ISOLATION AND CHARACTERIZATION OF NEW DELHI METALLO-β-LACTAMASE 1-PRODUCING *ENTEROBACTER CLOACAE* CLINICAL STRAIN IN CHONGQING, CHINA

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Abstract. Carbapenem resistance is an increasing clinical problem worldwide. From a total of 299 *Enterobacter cloacae* isolates collected from patients at the First Affiliated Hospital of Chongqing Medical University, Chongqing, China between 2007 and 2012, *E. cloacae* strain 413 from sputum of a 67-year-old female demonstrated carbapenemase production based on a modified Hodge test and contained a plasmid (pNDM-1) carrying bla_{NDM-1} encoding New Delhi metallo-β-lactamase 1. This was confirmed by both Southern blotting and direct DNA sequencing. Production of metallo-β-lactamase was determined by imipenem-EDTA E-test. PCR-based assay showed pNDM-1 also carried aac(6')-Ib-cr, blaCTX$_{-M-14}$, qnrA, qnrS, and rmtA, consistent with resistance of *E. cloacae* 413 to amoxicillin, cefoperazone, ceftazidime, penicillin, and piperacillin-tazobactam. *E. cloacae* 413 was sensitive to fosfomycin and polymyxin B. Pulsed-field gel-electrophoresis of *Xba*I-digested DNA revealed pNDM-1 belonged to B type. The plasmid could be transferred to *Escherichia coli* J53, conferring the same antibiogram profile. These findings highlight the spread of NDM-1-carrying bacteria and the necessity for rational use of antibiotics.

Keywords: *Enterobacter cloacae*, carbapenem, carbapenemase China, New Delhi metallo-β-lactamase 1

INTRODUCTION

With the wide clinical use of carbapenem antibiotics, carbapenem-resistant strains of pathogenic bacteria have gradually emerged. This resistance is becoming of high concern in China, particularly in Enterobacteriaceae species such as *Klebsiella pneumoniae* and *Escherichia coli* (Nordmann *et al*, 2011), and also in *Salmonella* (Huang *et al*, 2013) and *Acinetobacter baumannii* (Chang *et al*, 2015). In *Klebsiella pneumoniae*, resistance to carbapenem antibiotics was reported in 7.4% of isolates in two hospitals in China (Zhang *et al*, 2015). In Shanghai, the prevalence of carbapenem-resistant *K. pneumoniae* and imipenem-resistant *Citrobacter freundii* strains dramatically increased from 0.91% and 11.11%, respectively, in 2005, to 12.87% and 33.3%, respectively, in 2009 (Hu *et al*, 2012).

Carbapenemases belong to the β-lactamase family of enzymes with a broad

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range of β-lactam substrates (Queenan and Bush, 2007; Walsh, 2010). In Enterobacteriaceae, class A carbapenemase includes GES, KPC, SME, and IMI/NMC-A enzymes; Class B carbapenemase can inactivate penicillin, cephalosporins and carbapenems but not aztreonam, and is inhibited by metal chelators such as EDTA; Class D consists mainly of OXA and PSE enzymes (Queenan and Bush, 2007; Walsh, 2010). Thus far, acquired metallo-ß-lactamases (MBLs) fall into 11 main categories, namely, AIM-1, DIM-1, FIM-1, GIM-1, IMP family, KHM-1, NDM, SIM-1, SPM-1, TMB, and VIM family (Queenan and Bush, 2007; Walsh, 2010). In addition, MBLs are found in *Pseudomonas aeruginosa, Acinetobacter* spp, Enterobacteriaceae, and other common clinical pathogens (Queenan and Bush, 2007; Walsh, 2010).

