strains harboring extended-spectrum β -lactamase-producing *Enterobacteriaceae* (resistant to penicillins, cephalosporins and monobactams) and AmpC β -lactamases is a major treatment challenge.

Given the side-effects of antibiotics in newborns, carbapenems are often the only therapeutic option for treating severe infection caused by multidrug-resistant *Enterobacteriaceae* (4). In recent years, carbapenem-resistant *Enterobacteriaceae* in infants and children has become an emerging nosocomial pathogen worldwide.

Carbapenem-resistance is primarily mediated by the production of carbapenemases, which hydrolyze β -lactam of all classes, including the most potent class, carbapenems (5). Molecular structural classifications of carbapenemases include Ambler class A, B and D. The metallo- β -lactamases (MBLs) belong to class B carbapenemases that includes Verona integron-encoded MBL (VIM), IMP (IMP-type MBL) or New Delhi MBL (NDM) (6). Carbapenemases carried on mobile genetic elements, such as transposons or plasmids harboring resistance genes, can transfer into strains capable of efficient human to human transmission (7). The carbapenemase New Delhi metallo-beta-lactamase-1 (NDM-1) is one of the most recently reported MBLs. It was originally identified in India, but now has a global distri-

bution (8).

A few studies on spread of carbapenemase-producing *K. pneumonia* in newborns have been reported. In August 2011, a nosocomial outbreak of NDM-1-producing *K. pneumonia* was reported in Colombia involving six patients who were admitted to the neonatal unit of a general hospital in Bogota (9). An outbreak of carbapenemase-producing *K. pneumoniae* sequence type (ST) 258 was investigated in a neonatal intensive care unit (NICU) in Palermo, Italy and carbapenemase-producing *K. pneumoniae* was isolated from 10 out of 54 neonates admitted in the outbreak period (10). In China, an outbreak caused by imipenemase-4 (IMP-4)-producing *K. pneumonia* occurred in a neonatal intensive care unit in 2011 (11). Another outbreak of colonization by carbapenemase-producing *K. pneumoniae* occurred in a neonatal intensive care unit of Peking Union Medical College Hospital, China in 2015 (2).

2. Objectives

In this study, our efforts were focused on the 16 isolates from newborns because it poses a more serious problem than adults. The molecular epidemiology of clonally related metallo- β -lactamase-producing *K. pneumoniae* was investigated by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The strains were subjected to antimicrobial susceptibility testing and the expression of carbapenemases was investigated to understand the mechanisms responsible for the emergence of carbapenem resistance. Furthermore, plasmid typing was carried out to study the transferability of carbapenem resistance.

3. Methods

3.1. Ethics Statement

Samples were collected during routine check-ups by medical professionals. The study was approved by the Ethics Committee of Provincial Hospital affiliated with Shandong University and carried out in accordance with the approved guidelines.

3.2. Collection of Isolates

A total of 16 *K. pneumonia* isolates were recovered from newborns (age of 2 to 28 days) hospitalized in our hospital between September 2011 and June 2014. Hand and nasal swabs were collected from the doctors and nursing staff in the neonatal unit and intensive care unit (ICU), as well as the environmental samples cultured with a swab pre-moistened with sterile saline. The sites for

collecting environmental samples included the bed railing, stethoscope, weighing machine, milk powder, suction apparatus, wash basin, radiant warmer, washing area, electric switch, medicine tray and ventilator machine. All isolates were identified on a VITEK-2 compact system (bioMérieux, Marcy l'Etoile, France) and screened for carbapenemase production by the modified Hodge test (MTH) and ertapenem-EDTA double-disc synergistic test (12).

3.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was determined using the VITEK-2 system (bioMérieux, Marcy l'Etoile, France). The susceptibility of meropenem, imipenem, ertapenem, tigecycline, colistin and fosfomycin were verified by the E-test (bioMérieux, Marcy l'Etoile, France) method on a Mueller-Hinton agar (Oxoid, Hampshire, United Kingdom). All antibiotics were purchased from Sigma, St Louis, USA. The susceptibility data were interpreted according to the Clinical and Laboratory Standards Institute guidelines, except for colistin and tigecycline, which were interpreted by the 2011 European committee on antimicrobial susceptibility testing break points (available at http://www.eucast.org/clinical_breakpoints/). *Escherichia coli* ATCC25922 was used for quality control in antimicrobial susceptibility testing.

