Infection and Drug Resistance

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ORIGINAL RESEARCH

First Reported Nosocomial Outbreak Of NDM-5-Producing *Klebsiella pneumoniae* In A Neonatal Unit In China

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Purpose: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have emerged worldwide and also being a major threat to children and neonate. In this study, we describe a nosocomial outbreak of NDM-5-producing *Klebsiella pneumoniae* in neonatal unit of a teaching hospital in China from September 2015 to September 2016.

Patients and methods: We collected 12 carbapenem-resistant *K. pneumoniae* outbreak strains from 12 newborns and characterized these isolates for their antimicrobial susceptibility, clone relationships, and multi-locus sequence types using vitek-2 compact system, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). Resistant genes were detected by using PCR and sequencing. Plasmid conjugation experiment was carried out to determine the transferability of carbapenem resistance. PCR-based replicon typing (PBRT), S1 nuclease-PFGE, and southern blotting were conducted for plasmid profiling.

Results: All 12 *K. pneumoniae* isolates were resistant to carbapenems and carried $bla_{\text{NDM-5}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-11}}$. Furthermore, PFGE analysis showed that NDM-5-producing *K. pneumoniae* were clonally related and MLST assigned them to sequence type 337. Conjugative assays showed that plasmids harboring $bla_{\text{NDM-5}}$ gene were self-transmissible. Plasmid analysis suggested that all $bla_{\text{NDM-5}}$ gene located on a ~45 kb IncX3 type plasmid.

Conclusion: To the best of our knowledge, this is the first report of a clone outbreak of $bla_{\text{NDM-5}}$-carrying *K. pneumoniae* isolates from neonates. There is an urgent need for effective infection control measures to prevent $bla_{\text{NDM-5}}$ variants from becoming epidemic in the neonates in the future.

Keywords: *Klebsiella pneumoniae*, carbapenemases, $bla_{\text{NDM-5}}$, ST337, neonate, IncX3

Introduction

The continuous emergence of Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has become a serious public-health problem worldwide.[1] Among the newly emerging carbapenemases, New Delhi metallo-β-lactamase (NDM) has been considered as a major clinical challenge due to its ability to hydrolyzing all β-lactams antibiotics except monobactams and its rapid spread worldwide.[2] Since NDM-1 was first identified in a *K. pneumoniae* isolate from a Swedish patient who had traveled to New Delhi in 2008,[3] 21 new bla_{NDM} alleles have been discovered in global.[4] The rapid evolution of bla_{NDM} genes represents a huge challenge for clinical infection treatment. In 2011, NDM-5 was first identified in a multidrug-resistant *Escherichia coli* isolate in the UK.[5] The NDM-5 enzyme differs from the prevalent NDM-1 at the amino acid positions, which appears to confer elevated

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DovePress
http://doi.org/10.2147/IDR.S218945

resistance to carbapenems and extended-spectrum cephalosporins. Unlike the prevalent NDM-1 enzyme, the identification of NDM-5 enzymes is not common. Among the *Enterobacteriaceae*, reports of bla_{NDM-5}-harboring *K. pneumoniae* isolates are sporadic.[6–12] In this study, we investigate an outbreak of bla_{NDM-5}-positive *K. pneumoniae* strains in neonatal unit of a teaching hospital in China with the aim of determining the molecular basis of the emerging ST337 carbapenem-resistant *K. pneumoniae* isolates responsible for this outbreak. As far as we know, this is the first description of NDM-5-producing *K. pneumoniae* nosocomial outbreak in the neonatal ward.

Materials And Methods

Collection Of Clinical Isolates And Patients

The study was conducted in the Department of Neonatology at a teaching hospital in Jiangsu Province, China. There are 120 beds in the Department of Neonatology, with an estimated population of 5000 patient visits per year. From September 2015 to September 2016, 12 non-duplicated strains of carbapenem-resistant *K. pneumoniae* were isolated from inpatients in newborn medicine ward. Strain identification was performed by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany). In vitro antimicrobial susceptibility testing of isolates was analyzed with VITEK-2 compact system (bioMérieux, Marcy-l'Étoile, France). The electronic medical records of the newborns including patient demographics, neonatal birth information, antimicrobial treatment, and clinical outcomes were retrospectively reviewed.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of meropenem (MEM), imipenem (IPM), aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), cefotaxime (CTX), amoxicillin-clavulanate (AMC), sulbactam/cefoperazone (SCF), piperacillin-tazobactam (TZP), amikacin (AM), levofloxacin (LE), ciprofloxacin (CPFX), compound Sulfamethoxazole (SMZ-TMP), tigecycline (TG) and polymyxin B (PB) were measured by broth microdilution method. Antimicrobial susceptibility testing was interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI),[13] except for sulbactam/cefoperazone, tigecycline and colistin, which were interpreted based on the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria.[14]

