ORIGINAL RESEARCH

Emergence of an NDM-5-Producing *Escherichia coli* Sequence Type 410 Clone in Infants in a Children's Hospital in China

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Purpose: Outbreaks of infection due to carbapenem-resistant *Enterobacterales* (CRE), including New Delhi metallo-β-lactamase (NDM)-producing *Escherichia coli*, have been increasingly reported worldwide, primarily in adults and rarely in children. The goal of this study was to characterize an outbreak of infection caused by NDM-5-producing *E. coli* in a children's hospital in China.

Methods: A total of 86 CRE isolates were collected from 85 hospitalized children between June 2017 and May 2018. These isolates were subjected to multiple phenotypic and molecular tests, including in vitro antimicrobial susceptibility testing, PCR, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole-genome sequencing (WGS).

Results: Among the 86 CRE isolates, we identified 9 NDM-5-producing *E. coli* isolates, with 5 of them sharing the same PFGE pattern, same MLST type (ST410), same plasmid replicon type (IncFII), and nearly the same set of additional resistance genes. All 9 isolates were resistant to most antimicrobial agents, including carbapenems, cephalosporins, and levofloxacin, while being sensitive to trimethoprim/sulfamethoxazole, amikacin, tigecycline, and colistin. According to the clinical background, all 9 isolates were collected in a period of < 3 months from infants among whom there was overlap in the time of hospitalization. None of them had a travel history.

Conclusion: Our analysis suggests an outbreak of clonal dissemination, presumably due to nosocomial transmission. This study represents the first documented outbreak of NDM-5-producing *E. coli* mediated by IncFII in infants. Close monitoring is urgently needed to prevent and control the spread of this difficult-to-treat superbug.

Keywords: *Enterobacterales*, carbapenem resistance, *bla*$_{NDM-5}$, IncFII

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Introduction

Carbapenem-resistant *Enterobacterales* (CRE) have emerged as one of the major multidrug-resistant bacterial pathogens responsible for a variety of healthcare-associated infections.[1–3] CRE are very difficult to treat and have been referred as superbugs and nightmare bacteria, because they do not respond to commonly used antibiotics and are associated with high mortality. While there have been numerous reports of CRE infections including nosocomial outbreaks in adult patients worldwide, there are few reports of such infections in children.[4,5] A nationwide study in the USA reported that the frequency of CRE isolates in children (age range 1–17 years) increased from 0% in 1999–2000 to 0.47% in 2010–2011.[6] Despite this

increasing threat to children, little is known about the epidemiology, treatment, and prognosis of these infections in this population.

Among the heterogeneous forms of carbapenemases, New Delhi metallo-β-lactamase (NDM) is one of the most important enzymes accounting for carbapenem-resistance. Since the first NDM reported in 2009 from *Klebsiella pneumoniae*, a total of 24 variants of the NDM enzyme have been identified globally, and they are referred to as NDM-1 to NDM-24.[7] Among these variants, NDM-5, first identified in an *Escherichia coli* strain in the UK in 2011, has attracted extensive attention due to its increased resistance phenotype and rapid dissemination.[8] This enzyme is encoded by the bla_{NDM-5} gene, which can be carried and transferred among different incompatibility types of plasmids, such as IncX3 and IncFII.[9–11] These properties enable wide dissemination of bla_{NDM-5} through horizontal gene transfer among the members of *Enterobacterales*. Indeed, NDM-5-producing isolates have been identified worldwide,[8,9,12–17] not only from humans but also from animals such as dog and cow,[18,19] as well as hospital environments' sewage water.[20] Like all other CRE isolates, the vast majority of the human NDM-5 isolates have been identified in the adult population with only a few cases reported in children.[21,22] Reports of outbreaks of NDM-5-producing in neonates are rarer still.[23] There has been only one report of clonal dissemination involving NDM-5-producing *E. coli* (ST410) in 4 adults in a university hospital in China.[24] However, there has been no report of an outbreak of NDM-5-producing *E. coli* (ST410) in children.

In this report, we describe the first outbreak of NDM-5-producing *E. coli* isolates representing the sequence type 410 isolated from infants in a children's hospital in China.

Materials and Methods
Ethics Statement and Study Subjects

This retrospective study was carried out in accordance with the recommendations of the Ethics Committee of Hunan Children's Hospital (Changsha, Hunan Province, China) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The protocol was approved by the Ethics Committee of Hunan Children's Hospital (Changsha, Hunan Province, China). Written informed consent was obtained from the patients' guardians prior to the study.