3.4. Drug-Resistance Genes

Isolates were screened by polymerase chain reaction (PCR) for the presence of the molecular class A carbapenemases (*bla*_{KPC}, *bla*_{GES}, *bla*_{SME}, *bla*_{NMC}, and *bla*_{IMI}) (13-17), class B MBL (*bla*_{IMP}, *bla*_{VIM} and *bla*_{NDM}) (14, 18, 19), class D OXA β -lactamases (*bla*_{OXA-48}) (15), ESBL genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}), plasmid-mediated AmpC β -lactamase genes (*bla*_{ACC}, *bla*_{FOX}, *bla*_{MOX}, *bla*_{DHA}, *bla*_{CIT}, and *bla*_{EBC}) and integron (Int1, Int2, Int3) (20-22). All PCR-positive products were sequenced by Shanghai Majorbio Bio-Pharm technology Co. (Shanghai, China), and the results were compared to the sequences available from GenBank (www.ncbi.nlm.nih.gov/blast/).

3.5. Resistance Gene Transfer Experiments

Conjugation transfer assay was performed using azide-resistant *Escherichia coli* J53 as the recipient strain. Briefly, overnight cultures of the donor strain (100 mL) and recipient strain (200 mL) were mixed with 4 mL fresh Mueller-Hinton broth and incubated at 35°C for 24 hours. The mixture was inoculated on MacConkey agar plates containing sodium azide (100 μ g/mL) and imipenem (0.5 μ g/mL) at 35°C for 24 hours. Conjugation was confirmed by Indole testing (23). Transconjugants were subjected to PCR to determine the presence of the carbapenemase genes.

3.6. Plasmid Profiling

Bands of high-molecular-weight plasmids were visualized for each isolate by PFGE followed by the S1 nuclease (Takara, Tokyo, Japan) treatment of the genomic DNA embedded in agarose. The DNA fragments were separated on a Chef-DrIII apparatus at 14°C for 20 hours, with a voltage of 6 V/cm, and an initial and final pulse time of 2.16 and 63.8 seconds, respectively (24). The gel was recovered to purify the plasmid bands for further sequencing.

3.7. Genotypes and Homology of Drug-Resistance Isolates

DNA fingerprinting was performed by pulsed-field gel electrophoresis (PFGE). DNA fragments were separated on 1% agarose gels (SeaKem Gold Agarose, Rockland, ME) using a Chef Mapper apparatus (Bio-Rad, Hercules, CA). Electrophoresis was conducted in Tris-boric acid-EDTA (TBE) buffer (0.5 ×) at 14°C and a 6 V/cm gradient with alternating pulses at 120°C and a 2 - 40 seconds pulse time gradient for 22.5 hours. The *Salmonella enterica* serotype Braenderup H9812 was used as the DNA marker (24). Multi-locus sequence typing (MLST) was performed with seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) according to the protocol described on the *K. pneumoniae* MLST website

(<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>).

Experimentally determined DNA sequences were uploaded to the MLST database and allelic numbers and sequence types (STs) were obtained.

4. Results

4.1. Molecular Epidemiology of *K. pneumoniae*

On Sept 2, 2011, the first case of *K. pneumoniae* was identified from a sputum sample in the pediatric intensive care unit (PICU) of our hospital, China. The patient was diagnosed with pneumonia on admission and was admitted to PICU due to heart failure. After symptoms were relieved, the patient was transferred to the neonatal unit. Breathing machines and trachea cannula were used during the treatment period in the PICU. On Dec 30, 2011, the second case of *K. pneumoniae* was identified in the neonatal unit from a blood sample. Eight strains were isolated from the neonatal unit and the PICU since May 30, 2012, which prompted the initiation of active surveillance of all nurses, doctors and environments in the wards. However, negative surveillance results were obtained. The spread of metallo- β -lactamases-producing *K. pneumoniae* (CR-KP) was controlled temporarily by a restricted visitor policy and daily cleaning of all rooms. However, CR-KP strains were

isolated from newborns again two months later. Seven patients infected with *K. pneumoniae* were identified from Oct 2012 to Oct 2013.

In total, 16 isolates were obtained from the neonates in the neonatal unit and PICU in this hospital, during this period, including 9 boys and 7 girls. Table 1 lists the clinical characteristics of these 16 *K. pneumoniae*, including isolation date, specimen and ward distribution. Eighteen patients suffered from pneumonia, two with umbilical infection and one with coronary heart disease. There were 14 premature patients with low birth weight and five were intrauterine infection at birth. During hospitalization, all patients were treated with multiple courses of antimicrobial drugs including cloxacillin, cefthiamidine, ceftizoxime and cefoperazone/tazobactam. Among these patients, ten were treated with carbapenems (imipenem or meropenem).