New Delhi metallo-β-lactamase-1 (NDM-1) is a class B MBL that confers bacterial resistance to a broad range of β-lactam antibiotics, including carbapenem family, which currently form the backbone of treatment regimens for antibiotic-resistant bacterial infections (Kumarasamy *et al*, 2010; Bushnell *et al*, 2013). NDM-1 was first detected in *K. pneumoniae* (KP05-506) and *E. coli* (NF-NDM-1) isolated from urine and feces of a Swedish patient of Indian origin in 2008 (Yong *et al*, 2009). NDM-1 was later detected in bacteria present in environmental and clinical samples in 13 countries in Europe as well as in Asia, Australia and North and South America (Walsh *et al*, 2011). NDM-1 is most frequently identified in gram-negative bacteria such as *Acinetobacter* spp, *E. coli*, *Enterobacter* spp, and *K. pneumoniae,* and less frequently in *Citrobacter freundii, Enterobacter cloacae*, *K. oxytoca, Proteus mirabilis,* and *Providencia* spp (Thomson, 2010). Recently, carbapenemase-producing gram-negative bacteria were reported in Brazil and Russia, including 18 strains (17 *K. pneumoniae* and 1 *Acinetobacter nosocomialis*) and 11 isolates (9 *Enterobacter cloacae* and 2 *Morganella morganii*), raising concerns about the impact of resistance to this antibiotic on public health (Ageevets *et al*, 2014; Rozales *et al*, 2014). At present, such bacteria are susceptible only to polymyxins and tigecycline (Fomda *et al*, 2014).

The Chinese Center for Disease Control and Prevention reported that 11,298 NDM-1-producing strains of gram-negative bacilli were detected in 57 different hospitals from 18 provinces nationwide between January 2009 and September 2010, including four NDM-1-producing *A. baumannii* isolates (Chen *et al,* 2011). Guangzhou First People's Hospital isolated four strains of NDM-1-producing bacteria between 2008 and 2010, including one *K. ozaenae* and three *A. baumannii* strains (Yang *et al*, 2011). In 2012, a strain of NDM-1-producing *K. pneumoniae* was detected in a child's sputum specimen in a Hunan general hospital (Zhu *et al,* 2015). All these patients resided in mainland China and had never been to India.

The gene encoding NDM-1 is a member of a large gene family that encodes several carbapenemases (Trossman, 2014). Pulsed-field gel electrophoresis (PFGE) can be used to analyze the homology of the NDM-1 gene among isolates, and obtain a DNA profile for the strains (Qu *et al*, 2015; Yang *et al*, 2015). Horizontal gene transfer is thought to be the primary mechanism of bacterial antibiotic resistance, involving plasmids or integrons (Bercot *et al*, 2011; Castanheira *et al*, 2011; Bushnell *et al*, 2013). A recent study revealed that NDM-1 gene is flanked by short inverted repeat elements, which facilitate transposition and mobilization

of NDM-1 gene (Poirel *et al*, 2011a; Sun *et al*, 2015).

NDM-1-producing *E. cloacae* has been previously reported elsewhere in the world (Sun *et al*, 2010; Sun and Xue, 2011; Zou *et al*, 2012), and only very recently found in Shanghai and the Henan Province (China) (Hu *et al*, 2012; Liu *et al*, 2015). In this study, a strain of NDM-1-producing *E. cloacae* detected during a carbapenem resistance screening at the First Affiliated Hospital of Chongqing Medical University was subjected to trans-conjugation test, PFGE analysis, Southern blotting, resistance gene analysis, and antibiogram profiling to evaluate the strain's clinical characteristics and capacity for conjugal transfer. The current study highlights the global spread of NDM-1-carrying bacteria and the necessity for new treatment modalities.

MATERIALS AND METHODS

Bacterial isolation

A total of 299 distinct *E. cloacae* isolates were collected from clinical specimens (sputum, blood, urine, secretions, and bile) from patients in the First Affiliated Hospital of Chongqing Medical University, China, between January 2007 and February 2012. After bacteria were isolated using standard methods (Zhu *et al*, 2015), a VITEK 2 Compact Automated Bacterial Identification system (bioMerieux, Marcy l'Etoile, France) was used for biochemical identification. *E. cloacae* 413 was isolated from a sputum specimen of a 67-year-old female patient with pneumonia in 2012.

The study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval no. 2017-049). Written consent was obtained from all patients before participation.

Modified Hodge test

A modified Hodge test was used to screen for carbapenemase production (Girlich *et al*, 2012; Hammoudi *et al*, 2014). In brief, *E. coli* ATCC 25922, used as an indicator organism, was applied onto a Müller-Hinton (MH) agar plate (Becton Dickinson BBL, Le Pont de Claix, France) (1:10 dilution; original turbidity of 0.5 McFarland unit), and allowed to dry for 3 minutes. A 10-μg meropenem-susceptibility disk (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) was placed in the center of the test area. The test organism was streaked from the disk onto the plate edge, and incubated at 37°C for 16-18 hours. Then, the intersection of the test organism and *E. coli* ATCC 25922 was examined within the zone of inhibition of the carbapenem-susceptibility disk; the presence of a cloverleaf-shaped indentation of growth of test versus indicator strain was interpreted as carbapenemase production. *E. coli* ATCC 25922 growth was observed along with the test organism growth within the disk diffusion zone. In addition, *K. pneumoniae* K6 (ATCC 700603) and *E. cloacae* 267 were included as carbapenemase-negative strains.