Bacterial Strains

A total of 86 CRE isolates confirmed by a VITEK-2 automated microbiology analyzer (bioMérieux, Marcyl'Etoile, France) were collected between June 2017 and May 2018 from 85 infants (aged from 0 to 36 months) at the Hunan Children's Hospital in China. A single isolate was obtained from each patient except for one patient from whom 2 sequential isolates were available. All isolates were further identified by Matrix Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS; Bruker Daltonics GmbH, Germany). *E. coli* ATCC 25922 was obtained from the National Center for Clinical Laboratories, China, and used as a quality control strain.

Identification of the bla_{NDM-5} Gene

Genomic DNA in clinical isolates was extracted from overnight cultured strains using a boiling method.[25] To detect the bla_{NDM-5} gene in these DNA samples, PCR was performed using bla_{NDM-5}-specific primers as previously described.[8] Positive PCR products were subjected to direct Sanger sequencing. All bla_{NDM-5}-containing isolates were further characterized as described below.

Antimicrobial Susceptibility Testing and Detection of Metallo-Lactamases Phenotypes

Minimal inhibitory concentrations (MICs) of the following antibiotics against bla_{NDM-5}-positive isolates were determined using the broth microdilution method: piperacillin/ tazobactam (TZP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), aztreonam (ATM), ceftazidime (CAZ), ceftriaxone (CRO), levofloxacin (LEV), amikacin (AMK), colistin (CST), and trimethoprim/sulfamethoxazole (TMP-SMZ). The susceptibility breakpoints were defined according to the Clinical and Laboratory Standards Institute (CLSI) standards.[26] The MIC of tigecycline (TGC) was determined by E-test (bioMérieux, France) and interpreted in accordance with the US Food and Drug Administration (FDA) breakpoint (resistant breakpoint, 2 μg/mL). In all MIC tests, *E. coli* ATCC 25922 was used as a quality control strain.

The EDTA-modified carbapenem inactivation method (eCIM) combined with the modified carbapenem inactivation method (mCIM) was used to identify metallo-lactamases according to the standard procedures of CLSI.[26]

Determination of Genetic Relatedness

Genetic relatedness among bla_{NDM-5}-positive isolates was determined by pulsed-field gel electrophoresis (PFGE). In brief, bacterial cells harvested from stationary-phase culture were embedded in agarose gel plugs (Lonza Rockland, ME, USA) and lysed by proteinase K, followed by restriction digestion with *Xba*I (Promega, USA) for 18 h at 37°C. Electrophoresis was performed at 14°C for 19 h using the Bio-Rad CHEF III system (120° angle, 6 V/cm, with switch times of 6 s and 36 s). Gels were stained with GelRed (Biotium Inc.) and digitally captured under UV light. Cluster analysis was performed with BioNumerics software Version 5.1 (Applied Maths, Kortrijk, Belgium) using the Dice Similarity Coefficient. Isolates with >85% pattern similarities were considered to be from the same PFGE cluster.[27,28]

A subset of isolates representing different PFGE clusters were further analyzed by multilocus sequence typing (MLST) based on 7 housekeeping genes of *E. coli* (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) following the Institut Pasteur scheme.[29]

WGS-Based Deduction of Resistance Genes and Plasmids

WGS was performed to detect resistant genes and identify resistant plasmids in NDM-5-producing strains. Briefly, genomic DNA was extracted using the DNeasy UltraClean Microbial Kit (QIAGEN, GmbH, Germany) following the manufacturer's recommendations. Approximately 10 μg of DNA for each strain was used to construct 2 Illumina paired-end libraries with average insertion lengths of 500 bp and 2000 bp. Libraries were sequenced using an Illumina GA IIx sequencer (Illumina Inc., San Diego, CA, USA). Raw data were processed by removing the following: 1) reads with 5 bp of ambiguous bases, 2) reads with 20 bp of low-quality (≤ Q20) bases, 3) adapter contamination, and 4) duplicated reads. The final cleaned reads had an about $100 \times$ genome coverage for each strain. Genome assembly was performed using SOAPdenovo v1.05.[30] The resistant genes and typing of plasmids of all NDM-5-producing isolates were analyzed using Resfinder and PlasmidFinder tool, respectively, which were provided by the Centre for Genomic Epidemiology.[31]