Polymerase Chain Reaction (PCR) Amplifications And Sequencing

PCR using the primers mentioned in Table 1 allowed the detection of carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48} and bla_{IMP}),[15–19] common extended-spectrum β-lactamase (ESBL) genes ($bla_{CTX-M-1group}$, $bla_{CTX-M-2group}$, $bla_{CTX-M-8\ group}$, $bla_{CTX-M-9\ group}$, bla_{SHV} and bla_{TEM}) and AmpC genes (bla_{CIT}, bla_{MOX}, bla_{DHA}, bla_{EBC}, and bla_{FOX}).[20,21] DNA templates were prepared by alkaline lysis method using the kit (MoBio, USA). Amplifications were carried out in 50 μL final volume with 2 μL DNA, 25 μL 2X Taq Master Mix (TaKaRa, Dalian, China), and 2 μM of each primer (Shanghai Sangon, Shanghai, China). PCR conditions for all genes consisted of one cycle at 95°C for 5 mins, followed by 30 cycles at 94°C for 1 min, 72°C for 1 min, and one cycle at 72°C for 5 mins; the annealing step varied depending on the target gene as follows: 55°C/1 min for bla_{IMP}; 59°C/1 min for bla_{KPC}, bla_{VIM}, $bla_{CTX-M-2group}$, bla_{MOX}; 58°C/1 min for bla_{NDM}, $bla_{CTX-M-8group}$, bla_{TEM}; 62°C/1 min for bla_{OXA-48}, $bla_{CTX-M-1group}$, bla_{DHA}, bla_{FOX}; 64°C/1 min for $bla_{CTX-M-9group}$ and bla_{EBC}; 66°C/1 min for bla_{CIT}; 68°C/1 min for bla_{SHV}. Amplified genes were screened by electrophoresis on a 1.5% agarose gel. The positive amplicons were subjected to direct sequencing, and the sequences obtained were compared to reported sequences from GenBank (www.ncbi.nlm.nih.gov/GenBank) using *BLAST* searches.

Molecular Typing

The clonal relationships of the NDM-5 producing *K. pneumoniae* isolates were analyzed using Pulsed-field gel electrophoresis (PFGE) and whole genomic DNA was digested with XbaI restriction endonuclease (TaKaRa, Dalian, China). Salmonella enterica serotype Braenderup H9812 was used as a marker. The PFGE patterns were compared using BioNumerics software version 5.10, with a cutoff at 90% similarity to indicate identical PFGE types (pulsotypes). Multi-locus sequence typing was also performed for genotyping. Seven housekeeping genes (infB, pgi, mdh, phoE, gapA, tonB and rpoB) were amplified by using primer sequences described on the MLST website.[22] Sequence types (STs) were assigned using the MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

Table 1 Primers Used For Amplification Of The Resistance Genes Of *K. pneumoniae* In This Study

Resistance Gene	Primer	Size Of PCR Product (bp)	References
bla_{KPC}	F: ATG TCA CTG TAT CGC CGT C R: TTA CTG CCC GTT GAC GCC	882	15
bla_{NDM}	F: GAAGCTGAGCACCGCATTAG R: GGGCCGTATGAGTGATTGC	982	16
bla_{VIM}	F: GCMCTTCTCGCGGAGATTGA R: TGCGCAGCACCRGGATAGA	257	17
bla_{OXA-48}	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGATGGC	743	18
bla_{IMP}	F: CTACCGCAGCAGAGTCTTTG R: AACCAGTTTTGCCTTACCAT	587	19
$bla_{CTX-M1group}$	F: CAGCGCTTTTGCCGTCTAAGC F: GGCCCATGGTTAAAAAATCACTGC	945	20
$bla_{CTX-M2\ group}$	F: CTC AGA GCA TTC GCC GCT CA R: CCG CCG CAG CCA GAA TAT CC	848	20
$bla_{CTX-M8group}$	F: ACTTCAGCCACACGGATTCA R: CGA GTA CGT CAC GAC GAC TT	1024	20
$bla_{CTX-M9group}$	F: GTTACAGCCCTTCGGCGATGATTC R: GCGCATGGTGACAAAGAGAGTGCAA	881	20
bla_{SHV}	F: CGCCGGGTTATTCTTATTTGTCGC R: TCT TTCCGATGCCGCCGCCAGTCA	1017	20
bla_{TEM}	F: ATA AAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATCA	1080	20
bla_{CIT}	F: TGGCCAGAACTGACAGGCAA R: TTTCTCCTGAACGTGGCTGG	462	21
bla_{MOX}	F: GCTGCTCAAGGAGCACAGGAT R: CACATTGACATAGGTGTGGTGC	520	21
bla_{DHA}	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	21
bla_{EBC}	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	21
bla_{FOX}	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	190	21