Detection of metallo-β-lactamase (MBL) and gene

Imipenem-EDTA E-test was employed to detect MBL-producing bacteria (Khosravi *et al*, 2012). In brief, test bacteria were cultured overnight in MH broth at 37°C, and the culture was adjusted to 0.5 McFarland unit with sterile saline before application onto MH agar plates (Becton Dickinson BBL), which were dried for 10 minutes. An E-test MBL strip containing imipenem/imipenem + EDTA (IP/IPI) (Biomerieux) was placed on the MH agar plate and incubated at 37°C for 16-18 hours. A reduction in minimum inhibitory

concentration (MIC) in presence of EDTA ≥ 8-fold (IP/IPI ≥ 8) was interpreted as indicating MBL activity.

Six pairs of PCR primers designed to amplify the MBL genes were employed in a PCR-based assay (Table 1) (Yamane *et al*, 2004; Walsh *et al*, 2011). Plasmid DNA was extracted using Qiagen QIAamp DNA mini kit (Qiagen, Hilden, Germany). PCR mixture (50 μl) contained 25 μl Takara Premix *Taq* enzyme (Takara, Kyoto, Japan), 4 μl of DNA template, 1 μl of each primer (10 μM), and 19 μl of sterile ultrapure water. Thermocycling was conducted in Thermo Cycle S1000 PCR System (Bio-Rad, Hercules, CA) as follows: 94°C for 10 minutes; 35 cycles of 94°C for 60 seconds, 60°C for 30 seconds and 72°C for 30 seconds; a final step of 72°C for 10 minutes. Amplicons were separated by 1% agarose gel-electrophoresis, stained with ethidium bromide and documented using ChemiDoc XRS gel imaging system (Bio-Rad). Gel-purified amplicons (QIAquick PCR Purification Kit Protocol, Qiagen) were sequenced by Invitrogen (Shanghai, China). DNA sequences were aligned with those in the NCBI database (www.ncbi.nlm.nih.gov) using the BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence (of bla_{NDM-1}) was deposited in GenBank, accession no. JN860195.1. A dendrogram was generated using the DNAMAN software (Lynnon, San Ramon, CA).

Trans-conjugation test

Trans-conjugation test was performed using azide-resistant *E. coli* J53 Az^r (Antibiotics Institute of Shanghai Huashan Hospital, China) as recipient strain and NDM-1 MBL-producing *E. cloacae* 413 as the donor. Trans-conjugants with plasmid-encoded resistance were selected with sodium azide and ceftazidime. Donor and recipient strains were inoculated separately in 5 ml of LB broth (Sigma-Aldrich, St Louis, MO). After incubation at 37°C overnight, 500 μl each of donor and recipient bacterial cultures were inoculated in 5 ml of fresh LB medium, and cultured at 37°C. After 20 hours, 50 μl aliquot of cell suspension was applied to MH plates (Becton Dickinson BBL) containing sodium azide (100 μg/ml) and ceftazidime (2 μg/ml), and incubated at 37°C overnight. Bacterial colonies growing on plate were re-incubated on plates containing both compounds, and the strain that survived was named *E. coli* J53 (pNDM-1).

Conjugated *E. coli* J53 (pNDM-1) and *E. cloacae* 413 were inoculated separately in 5 ml of LB broth (Sigma-Aldrich), and incubated at 37°C overnight with shaking. Plasmids were extracted using an OMEGA small plasmid extraction kit (Omega Bio-Tek, Norcross, GA) and analyzed by 1% agarose gel-electrophoresis as described above. Extracted plasmids of *E. coli* J53 (pNDM-1) and *E. cloacae* 413 were used as templates for PCR amplification of bla_{NDM-1}, whose presence was assessed by electrophoresis and sequencing as described above.