Results

Clinical Characteristics of bla_{NDM-5}-Positive Isolates

Based on MALDI-TOF-MS analyses, the 86 CRE isolates confirmed by VITEK-2 were categorized into 5 species, including *K. pneumoniae* (56 isolates), *E. coli* (16 isolates), *Enterobacter aerogenes* (9 isolates), *Enterobacter cloacae* (3 isolates), and *Serratia marcescens* (2 isolates). Further analysis by PCR using bla_{NDM-5}-specific primers revealed that 9 of them were bla_{NDM-5}-carrying *E. coli*, which accounted for 10.5% of all isolates tested. These 9 *E. coli* isolates were collected within 3 months from 8 infants aged from 13 days to 15 months in the same hospital (Table 1). One infant had 2 isolates collected 2 days apart from blood and ascites, separately, while each of the other 7 infants had only one isolate collected from sputum (n = 5) or blood (n = 2). The diagnosed diseases for these infants included pneumonia (n = 4), sepsis (n = 2), megacolon (n = 1, the infant with 2 isolates available), and duodenal atresia (n = 1). None of them had a travel history to other cities. All 8 infants received antibiotic treatment. Only one patient showed no response and died, and the 7 others recovered as evidenced by the resolution of symptoms along with a normal infection index (Table 1).

The timeline of patient admission and sample collection is shown in Figure 1. The first 2 bla_{NDM-5}-carrying *E. coli* isolates were collected from patients in the intensive care unit (ICU) about 1 week apart, and then 7 isolates were collected one after another from 4 other departments over a period of approximately 2 months. There was overlap in the time of hospitalization among the patients.

Antimicrobial Susceptibility and Carbapenems Phenotypes

Based on MIC tests, all 9 bla_{NDM-5}-carrying *E. coli* isolates were resistant to a broad spectrum of antimicrobial agents, including carbapenems, cephalosporins, and levofloxacin. Nevertheless, they were all sensitive to trimethoprim/sulfamethoxazole, amikacin, tigecycline, and colistin (Table 2).

Based on the eCIM–mCIM combination test, all the 9 isolates were positive for MBL production.

PFGE and Genetic Relatedness Analysis

According to PFGE patterns, 9 bla_{NDM-5}-positive *E. coli* isolates were divided into 4 distinct types: type A (n = 5), type B (n = 2), type C (n = 1), and type D (n = 1). Based on MLST, these 9 isolates belonged to 3 distinct sequence types (STs), including ST410 (6-4-12-1-20-18-

Table 1 Clinical Background Information of bla_{NDM-5}-Positive *E. coli* Isolates

Patient ID	Isolate ID	Age/Sex	Department	Travel History	Date of Isolation	Sample Type	Diagnosis	Antimicrobial Treatment	Outcome
1	EC3	13 days/M	Surgical department	No	4/16/2018	Blood	Megacolon	Imipenem +Cefoperazone/ sulbactam	Recovered
1	EC30	15 days/M	Surgical department	No	4/18/2018	Ascites	Megacolon	Imipenem +Cefoperazone/ sulbactam	Recovered
2	EC10	4 months/F	ICU, Digestive department	No	2/6/2018	Blood	Sepsis	Ceftazidime, Imipenem	Death
3	EC18	16 days/M	Neonatal department	No	2/26/2018	Sputum	Sepsis	Imipenem	Recovered
4	EC20	3 months/F	Cardiovascular medicine	No	3/2/2018	Sputum	Pneumonia	Imipenem	Recovered
5	EC7	15 months/M	ICU	No	1/28/2018	Sputum	Pneumonia	Imipenem	Recovered
6	EC8	27 days/M	Neonatal department, Surgical department	No	2/28/2018	Sputum	Duodenal atresia	Imipenem, Meropenem	Recovered
7	EC27	6 months/F	Infectious disease department, ICU	No	4/3/2018	Sputum	Pneumonia	Imipenem	Recovered
8	EC82	8 months/F	Neonatal department, ICU	No	4/15/2018	Blood	Pneumonia	Imipenem +Cefoperazone/ sulbactam	Recovered

Abbreviation: ICU, intensive care unit; M, male; F, female.