Conjugation Assay

A conjugation assay was performed between the isolates harboring bla_{NDM-5} as donors and *E. coli* 600 (rifampicin-resistant strain) as a recipient.[23] Transconjugants were selected on Brain Heart Infusion agar containing 600 μg/mL rifampicin and 2 μg/mL imipenem for 24 hrs at 35°C and the presence of the bla_{NDM-5} gene was confirmed by PCR sequencing. Susceptibility testing of the transconjugants was performed as well.

Plasmid Analysis

Plasmid incompatibility types of the transconjugants were identified by PCR-based replicon typing as reported previously.[24,25] S1-PFGE and Southern blotting were performed to isolate and localize the resistant plasmid carrying bla_{NDM-5} gene. Briefly, bla_{NDM-5} positive isolates embedded in gold agarose gel plugs were digested with S1 nuclease (TaKaRa Biotechnology, Dalian, China) and plasmid DNA was electrophoresed on a CHEF-mapper XA (PFGE)

system (Bio-Rad, USA). Subsequently, the DNA fragments were blotted onto a positive-charged nylon membrane (Millipore, USA) and hybridized with bla_{NDM-5} specific probe according to the protocol of DIG High Prime DNA Labeling and Detection Starter Kit I (Roche Applied Sciences, Penzberg, Germany).

Results

Clinical Characteristics Of 12 *K. pneumoniae*

In September 2015, a premature baby was born in this hospital with neonatal pneumonia and neonatal respiratory distress syndrome (case 1). The first carbapenem-resistant *K. pneumoniae* strain was isolated from the sputum culture on September 10, 2015. The first baby's mother suffered from acute gastroenteritis in the 7th month of pregnancy and hospitalized in another hospital in Jiangsu. After 1 day, another neonate also had neonatal respiratory distress syndrome and was positive for carbapenem-resistant *K. pneumoniae* in sputum culture. No other isolates were detected between October and November. However, since December 2015, the isolation rate of the strain has increased significantly, and 10 strains have been isolated from December 2015 to September 2016. By September 2016, 12 cases had occurred in newborn medicine ward in total. All the 12 neonates were improved after receiving various antimicrobial therapies including moxalactam, ceftazidime, cefotaxime, amoxicillin-sulbactam, amoxicillin-clavulanate and piperacillin-tazobactam. The 12 NDM-5-positive *K. pneumoniae* strains were detected from urine (n=1), blood (n=2) and sputum (n=9), respectively. Eleven newborns had neonatal pneumonia, three had neonatal respiratory distress syndrome, and two neonatal sepsis. Four newborns were premature, one of whom were very low birth weight infant. The demographic and clinical profiles of the 12 newborns involved in the outbreak of NDM-5-positive *K. pneumoniae* isolates are shown in Table 2.

Antibiotic Susceptibility Testing And Detection Of Resistance Determinants

All 12 clinical isolates exhibited resistance to meropenem, imipenem, aztreonam, cefotaxime, ceftazidime, cefepime, sulbactam-cefoperazone, amoxicillin-clavulanate and piperacillin-tazobactam but remained susceptible to levofloxacin, ciprofloxacin, amikacin, compound sulfamethoxazole, tigecycline and polymixin B. All 12 isolates carried the bla_{NDM-5}, bla_{SHV-11}, and bla_{TEM-1} genes. The other