PFGE analysis and Southern blotting

Low melting agarose gel containing total bacterial DNA from donor *E. cloacae* 413 and conjugated *E. coli* J53 (pNDM-1) was treated with *Xba*I for 24 hours and electrophoresed at 6 V/cm) for 22 hours at 14°C in a CHEF-DR II PFGE system (Bio-Rad) with a pulse angle of 120° and pulse time varying from 4 to 40 seconds. After 1 hour staining in ethidium bromide, the gels images were recorded with a ChemiDoc XRS gel imaging system. DNA restriction patterns were analyzed and interpreted according to the criteria proposed by Tenover *et al* (1995).

Table 1
PCR primers used in the study.

Primer	Sequence	Amplicon size (bp)
MBL gene		
bla_{VIM}-F	5'-GATGGTGTTTGGTCGCATA-3'	390
bla_{VIM}-R	5'-CGAATGCGCAGCACCAG-3'	
bla_{IMP}-F	5'-CATGGTTTGGTGGTTCTTGT-3'	448
bla_{IMP}-R	5'-ATAATTTGGCGGACTTTGGC-3'	
bla_{GIM-1}-F	5'-TCGACACACCTTGGTCTGAA-3'	477
bla_{GIM-1}-R	5'-AACTTCCAACTTTGCCATGC-3'	
bla_{SPM-1}-F	5'-AAAATCTGGGTACGCAAACG-3'	271
bla_{SPM-1}-R	5'-ACATTATCCGCTGGAACAGG-3'	
bla_{SIM-1}-F	5'-TACAAGGGATTCGGCATCG-3'	570
bla_{SIM-1}-R	5'-TAATGGCCTGTTCCCATGTG-3'	
bla_{NDM-1}-F	5'-GGTTTGGCGATCTGGTTTTC-3'	621
bla_{NDM-1}-R	5'-CGGAATGGCTCATCACGATC-3'	
Antimicrobial resistance gene		
TEM-F	5'-TTCTTGAAGACGAAAGGGC-3'	1207
TEM-R	5'-ACGCTCAGTGGAACGAAAAC-3'	
SHV-F	5'-GCCCGGGTTATTCTTATTTGTCGC-3'	990
SHV-R	5'-TCTTTCCGATGCCGCCGCCAGTCA-3'	
CTX-M-F	5'-SCVATGTGCAGYACCAGTAA-3'	543
CTX-M-R	5'-CCGCRATATGRTTGGTGGTR-3'	
CMY-F	5'-GCTTACAGCCTCTTTCTCCAC-3'	1082
CMY-R	5'-CCTCGACACGGACAGGGTTA-3'	
qnrA-F	5'-AGAGGATTTCTCACGCCAGG-3'	580
qnrA-R	5'-TGCCAGGCACAGATCTTGAC-3'	
qnrB-F	5'-GGMATHGAAATTCGCCACTG-3'	264
qnrB-R	5'-TTTGCYGYYCGCCAGTCGAA-3'	
qnrS-F	5'-GCAAGTTCATTGAACAGGGT-3'	428
qnrS-R	5'-TCTAAACCGTCGAGTTCGGCG-3'	
Aac(6')-Ib-cr-F	5'-TTGCAATGCTGAATGGAGAG-3'	172
Aac(6')-Ib-cr-R	5'-CGTTTGGATCTTGGTGACCT-3'	
armA-F	5'-ATTTTAGATTTTGGTTGTGGC-3'	101
armA-R	5'-ATCTCAGCTCTATCAATATCG-3'	
npmA-F	5'-GGGCTATCTAATGTGGTG-3'	229
npmA-R	5'-TTTTTATTTCCGCTTCTTCGT-3'	
rmtA-F	5'-AAACTATTCCGCATGGTTC-3'	88
rmtA-R	5'-TCATGTACACAAGCTCTTTCC-3'	
rmtB-F	5'-ACTTTTACAATCCCTCAATAC-3'	171
rmtB-R	5'-AAGTATATAAGTTCTGTTCCG-3'	
rmtC-F	5'-CAGGGGTTCCAACAAGT-3'	246
rmtC-R	5'-AGAGTATATAGCTTGAACATAAGTAGA-3'	
rmtD-F	5'-GGAAAAGGACGTGGACA-3'	171
rmtD-R	5'-TCCATCGATTCCACAGG-3'	

F, forward; R, reverse.

Then, the DNA was transferred onto a nylon membrane (Hybond N+; Amersham Pharmacia Biotech, Orsay, France) and Southern hybridized with a bla_{NDM-1}-specific probe labelled using DIG High Prime DNA labelling kit (Roche, Sant Cugat del Vallès, Spain) (Southern, 2006).