7), ST167 (10-11-4-8-8-13-2), and STn (a new ST). Comparison of the results between PFGE and MLST revealed that PFGE types A and B corresponded to ST410 and ST167, respectively, while both types C and type D of PFGE corresponded to STn. Five bla_{NDM-5}-positive *E. coli* isolates sharing the same
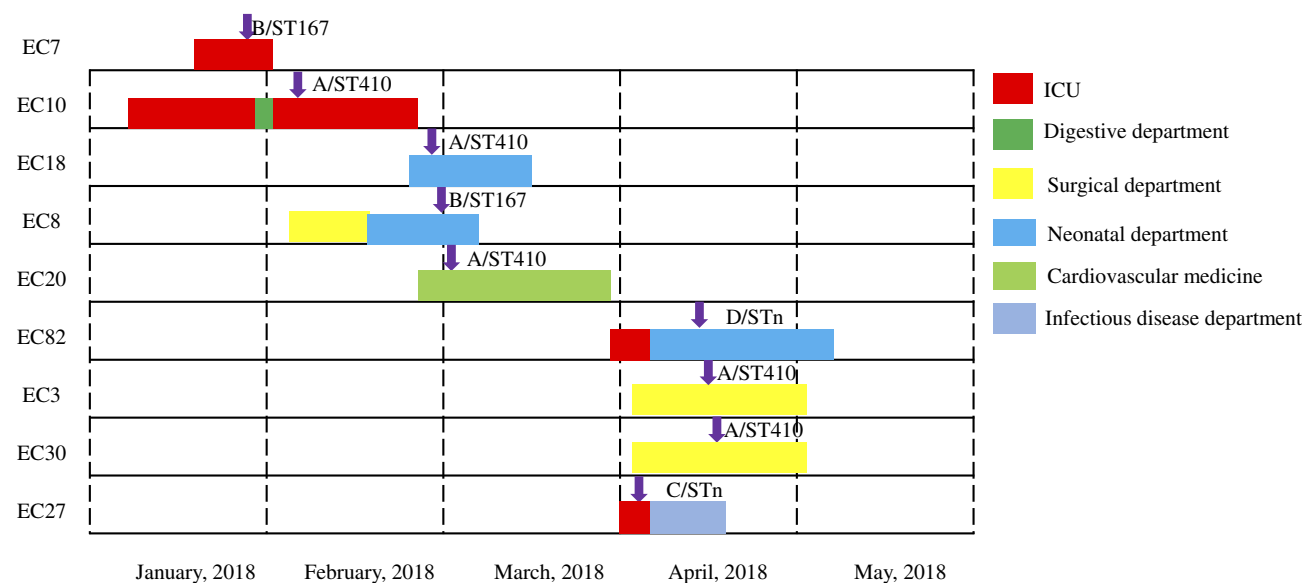

Figure 1 Timeline of outbreak of NDM-5-producing *E. coli* in 8 infants in a Children's hospital. Labels at the left side represent *E. coli* isolate IDs, including 2 (EC3 and EC30) obtained from the same patient. The different colors of the bars represent the different departments in which the patients were hospitalized (as shown at the top right), with the length of the bars representing the period of hospitalization. Four patients (for isolates EC10, EC8, EC27, and EC82) were hospitalized in 2 or more departments. The purple arrows indicate the dates of sampling.

Table 2 Antimicrobial Susceptibility of 9 NDM-5-Producing *E. coli* Isolates

Isolate ID	MIC (µg/mL)											
	TZP	MEM	IPM	ETP	CRO	CAZ	ATM	LEV	AMK	TMP-SMZ	TGC	CST
EC3	≥128	>32	>32	≥8	≥64	≥64	≥64	≥8	8	≤1/19	0.19	0.5
EC30	≥128	>32	>32	≥8	≥64	≥64	≥64	≥8	≤2	≤1/19	0.13	0.5
EC7	≥128	>32	≥16	≥8	≥64	≥64	≥64	≥8	≤2	≤1/19	0.19	1
EC8	≥128	>32	≥16	≥8	≥64	≥64	≥64	≥8	≤2	≤1/19	0.19	0.5
EC10	≥128	16	>32	≥8	≥64	≥64	≥64	≥8	4	≤1/19	0.19	1
EC18	≥128	16	4	≥8	≥64	≥64	≥64	≥8	8	≤1/19	0.19	≤0.25
EC20	≥128	>32	>32	≥8	≥64	≥64	≥64	≥8	8	≤1/19	0.19	0.5
EC27	≥128	16	8	≥8	≥64	≥64	≥64	≥8	≤2	≤1/19	0.19	0.5
EC82	≥128	>32	8	≥8	≥64	≥64	≥64	≥8	≤2	≤1/19	0.19	0.5

Abbreviations: TZP, piperacillin/tazobactam; MEM, meropenem; ETP, ertapenem; IPM, imipenem; CAZ, ceftazidime; CRO, ceftriaxone; ATM, aztreonam; LEV, levofloxacin; AMK, amikacin; TMP-SMZ, trimethoprim/sulfamethoxazole; TGC, tigecycline; CST, colistin.