Molecular typing by PFGE (Figure 1) identified 5 major PFGE groups (A to E), among which, type A is the predominant type; 11 isolates from the neonates belonged to type A and all of them were detected in the neonatal unit. The single strain belonging to type B and E was detected in the neonatal unit too. Three strains were isolated from the PICU, two belonged to type C and one to type E.

Genotyping was carried out by MLST, by amplifying and sequencing seven housekeeping genes. Four different STs were identified, including ST20, ST54, ST290 and ST705 (Figure 1). The MLST results were in good agreement with the PFGE profiles, with the ST54 being the most predominant type. All ST54 isolates were derived from the neonatal unit, out of which, only one strain belonged to PFGE type B and the rest was PFGE type A. ST290 was isolated from the neonatal unit and corresponding to PFGE type D. Two ST705 isolates were from the PICU, which were corresponding to PFGE type C; the other isolate from the PICU were PFGE type E.

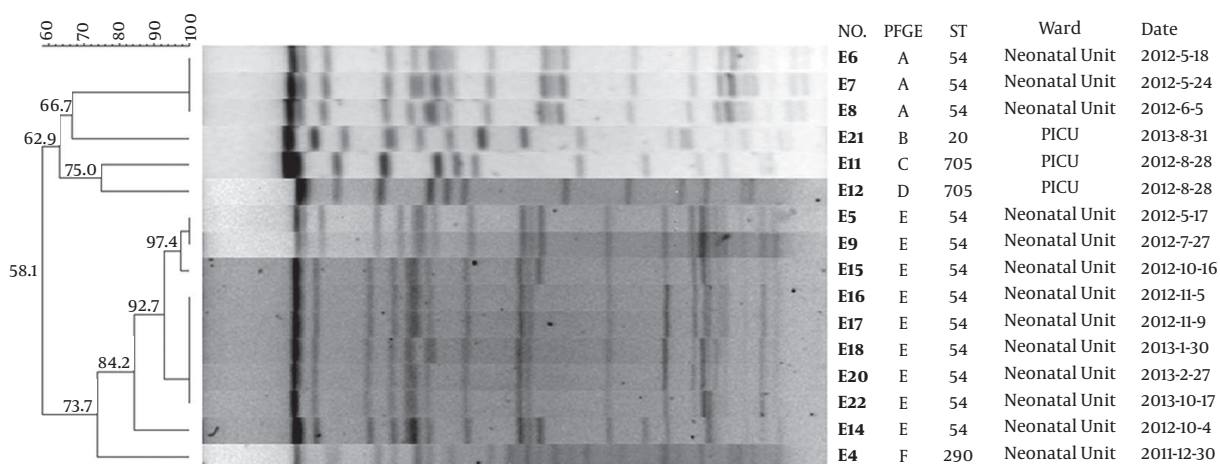
4.2. Antimicrobial Susceptibility and Carbapenem Resistance Mechanism

All isolates were resistant to multiple drugs, including cefotaxime, ceftazidime, cefepime, piperacillin-tazobactam and ertapenem. Tigecycline and colistin showed strong activity against *K. pneumoniae* with a susceptibility rate of 100% (Table 2). Three strains that showed low MICs of ertapenem were susceptible to meropenem and imipenem. All isolates from the neonatal unit were resistant to β -lactam antibiotics, but susceptible to trimethoprim-sulfamethoxazole, fosfomycin and amikacin. Strains E11 and E12 from the PICU belonging to ST705 were susceptible to aztreonam, but resistant to fosfomycin, which were the opposite of other strains. All isolates were resistant to ertapenem (MICs > 2 mg/L) and

Table 1. The Molecular Characteristics of 16 Metallo- β -Lactamases-Producing *K. pneumoniae* (CR-KP)