Bacterial plasmid resistance gene analysis

An OMEGA plasmid extraction kit (OMEGA) was used to extract pNDM-1-conjugated *E. coli* J53 and *E. cloacae* 413 plasmids. Primers for amplification of drug-resistance genes *aac(6')-Ib-cr*, *ArmA*, *CMY*, *CTX-M*, *npmA*, *qnrA*, *qnrB*, *qnrS*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *SHV*, and *TEM* are listed in Table 1 (Cattoir *et al*, 2007; Bercot *et al*, 2011; Poirel *et al*, 2011b). PCR mixture (50 μl) contained Takara Premix *Taq* enzyme, 4 μl of DNA template, 1 μl of each primer (10 μM), and 19 μl of sterile ultrapure water. Amplification of *rmtA*, *rmtB*, *rmtC*, *rmtD*, *aac(6')-Ib-cr*, *ArmA* was conducted as follows: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 54.5°C for 30 seconds and 72°C for 30 seconds; with a final step of 72°C for 5 minutes. Amplification of *qnrA*, *qnrB* was performed as follows: 95°C for 10 minutes; 35 cycles of 95°C for 60 seconds, 54°C for 60 seconds and 72°C for 60 seconds; and a final step of 72°C for 10 minutes. *CMY* thermocycling was conducted as follows: 98°C for 10 minutes; 35 cycles of 98°C for 40 seconds, 60°C for 40 seconds and 72°C for 30 seconds; with a final step of 72°C for 10 minutes. *CTX-M* thermocycling was performed as follows: 92°C for 10 minutes; 35 cycles of 92°C for 50 seconds, 54°C for 40 seconds and 70°C for 30 seconds; and a final step of 70°C for 10 minutes. Amplification of *npmA* was conducted as follows: 90°C for 10 minutes; 35 cycles of 90°C for 60 seconds, 54°C for 40 seconds and 70°C for 30 seconds; and a final step of 70°C for 10 minutes. *SHV* and *TEM* thermocycling were conducted as follows: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 70°C for 30 seconds; with a final step of 70°C for 5 minutes. Amplicons were isolated and sequenced as described above.

Antimicrobial susceptibility determination

Kirby-Bauer disk diffusion and agar plate dilution methods as recommended by Clinical and Laboratory Standards Institute were used to determine MIC values of imipenem and meropenem (CLSI, 2010). For strains with an inhibition zone diameter ≤ 21 mm in the disk diffusion test, the agar plate dilution method then was used to determine MIC values (≥ 2 μg/ml considered resistant to carbapenems). MIC values for NDM-1-producing *E. cloacae* 413, *E. coli* J53 Az^r and pNDM-1-conjugated *E. coli* J53Az^r were also determined of amoxicillin (AML), cefoperazone (CFP), ceftazidime (CAZ), ciprofloxacin (CIP), fosfomycin (FOS), gatifloxacin (GAT), imipenem (IPM), meropenem (MEM), penicillin (PG), piperacillin-tazobactam (TZP), polymyxin B (PB), and tobramycin (TOB).

RESULTS

Identification of carbapenemase-producing *E. cloacae* strains

In vitro antibiotic sensitivity testing of 299 clinical *E. cloacae* strains revealed that 14 strains were resistant to carbapenems, and isolated mostly from elderly (41-93 years old) and male patients (10). Specimens were collected from wound secretions (4 strains), urine (4), sputum (4), and blood (2). Between 2007 and 2012, the prevalence rates of carbapenem resistance in *E. cloacae* clinical isolates increased annually: 0.33%, 0%, 0.33%, 1.67%, and 1.67% in 2007, 2008, 2009, 2010 and 2011, respectively. Of the 14