PFGE pattern (type A) and the same MLST type (ST410).

Characterization of Plasmid Replicon Types and Resistance Genes Based on WGS Data

When contigs assembled using Illumina reads from all 9 bla_{NDM-5}-carrying isolates were analyzed by PlasmidFinder, 3 different types of plasmids were detected, including IncFII (n = 5), IncX3 (n = 3), and IncN (n = 1). Correlations of these plasmid types with PFGE and MLST types are illustrated in Figure 2. Of note, all 5 isolates with the same PFGE and MLST types also showed the same plasmid type (IncFII).

Analysis of contigs also confirmed the presence of the bla_{NDM-5} gene in all nine isolates. In addition, other β-lactamase genes were detected, including bla_{TEM-1}, $bla_{CTX-M-15}$, $bla_{OXA-320}$, bla_{CMY-2}, and bla_{CMY-62}, each of which was detected in at least 5 isolates. Plasmid-mediated $aac(6')$-Ib-cr was detected in 8 isolates. The remaining genes were infrequent, with each detected in no more than 3 isolates (Figure 2). Of note, all 5 isolates with the same PFGE, MLST and plasmid types also contained the same set of resistance genes except for one (EC10), which did not carry $bla_{OXA-320}$.

Discussion

In this study, we performed VITEK-2 and MALDI-TOF-MS analyses on a panel of 86 *Enterobacterales* isolates collected from children between June 2017 and May 2018 in a single Children's hospital in China. Further PCR analysis revealed 9 of these 86 isolates to be bla_{NDM-5}-
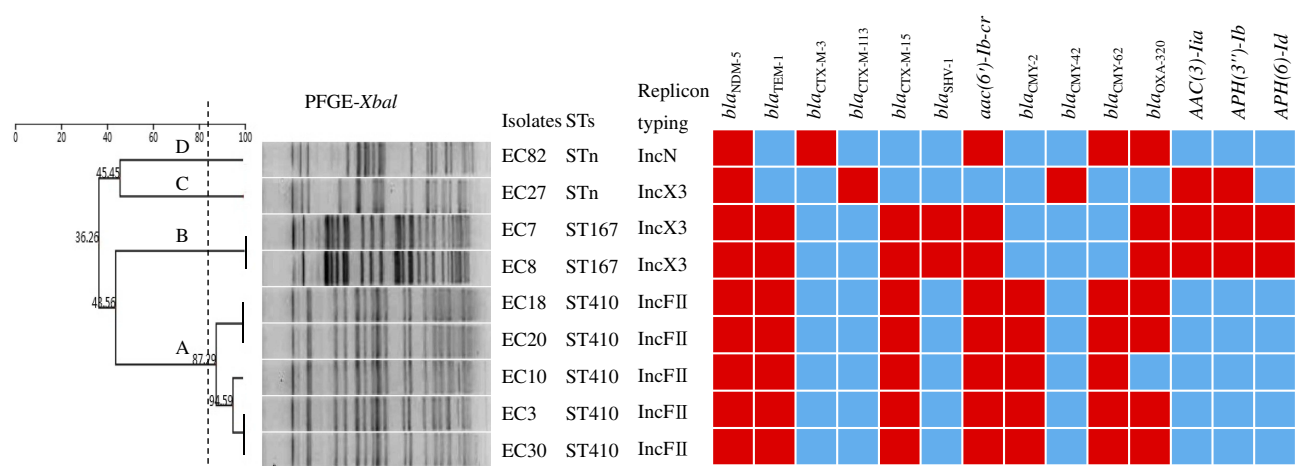

Figure 2 Dendrogram of 9 NDM-5-producing *E. coli* isolates based on pulsed-field gel electrophoresis (PFGE) patterns (indicated as A to D above the branches in the left) and their correlations with multilocus sequence typing (STs), replicon typing, and distribution of resistance genes. The dashed line in the left dendrogram represents 85% pattern similarity; isolates with >85% similarity were considered to have the same homogeneity. The red and blue squares indicate the presence and absence of the resistance genes indicated at the top, respectively.