Isolate No.	Patient Age (d)	Sex	Ward	Isolate Date	Specimen	PFGE Pattern	ST	EDTA	HIM	Resistance Gene
E4	10	M	Neonatal unit	2011-12-30	blood	D	290	+	-	IMP-8, TEM-1, CTXM-14
E5	28	F	Neonatal unit	2012-5-17	sputum	A	54	+	-	NDM-1, TEM-1, CTXM-14, DHA-1
E6	23	F	Neonatal unit	2012-5-18	sputum	A	54	+	-	NDM-1, TEM-1, CTXM-14, DHA-1
E7	18	M	Neonatal unit	2012-5-24	sputum	A	54	+	-	NDM-1, TEM-1, CTXM-14, DHA-1
E8	2	F	Neonatal unit	2012-6-5	sputum	A	54	+	-	NDM-1, TEM-1, CTXM-14, DHA-1
E9	13	M	Neonatal unit	2012-7-27	sputum	A	54	+	-	NDM-1, TEM-1, CTXM-14, DHA-1
E11	13	M	PICU	2012-8-28	sputum	C	705	+	+	IMP-4, TEM-1, CTXM-14
E12	5	M	PICU	2012-8-28	sputum	C	705	+	-	IMP-4, TEM-1, CTXM-14
E14	14	F	Neonatal unit	2012-10-4	sputum	B	54	+	-	IMP-4, TEM-1, CTXM-15, DHA-1
E15	1	F	Neonatal unit	2012-10-16	sputum	A	54	+	-	IMP-4, TEM-1, CTXM-15, DHA-1
E16	3	M	Neonatal unit	2012-11-5	umbilicus	A	54	+	-	IMP-4, TEM-1, CTXM-15
E17	4	F	Neonatal unit	2012-11-9	sputum	A	54	+	-	IMP-4, TEM-1, CTXM-15, DHA-1
E18	10	M	Neonatal unit	2013-1-30	sputum	A	54	+	-	IMP-4, TEM-1, CTXM-15
E20	16	M	Neonatal unit	2013-2-27	umbilicus	A	54	+	-	IMP-4, TEM-1, CTXM-15, DHA-1
E21	25	F	PICU	2013-8-31	sputum	E	20	+	-	NDM-1, TEM-1, CTXM-15, DHA-1
E22	17	M	Neonatal unit	2013-10-17	sputum	A	54	+	-	IMP-4, TEM-1, CTXM-15, DHA-1

Abbreviations: CTXM-14, CTX-M-14 Lactamase; CTXM-15, CTX-M-15 Lactamase; DHA-1, DHA-1 Beta-Lactamase; EDTA, EDTA Synergistic Test; F, Female; HIM, Modified Hodge Test; IMP-4, Imipenemase-4, IMP-8, Imipenemase-8; M, male; NDM-1, New Delhi Metallo-Beta-Lactamase-1; PICU, Pediatric Intensive Care Unit; ST, Sequence Type; TEM-1, TEM-1 Beta-Lactamase.

Figure 1. Dendrogram Analysis of *K. pneumoniae* Strains

The dendrogram was generated with the Fingerprinting II Informatix software package (Bio-Rad Laboratories, Hercules, CA) showing fingerprint relationships (XbaI-PFGE) for 16 *K. pneumoniae* strains. The phylogenetic tree was constructed using the Dice coefficient and UPGMA clustering. A genetic similarity index scale is shown in the left of the dendrogram. The PFGE types, sequence type, strain number, collection date and ward distribution are included in each PFGE lane.

were positive in the EDTA synergistic test. Five of these were positive or weakly positive in the Modified Hodge test (MHT).

Among the carbapenem resistant *K. pneumoniae* strains, 6 harbored the *bla*_{NDM-1} gene and 12 harbored the *bla*_{IMP-4} gene. In particular, the E10 strain carried both genes. Other β -lactamases (*bla*_{CTX-M-15}, *bla*_{CTX-M-14}, *bla*_{DHA-1} and *bla*_{TEM-1}) were also present in 7 (41.2%), 7 (41.2%), 12 (70.6%) and 17 (100%) strains, respectively. *Kleb-*

siella pneumoniae ST705 harbored the same resistant genes including *bla*_{IMP-4}, *bla*_{TEM-1}, and *bla*_{CTX-M-14} as well as the class 1 gene cassette.

4.3. Conjugative Transfer of Carbapenem Resistance and Plasmid Typing

PFGE analysis revealed that all *K. pneumoniae* ST54 strains contained three plasmids of ~300 kb, ~200 kb and ~140 kb. The ST705 strain contained two plasmids of

Table 2. Minimum Inhibitory Concentrations of the 16 Metallo- β -Lactamases-Producing *k. pneumoniae* (mg/L)