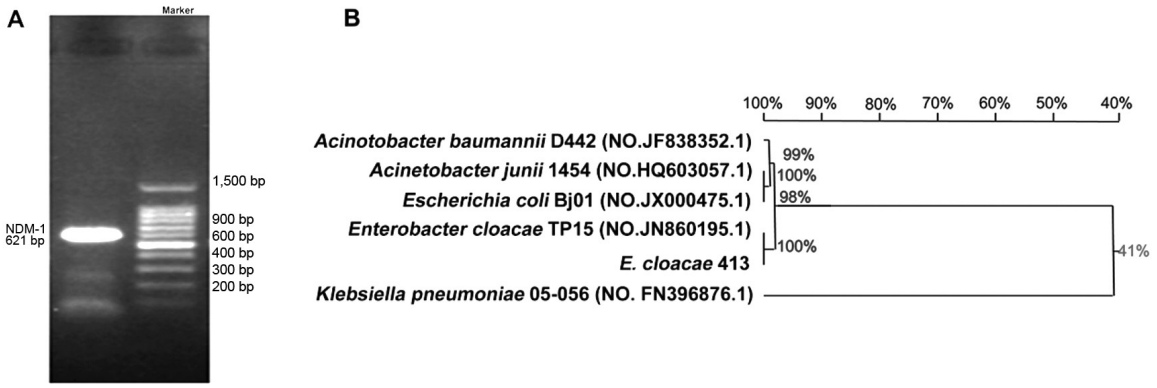

Fig 1–Identification of bla_{NDM-1} in a plasmid from *Enterobacter cloacae* 413 strain. A. Gel-electrophoresis of amplicons obtained using bla_{NDM-1}-specific primers listed in Table 1. B. Comparison of 621 bp *E. cloacae* 413 bla_{NDM-1} sequence. Scale represents percent similarity.

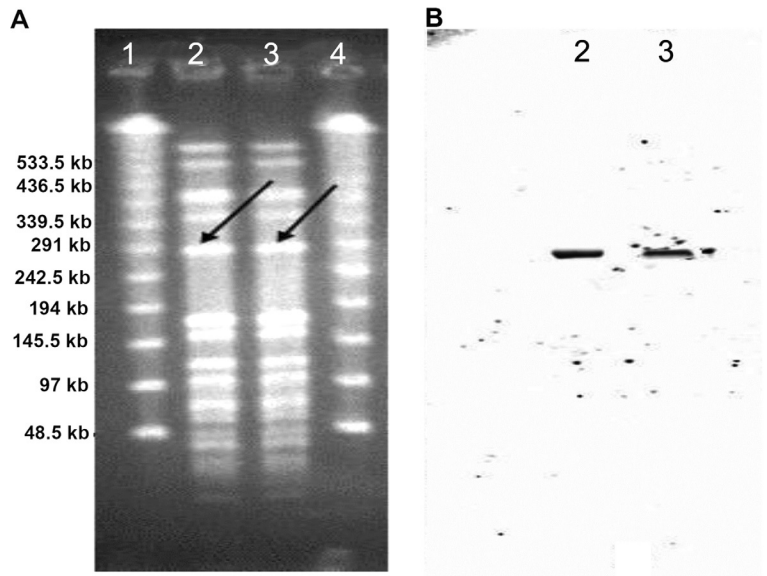

Fig 2–Pulsed-field gel electrophoresis (A) and Southern blot (B) of *Enterobacter cloacae* 413 and pNDM-1 conjugated *Escherichia coli* J53 DNA. Total extracted DNA was digested with *Xba*I and Southern blotting was performed with DIG-labelled 621 bp *E. cloacae* 413 bla_{NDM-1} fragment. Lanes 1 and 4, Lambda PFG size markers (New England Biolabs, Beijing, China); lane 2, *E. cloacae* 413; lane 3, pNDM-1 conjugated *E. coli* J53. Arrows indicate bands corresponding to those in panel B.

carbapenem-resistant *E. cloacae* strains, 6 produced carbapenemase (strains no. 130, 282, 343, 401, 408 and 413) and exhibited a positive phenotype in a modified Hodge test (data not shown).

Detection of bla_{NDM-1}-positive *E. cloacae* strain

PCR-based detection of carbapenemase-encoding gene failed to detect bla_{VIM}, bla_{IMP}, bla_{GIM-1}, or bla_{SPM-1}; however, bla_{NDM-1} amplicon (621 bp) was detected in one *E. cloacae* strain (no. 413) (Fig 1A) with a sequence identical to that of *E. cloacae* TP15 (Fig 1B). Imipenem-EDTA E-test confirmed that *E. cloacae* 413 was more sensitive to imipenem in the presence of EDTA, with an IP/IPI ≥ 16 (data not shown).