Table 2 Clinical Features Of The Neonates In This Study

Case	Sex	Pregnancy Duration (wk)	Birth wt (g)	Dates Of Hospital Stay	Date Isolate Identified	Specimen	Type(s) Of Infections	Antimicrobial Therapy	Clinical Outcome
1	M	35	3000	1 Sep–17 Sep 2015	10 Sep 2015	Sputum	NP, NRDS	MOX	Improvement
2	M	36	1600	2 Sep–28 Sep 2015	11 Sep 2015	Sputum	NRDS	MOX+CAZ	Improvement
3	M	40	3400	18 Dec 2015–13 Jan 2016	23 Dec 2015	Sputum	NP	CAZ	Improvement
4	F	42	3100	22 Dec 2015–6 Jan 2016	24 Dec 2015	Sputum	NP	CTX	Improvement
5	M	39	3500	30 Dec 2015–8 Jan 2016	31 Dec 2015	Sputum	NP	MOX+AMS	Improvement
6	F	40	3300	29 Dec 2015–7 Jan 2016	1 Jan 2016	Urine	NP	MOX+AMS	Improvement
7	F	40	3300	18 Jan –7 Feb 2016	26 Jan 2016	Sputum	NP	MOX+AMS	Improvement
8	M	39	3450	7 Feb –14 Mar 2016	8 Mar 2016	Blood	NP, NS	MOX +AMC	Improvement
9	F	38	3300	20 Jul–2 Aug 2016	29 Jul 2016	Sputum	NP, NRDS	CAZ	Improvement
10	M	34	1500	22 Aug–2 Oct 2016	27 Aug 2016	Sputum	NP, NRDS	CAZ+TZP	Improvement
11	M	35	2590	3 Sep–14 Sep 2016	4 Sep 2016	Sputum	NP	CAZ	Improvement
12	F	40	3000	10 Sep–20 Sep 2016	18 Sep 2016	Blood	NP, NS	MOX +AMC	Improvement

Abbreviations: M, male; F, female; NP, neonatal pneumonia; NRDS, neonatal respiratory distress syndrome; NS, neonatal sepsis; MOX, moxalactam; CAZ, ceftazidime; CTX, cefotaxime; AMS, amoxicillin-sulbactam; AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam.

resistance genes present in clinical *K. pneumoniae* isolates were not detected. The antibiotic resistance characteristics of the 12 NDM-5-positive *K. pneumoniae* isolates and their corresponding transconjugants are shown in Table 3.

Molecular Typing

PFGE typing revealed that all 12 *K. pneumoniae* isolates in this outbreak shared highly similar PFGE patterns, which suggests that the 12 isolates were clonally related (Figure 1). MLST analysis showed that these 12 strains, KP1–12, belonged to ST337 with the allelic profile 2-1-11-1-1-1-13.

Conjugation And Plasmid Analysis

In our study, all of plasmids carried $bla_{\text{NDM-5}}$ gene from the donor carbapenem-resistant *K pneumoniae* strains were successfully transferred to the recipient *Escherichia coli* EC600. The corresponding transconjugants are termed EC600-1–EC600-12, respectively. The MIC values of 12 the transconjugants were tested, and all transconjugants exhibited resistance to carbapenem and enzyme inhibitors (Table 3). As shown in Figure 2, S1-PFGE and Southern blot hybridization using a DIG-labeled $bla_{\text{NDM–5}}$ with incompatibility group-specific probe revealed that $bla_{\text{NDM-5}}$ was located on the nearly same size (~45 kb) IncX3 plasmids.

Discussion

Carbapenem-resistant *Enterobacteriaceae* are an emerging problem, which spreads among our most vulnerable populations, children.[26] Many carbapenem resistance genes, such as $bla_{\text{KPC-2}}$,[21] $bla_{\text{NDM-1}}$,[27] $bla_{\text{OXA-48}}$,[28] $bla_{\text{OXA-232}}$ and $bla_{\text{IMP-38}}$,[29,30] have been found in *K. pneumoniae* outbreak strains in the newborn. Although NDM-1 enzyme was previously reported as the most prevalent type of carbapenemase in children,[31] our current results suggested that the emergence of NDM-5-producing *K. pneumoniae* caused nosocomial outbreak in neonatal unit. Our research team previously found high prevalence of $bla_{\text{NDM-5}}$ variants among carbapenem-resistant *E. coli* in Northern Jiangsu Province.[32] Based on analysis of previous articles, this is the first identification of $bla_{\text{NDM-5}}$-harboring *K pneumoniae* outbreak strains in the neonatal infection. In this study, we aimed to identify the microbial resistance characteristics and transmission mechanism of CPKP infections caused by NDM-5-producing *K. pneumoniae* isolates, which may help to prevent the $bla_{\text{NDM-5}}$ from becoming epidemic in neonates.