Isolate No.	EPT	IMP	MEM	SXT	TZP	KZ	CTN	CAZ	CRO	FEP	AZM	AK	GN	TOB	CIP	LEV	TGC	CO	FOS
E4	16	3	2	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	1	1	0.75	0.125	64
E5	> 32	> 32	> 32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	1	1	0.5	0.125	16
E6	> 32	32	> 32	≤ 1	128	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	1	1	0.5	0.125	32
E7	> 32	16	> 32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	1	1	0.75	0.125	32
E8	> 32	> 32	32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	0.5	0.5	0.5	0.125	16
E9	> 32	> 32	> 32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	32	≥ 64	≤ 2	≥ 16	8	1	0.5	0.75	0.125	24
E11	32	8	16	≥ 16	64	≥ 64	≥ 64	≥ 64	≥ 64	16	2	≤ 2	≥ 16	8	1	1	1.5	0.125	1024
E12	> 32	> 32	> 32	≥ 16	64	≥ 64	≥ 64	≥ 64	≥ 64	16	2	≤ 2	≥ 16	8	1	1	0.75	0.125	1024
E14	> 32	4	32	≤ 1	128	≥ 64	≥ 64	≥ 64	≥ 64	32	≥ 64	≤ 2	≥ 16	8	1	0.5	0.75	0.25	12
E15	4	1	0.75	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	32	≥ 64	≤ 2	≥ 16	8	1	0.5	0.5	0.125	8
E16	32	2	32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≤ 2	≥ 16	8	≤ 0.25	≤ 0.25	0.75	0.125	12
E17	32	32	32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≤ 2	≥ 16	8	≤ 0.25	≤ 0.25	0.5	0.125	16
E18	4	0.5	1	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	32	≥ 64	≤ 2	≥ 16	8	≤ 0.25	≤ 0.25	0.5	0.125	24
E20	> 32	> 32	32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≤ 2	≥ 16	8	≤ 0.25	≤ 0.25	0.5	0.125	24
E21	> 32	16	> 32	≤ 1	128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≤ 2	≤ 1	8	1	1	0.75	0.125	64
E22	> 32	32	> 32	≤ 1	64	≥ 64	≥ 64	≥ 64	≥ 64	16	≥ 64	≤ 2	≥ 16	8	≤ 0.25	≤ 0.25	0.5	0.125	32

Abbreviations: AK, Amikacin; ATM, Aztreonam; CAZ, Cefazidime; CIP, Ciprofloxacin; CN, Gentamicin; CO, Colistin; CRO, Ceftriaxone; CTN, Cefatan; EPT, Ertapenem; FEP, Cefepime; FOS, Fosfomicin; IMP, Imipenem; KZ, Cefazolin; LEV, Levofloxacin; MEM, Meropenem; SXT, Trimethoprim-Sulfamethoxazole; TGC, Tigecycline; TOB, Tobramycin; TZP, Piperacillin-Tazobactam.

~ 260 kb and ~ 55 kb. Conjugation experiments of *K. pneumoniae* ST54 failed to demonstrate the transferability of carbapenem-resistance from the isolates to the recipient. On the other hand, the *K. pneumoniae* ST705 transconjugants acquired a single high-molecular-weight plasmid of ~ 55 kb. Gel recovery was used to purify the three plasmid bands of the *K. pneumoniae* ST54 strain and the one plasmid band of the *K. pneumoniae* ST705 transconjugants for further sequencing. The results showed that the *bla*_{NDM-1} and *bla*_{IMP-4} genes carried by the *K. pneumoniae* ST54 strains were located on the 300 kb and 200 kb plasmids; the *bla*_{IMP-4} gene carried by the *K. pneumoniae* ST705 strain was located on the 55kb plasmid. All *E. coli* transconjugants had significantly reduced carbapenem susceptibility and positive EDTA synergistic tests. The recipient carried the same carbapenemase genes as the donor shown by sequencing.

5. Discussion

In this study, we investigated the molecular epidemiology of 16 isolates from newborns in a hospital in Shandong, China. *Klebsiella pneumoniae* is an opportunistic pathogen posing to great public health threat, especially to newborns. Logan reviewed the clinical history of children with *K. pneumoniae* infection and noted that the most common condition was pulmonary disease (30%) and premature birth (27%) (7). According to our clinical data, 18 of 21 newborns suffered pulmonary infection. The most common risk factors contributing to newborn carbapenem-resistant *K. pneumoniae* infection were prematurity, low

body weight, intrauterine infection and extensive use of antimicrobial drugs.

There were 14 premature patients with low birth weight, among which, 5 had intrauterine infection at birth. All patients were treated with multiple courses of β -lactam antibiotics including carbapenems. In this study, two patients died and six showed no improvement after treatment. The spread of carbapenem-resistant *K. pneumoniae* in a hospital is a complex event involving several modes including dissemination of several unrelated strains, the propagation of a single clone from patient to patient and from the environment to patients. There are few reports of outbreaks due to NDM-1-producing *Enterobacteriaceae* (25-28). Patients with infection and asymptomatic colonization are at risk of invasive infection that often leads to a CRE outbreak in hospitals and communities (29).