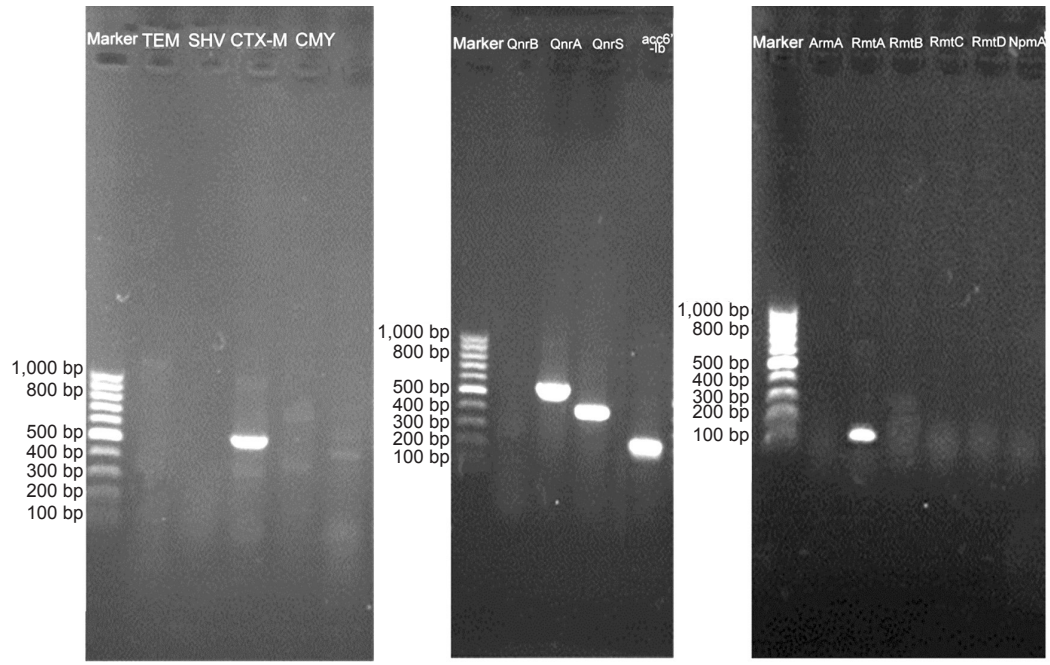

Fig 3–Gel-electrophoresis of amplicons of antimicrobial resistance genes from pNDM-1 of *Enterobacter cloacae* 413. Gene specific primers and expected amplicon sizes are listed in Table 1.

Trans-conjugation of *E. cloacae* strain 413 and analysis of trans-conjugated plasmid

A trans-conjugation experiment was performed to assess transmission of bla_{NDM-1} from *E. cloacae* 413 to azide-resistant *E. coli* J53 Az^r. Plasmid (pNDM-1) was detected in a conjugated *E. coli* J53 (azide and ceftazidime resistant) clone and *E. cloacae* 413, both of ~20 kb (super-coiled) in size and each carrying $bla_{NDM-1,}$ as demonstrated by the correct amplicon size and sequence (data not shown).

PFGE typing and Southern blotting

Total DNA specimens of *E. cloacae* 413 and conjugated *E. coli* J53, including their respective pNDM-1, were digested with *Xba*I and subjected to PFGE followed by Southern blotting. The presence of bla_{NDM-1} was revealed on a 291 kbp fragment from both types of cells (Fig 2). The PFGE profile was of type B according to the criteria proposed by Tenover *et al* (1995).

Plasmid-carried antimicrobial resistance genes of *E. cloacae* 413 and conjugated *E. coli* J53, and antimicrobial susceptibility

PCR-based analysis of a variety of antimicrobial resistance genes carried by pNDM-1 of *E. cloacae* 413 revealed the presence of aac(6')-Ib-cr (encoding aminoglycoside acetyl transferase), bla_{CTX-M}, rmtA (conferring resistance to aminoglycoside antibiotics), qnrA, and qnrS (latter two conferring quinolone resistance) (Fig 3). Similar results were obtained for pNDM-1 of conjugated *E. coli* J53 (data not shown). The identities of these genes were confirmed by direct sequencing of amplicons.

MICs of antimicrobial agents against *E. cloacae* 413 and conjugated *E. coli* J53 indicated resistance to amoxicillin, cefoperazone, ceftazidime, penicillin, and piperacillin-tazobactam (Table 2), consistent with the set of antimicrobial genes carried by pNDM-1.