Previous study found that premature birth and very low birth weight are associated with neonatal nosocomial infections with carbapenem-resistant *Enterobacteriaceae*.[33] In this study, 33.3% and 8.3% of the 12 newborns had premature birth and very low birth weight, respectively. During the study period, 7.0% and 3.15% of all patients in the neonatal unit had premature birth and very low birth weight, respectively. Newborns at high risk should be of the greatest concern in the future when performing procedures to prevent CRKP infection. Compared to adults, children have more limited therapeutic options.[34] In our study, all the clinical strains were susceptible to tigecycline and colistin in vitro. However, tigecycline is not recommended for children due to the risk of dental staining and colistin is not currently used in clinical treatment of children in China. Besides, aminoglycosides and fluoroquinolones are also rarely used for children due to their nephrotoxicity and ototoxicity.[35] Clinical data show that all the 12 neonates were improved after receiving various antimicrobial therapies including moxalactam, ceftazidime, cefotaxime, amoxicillin, sulbactam, amoxicillin-clavulanate and piperacillin-tazobactam. In this study, 12 newborns responded to treatment with these agents, which seems inconsistent with the antibiotic resistance characteristics of the clinical isolates. We assumed when these agents are combined, synergistic bactericidal action against pathogens may be achieved in vivo.[36] The isolates detected from sputum samples were presumed to be the flora colonized in the respiratory tract rather than the real infectious pathogen.

PFGE analysis showed that 12 clinical strains shared highly similar PFGE patterns in this outbreak. This implied that the outbreak of NDM-5-producing *K. pneumoniae* was because of clone spread and the first newborn might be the source of this outbreak. The first baby's mother was hospitalized for acute gastroenteritis during pregnancy and had high-risk factors for intrauterine infection. Although the NDM-5-producing *K. pneumoniae* strain was not detected from the mother, we assumed that a small number of these strains might potentially have been colonized in the mother's body and infected the fetus through the fetal circulation. Then, the CRKP isolate spread among patients in the neonatal ward, more research would be needed to uncover it in the future. It is noteworthy that blaNDM-5 were not only reported in clinical specimens but also in hospital environmental such as the incubator water and the sharing of breast milk.[37,38] Hence, we cannot deny that the hospital environment may be the diffusion reservoirs of NDM-5-producing bacteria. Regrettably, the origin of the NDM-5-producing strains in our study is still unknown. Personal contact

Table 3 Antibiotic Resistance Characteristics Of The 12 NDM-5-Positive *K. pneumoniae* Isolates And Their Corresponding Transconjugants

Isolate No.	Antimicrobial Resistance Genes			Minimum Inhibitory Concentration (μg/mL)												
		MEM	IPM	ATM	CTX	CAZ	FEP	AM	CPFX	LE	AMC	TZP	SCF	SMZ-TMP	TG	PB
KP1	$bla_{NDM-5, SHV-11, TEM-1}$	8	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP2	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	16	≥64	≥64	≥32	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
KP3	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	1	1	≥32	≥128	≥64	≤20	2	≤0.5
KP4	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP5	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	2	≤0.5
KP6	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP7	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP8	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	1	1	≥32	≥128	≥64	≤20	2	≤0.5
KP9	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP10	$bla_{NDM-5, SHV-11, TEM-1}$	8	≥16	≥64	≥64	≥64	≥32	≤2	≤0.25	≤0.25	≥32	≥128	≥64	≤20	2	≤0.5
KP11	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
KP12	$bla_{NDM-5, SHV-11, TEM-1}$	≥16	≥16	≥64	≥64	≥64	≥32	≤2	0.5	1	≥32	≥128	≥64	≤20	2	≤0.5
EC600-1	bla_{NDM-5}	8	≥16	≤1	≥64	≥64	4	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-2	bla_{NDM-5}	8	8	≤1	≥64	≥64	4	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-3	bla_{NDM-5}	8	≥16	≤1	≥64	≥64	4	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-4	bla_{NDM-5}	≥16	≥16	≤1	≥64	≥64	8	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-5	bla_{NDM-5}	8	≥16	≤1	≥64	≥64	8	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-6	bla_{NDM-5}	≥16	≥16	≤1	≥64	≥64	8	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-7	bla_{NDM-5}	4	≥16	≤1	≥64	≥64	8	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-8	bla_{NDM-5}	≥16	≥16	≤1	≥64	≥64	4	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-9	bla_{NDM-5}	4	≥16	≤1	≥64	≥64	4	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-10	bla_{NDM-5}	8	≥16	≤1	≥64	≥64	4	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-11	bla_{NDM-5}	4	≥16	≤1	≥64	≥64	8	≤2	0.5	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600-12	bla_{NDM-5}	8	≥16	≤1	≥64	≥64	4	≤2	≤0.25	≤0.12	≥32	≥128	≥64	≤20	≤0.5	≤0.5
EC600		≤1	≤1	≤1	≤1	≤1	≤1	≤2	≤0.25	≤0.12	2	2	2	≤20	≤0.5	≤0.5