In this study, genotyping was carried out by MLST, and the results showed 12 of 13 *K. pneumoniae* isolates from the neonatal unit belonged to the same sequence type, ST54. These isolates displayed the same plasmid profile and antibiotic susceptibility. To understand the transmission mechanisms, we examined the location of the *bla*_{IMP-4} and *bla*_{NDM-1} genes on the plasmids. We found that the *bla*_{IMP-4} gene in the ST705 isolates was transferrable to *E. coli* recipients through conjugation, while the plasmids in ST54 isolates failed to transfer. The carbapenem-resistant *K. pneumoniae* strains harbored different types of the *bla*_{CTX-M} genes; the NDM-1-producing strains carried the *bla*_{CTX-M-14} gene, while the IMP-4-producing strains

carried the *bla*_{CTX-M-15} gene. The ST705 strains were isolated from the PICU, which exhibited a different pattern of antibiotic susceptibility. Specifically, the ST705 were susceptible to aztreonam, but resistant to trimethoprim-sulfamethoxazole and fosfomycin. The sequencing data showed that both ST types harbored the same resistance genes, including *bla*_{IMP-4}, *bla*_{TEM-1}, and *bla*_{CTX-M-14} as well as the class 1 gene cassette.

Metallo- β -lactamase (MBL)-producing *K. pneumoniae* is resistant to all β -lactam agents except aztreonam (30). The ST54 isolates in this study were aztreonam-resistant because they harbored the extended spectrum β -lactamase CTX-M-15. On the other hand, although the ST705 strains also harbored an extended spectrum β -lactamase, these strains were susceptible to aztreonam because the ESBL (CTX-M-14) is less active in hydrolytic activity than CTX-M-15 (31).

5.1. Conclusion

In summary, we investigated the molecular epidemiology of 16 isolates from newborns in a hospital in Shandong, China. The molecular typing was carried out by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Four ST types were identified, including ST54, ST705, ST20 and ST290. The expression of carbapenemases and the plasmid transferability between ST54 and ST705 were investigated to understand the difference of the antimicrobial susceptibility. To the best of our knowledge, this study is the first report of an outbreak of NDM-1-producing *K. pneumoniae* in newborns in China. The emergence of the carbapenem-resistant *K. pneumoniae* isolates underscores the difficulty in eliminating multidrug-resistance bacteria. The outcome of neonatal infections can be improved if the symptoms are identified early and appropriate measures are applied.

Acknowledgments

This study was supported by Shandong province science and technology development program of China (NO. 2012G0021839), the national natural science foundation of China (NO. 81401696), and Shandong provincial primary research and development plan (2016GSP201078).

Footnotes

Authors' Contribution: Study concept and design, Yan Jin, Chunzhong Dong and Yun Liu; acquisition of data, Yan Jin, Chunhong Shao, and Yun Liu; analysis and interpretation of data, Yan Jin, Yong Wang and Yun Liu; drafting of the manuscript, Yan Jin and Yun Liu.

Conflicts of Interest: The authors declare no potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding/Support: This study was supported by Shandong province science and technology development program of China (NO. 2012G0021839), the national natural science foundation of China (NO. 81401696), and Shandong provincial primary research and development plan (2016GSP201078).