DISCUSSION

Carbapenem resistance of microbes of clinical relevance is an increasing public health problem worldwide (Guerra *et al*, 2014). We isolated from sputum of a 67-year-old female admitted to a hospital in Chongqing, China, an NDM-1-producing *E. cloacae* strain, in which bla_{NDM-1} is located on a large plasmid (supercoil size ~20 kbp) that also carried 5 other genes conferring resistance to amoxicillin, cefoperazone, ceftazidime, penicillin, and piperacillin-tazobactam. The plasmid could be transferred to *E. coli* that subsequently displayed antimicrobial resistance pattern similar to that of the donor *E. cloacae* strain. However, the *E. cloacae* strain was still sensitive to fosfomycin and polymyxin B.

NDM-1-producing *E. cloacae* has been previously found only in India (Kumarasamy *et al*, 2010; Castanheira *et al*, 2011; Bushnell *et al*, 2013), but recently its presence on mainland China, notably in Shanghai and the Henan Province, was reported (Liu *et al*, 2015; Qu *et al*, 2015), suggesting that robust antibiotic resistance surveillance and infection control measures should be implemented to fight such microorganisms. Therefore, the current report is the third of this kind. The patient had never been to India or Pakistan; however, it is worth noting that individuals from the latter regions reside in Chongqing. The actual prevalence of NDM-1-producing bacteria in China is unknown. In Brazil, nine *E. cloacae* complex and two *Morganella morganii* were reported (Rozales *et al*, 2014).

In this study, the patient improved after treatment with moxifloxacin and cefoperazone, although the strain was probably resistant to these two antibiotics. At the time, no other antibiotics were

Table 2
Antimicrobial susceptibility of *Enterobacter cloacae* 413, pNDM-1-conjugated *Escherichia coli* J53 and *E. coli* J53.

Bacterium	Minimum inhibitory concentration (μg/ml)										
	AML	TZP	PG	CFP	CAZ	TOB	GAT	CIP	FOS	PB	IPM	MEM
E. cloacae 413	>256	>256	>256	>256	>256	8	16	64	8	2	16	8
E. coli J53[c]	>256	>256	>256	>256	>256	8	16	64	8	2	16	8
E. coli J 53	4	1	4	1	<1	1	<1	<1	2	2	<0.5	<0.5

AML, amoxicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CFP, cefoperazone; FOS, fosfomycin; GAT, gatifloxacin; IPM; imipenem; MEM, meropenem; PB, polymyxin B; PG, penicillin; TOB, tobramycin; and TZP, piperacillin-tazobactam. *E. coli* J53[c], pNDM-1-conjugated *Escherichia coli* J53.

available and the latters were used. There was no evidence of *E. cloacae* lung colonization; hence, the pathogenicity of *E. cloacae* remains an open question. However, the facile ability to transfer its plasmid carrying multiple antimicrobial resistant genes, including those to carbapenems, cannot be overlooked.

On 28 September, 2010, the Chinese Ministry of Health issued the "Pan-drug resistant NDM-1 Producing Enterobacteriaceae Infections Treatment Guidelines (Trial Version)" for such refractory infections (Ministry of Health of China, 2010). These guidelines indicate that for mild to moderate infections, single or combined effective drugs can be administered. For severe and serious infections, clinicians should choose effective or relatively effective antibiotic drug combinations, according to antibiogram results, and closely monitor the patient's response to therapy. Patients not improving may require tigecycline and polymyxin.

Once NDM-1 bacterial infection occurs, multiple drug resistance renders treatment extremely difficult. It is therefore important to trace the origin of the drug resistance gene and promote the monitoring, prevention, and control of drug-resistant bacteria. In addition, prescription of antibiotics should be chosen carefully. Furthermore, the medical personnel should enforce strict disinfection measures and strengthen hospital infection prevention and control practices, which are very important in preventing the spread of drug-resistant bacteria.

In conclusion, this report identified in mainland China an NDM-1-producing clinical *E. cloacae* strain resistant to carbapenems, quinolones and aminoglycosides. This array of antimicrobial resistance could be transferred to other bacteria (*eg*, *E. coli*). These findings highlight the global spread of NDM-1-carrying bacteria and the necessity for evidence-based use of antibiotics.

ACKNOWLEDGEMENTS

The authors thank Chengwei Liu and Bei Jia for their assistance in PCR and DNA sequencing experiments. The study was funded by the Chongqing Municipal Committee of Science and Technology Education Project (KJ100311).

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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