Abbreviations: MEM, meropenem; IPM, imipenem; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; AM, amikacin; CPFX, ciprofloxacin; LE, levofloxacin; AMC, amoxicillin-clavulanate; TZP, piperacillin/tazobactam; SCF, sulbactam/cefoperazone; SMZ-TMP, compound Sulfamethoxazole; TG, tigecycline; PB, polymixin B.

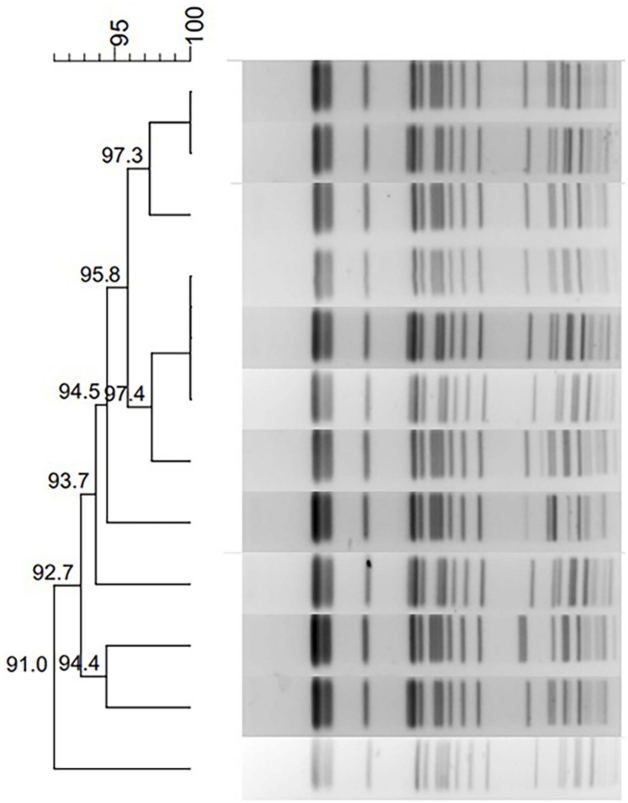

Figure 1 Dendrogram of PFGE profiles of 12 *bla*$_{NDM-5}$-positive *Klebsiella pneumoniae* isolates.

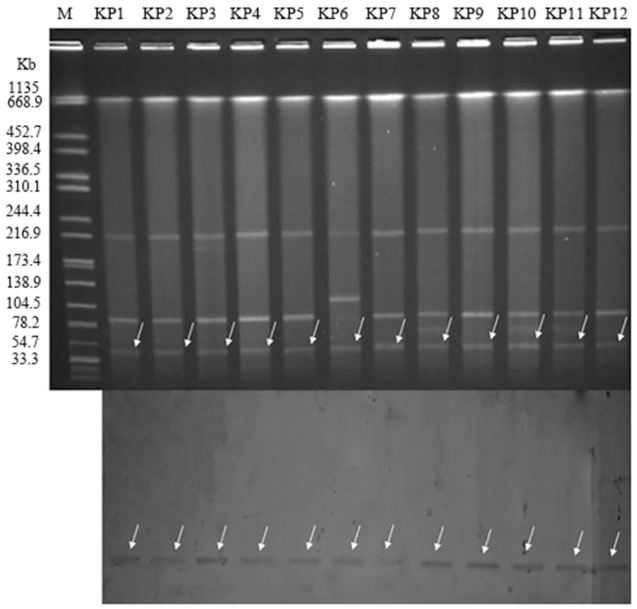

Figure 2 *bla*$_{NDM-5}$-carrying plasmid analysis (Top: S1-nuclease PFGE patterns; Bottom: Southern hybridization of the *bla*$_{NDM-5}$ probe, which was hybridized to the roughly 45 kb plasmid to confirm the presence of the resistant plasmid in the 12 ST337 carbapenem-resistant *K. pneumoniae* strains). Lane M: marker (*Salmonella* H9812); KP1 to KP12: *Klebsiella pneumoniae* outbreak strains.