References

- Bennet R, Eriksson M, Zetterstrom R. Bacterial etiology of neonatal septicemia in relation to prior antibiotic treatment. *Acta Paediatr Scand.* 1987;76(4):673-4. doi: 10.1111/j.1651-2227.1987.tb10543.x. [PubMed: 3630687].
- Zhou J, Li G, Ma X, Yang Q, Yi J. Outbreak of colonization by carbapenemase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit: Investigation, control measures and assessment. *Am J Infect Control.* 2015;43(10):1122-4. doi: 10.1016/j.ajic.2015.05.038. [PubMed: 26149749].
- Ruppe E, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Ann Intensive Care.* 2015;5(1):61. doi: 10.1186/s13613-015-0061-0. [PubMed: 26261001].
- Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment Options for Carbapenem-Resistant Enterobacteriaceae Infections. *Open Forum Infect Dis.* 2015;2(2):ofv050. doi: 10.1093/ofid/ofv050. [PubMed: 26125030].
- Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev.* 2007;20(3):440-58. doi: 10.1128/CMR.00001-07. [PubMed: 17630334] table of contents.
- Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci.* 1980;289(1036):321-31. [PubMed: 6109327].
- Logan LK. Carbapenem-resistant enterobacteriaceae: an emerging problem in children. *Clin Infect Dis.* 2012;55(6):852-9. doi: 10.1093/cid/cis543. [PubMed: 22700827].
- Bushnell G, Mitrani-Gold F, Mundy LM. Emergence of New Delhi metallo-beta-lactamase type 1-producing enterobacteriaceae and non-enterobacteriaceae: global case detection and bacterial surveillance. *Int J Infect Dis.* 2013;17(5):e325-33. doi: 10.1016/j.ijid.2012.11.025. [PubMed: 23332300].
- Escobar Perez JA, Olarte Escobar NM, Castro-Cardozo B, Valderrama Marquez IA, Garzon Aguilar MI, Martinez de la Barrera L, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. *Antimicrob Agents Chemother.* 2013;57(4):1957-60. doi: 10.1128/AAC.01447-12. [PubMed: 23357776].
- Giuffre M, Bonura C, Geraci DM, Saporito L, Catalano R, Di Noto S, et al. Successful control of an outbreak of colonization by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* sequence type 258 in a neonatal intensive care unit, Italy. *J Hosp Infect.* 2013;85(3):233-6. doi: 10.1016/j.jhin.2013.08.004. [PubMed: 24074641].
- Yu F, Ying Q, Chen C, Li T, Ding B, Liu Y, et al. Outbreak of pulmonary infection caused by *Klebsiella pneumoniae* isolates harbouring blaIMP-4 and blaDHA-1 in a neonatal intensive care unit in China. *J Med Microbiol.* 2012;61(Pt 7):984-9. doi: 10.1099/jmm.0.043000-0. [PubMed: 22466031].
- Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect.* 2001;7(2):88-91. [PubMed: 11298149].