Table 3 Previously Reported *E. coli* Isolates Carrying NDM-5 and ST410

Year of Isolation	Location	Isolate IDs	Host	Replicon Typing	Reference
2014	Egypt	Ec7, Ec44	Human	NT	[38]
2014–2015	Saudi Arabia	UPEC-RIY-7, UPEC-RIY-8, UPEC-RIY-9, UPEC-RIY-10	Human	–	[39]
2016	China	E47, E12	Human	NT	[40]
2016	Myanmar	N73	Human	–	[41]
2017	South Korea	CC1702-1, CC1706-1	Human	IncFIA/B	[42]
2017	South Korea	Z0117EC0028, Z0117EC0033, Z0117EC0035, Z0117EC0037	Dog	IncX3	[43]
2017–2018	China	CREC-10, CREC-11, CREC-12, CREC-21	Human	IncX3	[24]
2018	South Korea	CR-ECO13	Human	–	[44]
2018	China	EC3, EC10, EC18, EC20, EC30	Human	IncFII	This study

Abbreviations: "-", not reported; NT, not typed.

carrying *E. coli*. These 9 *E. coli* isolates were collected within 3 months from 8 infants aged from 13 days to 15 months (Table 1). Subsequent molecular and phenotypic characterization suggests an outbreak due to clonal dissemination.

The possibility of clonal dissemination was supported by the following observations. First, based on DNA analysis by multiple approaches (Figure 2), all 9 *E. coli* isolates carried bla_{NDM-5}, with 5 of them (isolates EC3, EC10, EC18, EC20, and EC30) sharing the same PFGE pattern (type A), the same MLST type (ST410), the same plasmid replicon type (IncFII), and nearly the same set of resistance genes (including bla_{TEM-1}, $bla_{CTX-M-15}$, $aac(6')$-*Ib-cr*, bla_{CMY-2}, bla_{CMY-62}, and $bla_{OXA-320}$). Two other isolates (EC7 and EC8) also showed the same molecular profile. In addition, eCIM–mCIM confirmed the production of metallo-lactamase in all 9 isolates. These results suggest that these isolates, especially the 5 isolates with the same molecular profile, originated from the same clone.

Second, in vitro MIC tests revealed that all 9 isolates were resistant to a broad spectrum of antimicrobial agents, including carbapenems, third-generation cephalosporins, β-lactam enzyme-inhibitor, and levofloxacin, while they were all sensitive to trimethoprim/sulfamethoxazole, amikacin, tigecycline, and colistin (Table 2).

Third, according to the clinical background and epidemiological information, all 9 isolates were collected over a period of less than 3 months from patients in the same hospital, and there was overlap in the time of hospitalization among these patients (Figure 1). None of these patients had a travel history, although that of their parents is unknown. These findings support the possibility of a nosocomial transmission. Based on the outbreak timeline, it seems that the outbreak started in the ICU and then was transmitted to other departments, although we were unable to identify the original bacterial source or how it was transmitted from one patient to another.

Infections due to NDM-5-producing *E. coli* ST410 have been mainly reported in adults in Asia, as summarized in Table 3. Recently, Sun et al reported an outbreak involving 4 NDM-5-producing *E. coli* ST410 isolates in adults in a university hospital in China.[24] Our present study is the first to document an outbreak of NDM-5-producing *E. coli* ST410 in infants. The dominance of the ST410 isolate in our study appears to be different from the previous reports in China showing a dominance of ST167,[11,24,32–34] though this difference may be insignificant due to the small sample sizes in all of these studies. While only 2 ST167 isolates were identified in our study, this type of NDM-5-producing *E. coli* is widely disseminated over the world[16,35–37] and has been found to be the predominant type in China.[11,24,32–34]

As in many bacterial species, mobile elements play a major role in the transmission of bla_{NDM} in a variety of *Enterobacterales* species. In this study, 5 out of 9 *E. coli* isolates carried IncFII plasmids, which may account for the clonal spread of bla_{NDM-5} gene in infants, as has been reported previously for outbreaks in adult patients.[45,46] Our study is the first to show a clonal spread of NDM-5-producing *E. coli* isolates carrying IncFII plasmids in infants. We also detected IncX3 plasmids in 3 NDM-5-producing *E. coli* isolates. This type of plasmid has been also previously reported in China[24,32,47] and other countries.[17,38,42,48]

Outbreaks of CRE can have a potentially devastating impact on children, especially infants. The outbreak described in this report highlights the need to develop better strategies for determining susceptibility to CRE so

that appropriate treatment can be initiated once diagnosed. This outbreak also highlights the importance of improving our knowledge of epidemiological factors that might enhance CRE transmission to susceptible children. Such knowledge is essential for the development of effective control and prevention measures.

Disclosure

The authors declare no conflicts of interest.

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