between the caregivers and the newborns hospitalized in the same ward is the most likely route of the transmission of the isolates. Hospital infection department took strict measures to control the outbreak in the hospital. First, pre-screening of carbapenem-resistant *Enterobacteriaceae*(CRE) in sputum samples and rectal swabs were introduced before admission to the neonatal medical ward. Second, strict isolation procedures were implemented for patients with CRE infection. Third, it was necessary for medical staff who contact with patients infected with CRE to go through a disinfection procedure. Finally, the neonatal medical wards where newborns with CRE infection stayed were thoroughly sterilized after the discharge of the patients. The sterilized ward left unoccupied for more than 2 weeks before new patients were admitted. Since the implementation of these strict measures in this neonatal medical ward, no infections due to ST337 NDM-5-producing *K. pneumoniae* isolates have occurred as of May 2017.

Several countries, such as India (ST648),[39] Denmark (ST1284),[40] Poland (ST418),[41] Spain (ST418),[42] Japan (ST540),[43] and Singapore (ST231),[10] have reported infections caused by NDM-5-positive strains since the initial identification of this carbapenemase type in UK (ST648).[5] In China, ST167 carbapenemase-producing *Escherichia coli* is main reservoir of *bla*$_{NDM-5}$.[44] This is the first identification of NDM-5-producing *K. pneumoniae* isolate belonged to ST337, which related to CC37, and have only been identified in KPC-2-producing *K. pneumoniae* in carbapenem-resistant *Enterobacteriaceae* family.

The conjugation experiment showed that the carbapenem resistance phenotype of the 12 clinical isolates could be successfully transferred to the recipient strain EC600. All 12 transconjugants only carried the *bla*$_{NDM-5}$, and the *bla*$_{SHV-11}$ and *bla*$_{TEM-1}$ were not detected. The plasmid analysis showed that all *bla*$_{NDM-5}$ were located on the IncX3 plasmids of the same size (with approximate size 45 kb). Notably, IncX3-type plasmids of 45 kb carrying *bla*$_{NDM-5}$ genes have been identified in India,[6] Australia and Denmark.[38,39] Furthermore, previous studies revealed that the IncX3-type plasmids played a significant role in the dissemination of the *bla*$_{NDM-5}$ gene among *Enterobacteriaceae* in China.[45] Therefore, there is an urgent need to adopt effective measures to control the spread of this resistant plasmid type.

Although international travel previously reported contributed to the global spread of NDM enzyme,[3] all patients in our study who carry *bla*$_{NDM-5}$ gene have never been

abroad. It was assumed that these NDM-5-producing isolates were autochthonous clones that had obtained the plasmid carrying bla_{NDM-5}.[44] Notably, recent studies in China have revealed that NDM-5-producing *Enterobacteriaceae* was identified from pigs, dairy cows, and vegetables.[46–48] And, animals and plants were already important sources of drug-resistant strains. There is an urgent need for more epidemiological studies in the future to better understand mechanisms of emergence and dissemination of bla_{NDM-5} gene in China.

Conclusion

Our study first described the outbreak of ST337 NDM-5-producing *K. pneumoniae* isolates from neonates in China. Moreover, these outbreak strains were also detected to carry bla_{SHV-11} and bla_{TEM-1}. Their resistance profile may help pediatricians promote the prudent use of antibiotics in child health care. Worldwide surveillance of these ST337 NDM-5 producing *K. pneumoniae* isolates from neonates and implementation of stricter control measures are urgently needed to prevent these multiple-resistant strains from further disseminating in neonatal ward.

Ethical Approval

Clinical research ethics committee of the Affiliated Hospital of Xuzhou Medical University approved the study as all samples collected in this work were initially used to diagnose for patient care without increasing the patient's medical costs and suffering.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (81871734, 81471994), Jiangsu Provincial Natural Science Foundation (BK20151154), Jiangsu Provincial Medical Talent (ZDRCA2016053), Six Talent Peaks Project of Jiangsu Province (WSN-135), and Advanced Health Talent of Six-One Project of Jiangsu Province (LGY2016042).

Disclosure

The authors report no conflicts of interest in this work.

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