13. Aubron C, Poirel L, Ash RJ, Nordmann P. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. *Emerg Infect Dis*. 2005;**11**(2):260–4. doi: [10.3201/eid1102.030684](https://doi.org/10.3201/eid1102.030684). [PubMed: [15752444](https://pubmed.ncbi.nlm.nih.gov/15752444/)].
14. Bradford PA, Bratu S, Urban C, Visalli M, Mariano N, Landman D, et al. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin Infect Dis*. 2004;**39**(1):55–60. doi: [10.1086/421495](https://doi.org/10.1086/421495). [PubMed: [15206053](https://pubmed.ncbi.nlm.nih.gov/15206053/)].
15. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J Antimicrob Chemother*. 2010;**65**(3):490–5. doi: [10.1093/jac/dkp498](https://doi.org/10.1093/jac/dkp498). [PubMed: [20071363](https://pubmed.ncbi.nlm.nih.gov/20071363/)].
16. Queenan AM, Torres-Viera C, Gold HS, Carmeli Y, Eliopoulos GM, Moellering RJ, et al. SME-type carbapenem-hydrolyzing class A beta-lactamases from geographically diverse *Serratia marcescens* strains. *Antimicrob Agents Chemother*. 2000;**44**(11):3035–9. [PubMed: [11036019](https://pubmed.ncbi.nlm.nih.gov/11036019/)].
17. Radice M, Power P, Gutkind G, Fernandez K, Vay C, Famiglietti A, et al. First class A carbapenemase isolated from enterobacteriaceae in Argentina. *Antimicrob Agents Chemother*. 2004;**48**(3):1068–9. doi: [10.1128/AAC.48.3.1068-1069.2004](https://doi.org/10.1128/AAC.48.3.1068-1069.2004). [PubMed: [14982814](https://pubmed.ncbi.nlm.nih.gov/14982814/)].
18. Tijet N, Alexander DC, Richardson D, Lastovetska O, Low DE, Patel SN, et al. New Delhi metallo-beta-lactamase, Ontario, Canada. *Emerg Infect Dis*. 2011;**17**(2):306–7. doi: [10.3201/eid1702.101561](https://doi.org/10.3201/eid1702.101561). [PubMed: [21291614](https://pubmed.ncbi.nlm.nih.gov/21291614/)].
19. Zhao WH, Chen G, Ito R, Hu ZQ. Relevance of resistance levels to carbapenems and integron-borne blaIMP-1, blaIMP-7, blaIMP-10 and blaVIM-2 in clinical isolates of *Pseudomonas aeruginosa*. *J Med Microbiol*. 2009;**58**(Pt 8):1080–5. doi: [10.1099/jmm.0.010017-0](https://doi.org/10.1099/jmm.0.010017-0). [PubMed: [19528141](https://pubmed.ncbi.nlm.nih.gov/19528141/)].
20. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem*. 1995;**226**(2):235–40. doi: [10.1006/abio.1995.1220](https://doi.org/10.1006/abio.1995.1220). [PubMed: [7793624](https://pubmed.ncbi.nlm.nih.gov/7793624/)].
21. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother*. 2001;**45**(3):723–6. doi: [10.1128/AAC.45.3.723-726.2001](https://doi.org/10.1128/AAC.45.3.723-726.2001). [PubMed: [11181350](https://pubmed.ncbi.nlm.nih.gov/11181350/)].
22. Levesque C, Piche L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother*. 1995;**39**(1):185–91. doi: [10.1128/AAC.39.1.185](https://doi.org/10.1128/AAC.39.1.185). [PubMed: [7695304](https://pubmed.ncbi.nlm.nih.gov/7695304/)].
23. Maslow JN, Brecher SM, Adams KS, Durbin A, Loring S, Arbeit RD. Relationship between indole production and differentiation of Klebsiella species: indole-positive and -negative isolates of Klebsiella determined to be clonal. *J Clin Microbiol*. 1993;**31**(8):2000–3. [PubMed: [8370726](https://pubmed.ncbi.nlm.nih.gov/8370726/)].
24. Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, Graves L, et al. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J Clin Microbiol*. 2005;**43**(3):1045–50. doi: [10.1128/JCM.43.3.1045-1050.2005](https://doi.org/10.1128/JCM.43.3.1045-1050.2005). [PubMed: [15750058](https://pubmed.ncbi.nlm.nih.gov/15750058/)].
25. Calfee D, Jenkins SG. Use of active surveillance cultures to detect asymptomatic colonization with carbapenem-resistant Klebsiella pneumoniae in intensive care unit patients. *Infect Control Hosp Epidemiol*. 2008;**29**(10):966–8. doi: [10.1086/590661](https://doi.org/10.1086/590661). [PubMed: [18754738](https://pubmed.ncbi.nlm.nih.gov/18754738/)].
26. Centers for Disease C. Carbapenem-resistant Enterobacteriaceae containing New Delhi metallo-beta-lactamase in two patients - Rhode Island, March 2012. *MMWR Morb Mortal Wkly Rep*. 2012;**61**(24):446–8. [PubMed: [22717513](https://pubmed.ncbi.nlm.nih.gov/22717513/)].
27. Gaibani P, Ambretti S, Berlingeri A, Cordovana M, Farruggia P, Panico M, et al. Outbreak of NDM-1-producing Enterobacteriaceae in northern Italy, July to August 2011. *Euro Surveill*. 2011;**16**(47):20027. [PubMed: [22152705](https://pubmed.ncbi.nlm.nih.gov/22152705/)].
28. Roy S, Singh AK, Viswanathan R, Nandy RK, Basu S. Transmission of imipenem resistance determinants during the course of an outbreak of NDM-1 *Escherichia coli* in a sick newborn care unit. *J Antimicrob Chemother*. 2011;**66**(12):2773–80. doi: [10.1093/jac/dkr376](https://doi.org/10.1093/jac/dkr376). [PubMed: [21930573](https://pubmed.ncbi.nlm.nih.gov/21930573/)].
29. Borer A, Saidel-Odes L, Riesenber K, Eskira S, Peled N, Nativ R, et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. *Infect Control Hosp Epidemiol*. 2009;**30**(10):972–6. doi: [10.1086/605922](https://doi.org/10.1086/605922). [PubMed: [19712030](https://pubmed.ncbi.nlm.nih.gov/19712030/)].
30. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother*. 2009;**53**(12):5046–54. doi: [10.1128/AAC.00774-09](https://doi.org/10.1128/AAC.00774-09). [PubMed: [19770275](https://pubmed.ncbi.nlm.nih.gov/19770275/)].
31. Wang P, Hu F, Xiong Z, Ye X, Zhu D, Wang YF, et al. Susceptibility of extended-spectrum-beta-lactamase-producing Enterobacteriaceae according to the new CLSI breakpoints. *J Clin Microbiol*. 2011;**49**(9):3127–31. doi: [10.1128/JCM.00222-11](https://doi.org/10.1128/JCM.00222-11). [PubMed: [21752977](https://pubmed.ncbi.nlm.nih.gov/21752977/)].