

Detection of New Delhi Metallo-Beta-Lactamase (Encoded by *bla_{NDM-1}*) in *Enterobacter aerogenes* in China

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Background: The increase in *bla_{NDM-1}* in *Enterobacteriaceae* has become a major concern worldwide. In previous study, we investigated clonal dissemination and mechanisms of resistance to carbapenem in China. **Methods:** We carried out retrospective surveillance for *bla_{NDM-1}* among carbapenem-resistant enterobacter strains, which were isolated from patients at our hospital by bacterial strains selection, antimicrobial susceptibility testing, species identification, and molecular detection of

resistance gene. **Results:** We found three *bla_{NDM-1}*-positive isolates which were identified as *Enterobacter aerogenes* in clinical patients in China. The *bla_{NDM-1}*-positive *Enterobacter aerogenes* isolates were first found. **Conclusion:** It is important to mandate prudent usage of antibiotics and implement infection control measures to control the spread of these resistant *bla_{NDM-1}*-positive strains. *J. Clin. Lab. Anal.* 31:e22044, 2017. © 2016 Wiley Periodicals, Inc.

Key words: *bla_{NDM-1}*; *enterobacter aerogenes*; New Delhi metallo-beta-lactamase; resistance

INTRODUCTION

The increase of the novel carbapenem resistance mechanism of New Delhi metallo-beta-lactamase (*bla_{NDM-1}*) in *Enterobacteriaceae* has become a major concern worldwide (1). The *bla_{NDM-1}* gene was first reported in 2009 in a *Klebsiella pneumonia* isolated in a Swedish hospital from a patient previously hospitalized in a medical institution in India (2). Since then, the isolation of bacterial species carrying the *bla_{NDM-1}* gene has been reported in several countries, usually from patients who travelled to the Indian subcontinent.

There were several studies which demonstrated the high similarity of the genomic region surrounding *bla_{NDM-1}* gene among members of *Enterobacteriaceae* (1–3). Moreover, *bla_{NDM-1}*-positive plasmids were revealed by complete genome sequencing in *E. coli* that was indicated a broad host range and multiple mobile genetic elements, and highlighted the potential for the extensive spread of this determinant of resistance.

There were some previous reports on *bla_{NDM-1}* gene which was focused on its epidemiological dissemination in different countries, including India, China, the

United States, Germany, France, Canada, Austria, Africa, and the United Kingdom (1, 4). However, there was no *bla_{NDM-1}*-producing *Enterobacter aerogenes* detected in these studies. In the current study, we investigated clonal dissemination and mechanisms of resistance to carbapenem on tumor patients in China.

MATERIALS AND METHODS

Bacterial Strains

We carried out retrospective surveillance for *bla_{NDM-1}* among carbapenem-resistant *Enterobacter* strains which were isolated from patients at the Affiliated Tumor Hospital of Zhengzhou University in China. There are 2,600 beds in this tumor hospital and

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a total of 14,825 tumor patients were detected for *bla*_{NDM-1} gene by Kirby–Bauer diffusion test and PCR amplification from January 2010 to June 2014. The research protocol was approved by the institutional ethics committee of the bioscience of Zhengzhou University. All the participants signed an individual informed consent after receiving a detailed explanation of the research. All the data were processed respecting the confidentiality of the participants.

Antimicrobial Susceptibility Testing

MICs of 17 antibiotics, together with the *MBL* phenotype test, were determined using the *E*-test. The susceptibility results were interpreted according to the CLSI guidelines (5). The MIC breakpoint of cefoperazone for *Enterobacteriaceae* was used for cefoperazone/sulbactam (6). Due to the absence of CLSI criteria, the BSAC breakpoint and the FDA breakpoint for *Enterobacteriaceae* were applied for aztreonam and tigecycline, respectively (5). Quality controls were included using standard strains (*E. coli* ATCC 25922).

Species Identification

The *bla*_{NDM-1}-positive isolates were distinguished by the automated bacterial identification susceptibility analyzers. The metalloenzyme phenotype of isolates was determined using the *E*-test. Further differentiations within the strains were performed using the 16S rRNA gene intergenic spacer (ITS) sequence to differentiate the strains. The isolates were screened for the species identification by PCR with primers 16s-F-(5'-AGTTTGATCTGGCTCAG-3') and 16s-R-(5'-GGAC-TACAGGGTATCTAAT-3').

In order to distinguish the source of the strains, the isolates were detected by PCR with primer ERIC2-5'-AAGTAAGTGACTGGGGTGAGCG-3'. PCR experiments were performed according to standard conditions with an annealing temperature of 45°C.

Test strains were adjusted to the McFarland 0.5 standard and used to inoculate Mueller-Hinton agar plates. Depending on the test, a 10-μg imipenem (IPM) disk was placed on the plate, and a blank filter paper disk was placed at a distance of 10 mm (edge to edge). To the blank disk, 10 μl of a 0.5 M EDTA solution (ca. 1,900 μg of disodium salt, dihydrate) was added. After overnight incubation, the presence of even a small synergistic inhibition zone (4 mm) was interpreted as positive.

Molecular Detection of Resistance Genes

The isolates were detected for the presence of *bla*_{NDM-1} by PCR with primers NDM-1-U-(5'TC

GCATAAAACGCCTCTG-3') and NDM-1-L-(5'-GAAACTGTCTG CACCTCAT-3'). PCR experiments were performed according to standard conditions with an annealing temperature of 55°C.

RESULTS

Clinical Features of the *bla*_{ndm-1}-Positive Strains

From a total of 14,825 clinical tumor patients, there were 42 patients who were infected with *Enterobacter aerogenes*. We then found three *bla*_{NDM-1}-positive *Enterobacter aerogenes* isolates. According to the epidemiological data for the three corresponding patients, there were two isolates, which were found from venous indwelling tube and another was from abdominal drainage. The three patients who only underwent surgical treatment without radiotherapy and chemotherapy drugs were diagnosed with lymphoma, colorectal cancer, and gastric cancer, respectively. The histories of the patients were confirmed as lacking any foreign travel. However, they had lived in the same ward at the same time (Table 1). The patients received empirical antibiotic treatment, including ceftriaxone, meropenem, imipenem, piperacillin/tazobactam, ampicillin/sulbactam, amikacin, levofloxacin, or trimethoprim/sulfamethoxazole, during their hospitalization.

Antimicrobial Susceptibility Testing

The results of antimicrobial susceptibility testing had been showed that three isolates were sensitive only to LVX (levofloxacin), CIP (ciprofloxacin), and PB (polymyxin B) (5). However, two isolates (0711 and 0807) were intermediary to IPM (imipenem) and MEC (meropenem). The three isolates were resistant to other antibiotics (Table 2).

Species Identification

The three *bla*_{NDM-1}-positive isolates were diagnosed as *Enterobacter aerogenes* using BD PhoenixTM-100 automated bacterial identification susceptibility analyzers system. The three isolates are consistent with *Enterobacter aerogenes* by using 16s rRNA sequence alignment. So we believed that three bacteria were *Enterobacter aerogenes*.

All the metalloenzyme phenotypes of three isolates were positive. The 0711, 0807, and 1311 isolates measured 20 mm, 7 mm, and 10 mm in the IPM *E*-test, respectively. After the antimicrobial papers that add EDTA, the inhibition zone diameters were 28 mm, 12 mm, and 16 mm, respectively. Because of the difference of diameter above 4 mm, this indicated that three

TABLE 1. Clinical Features of the *bla*_{NDM-1}-Positive Strains

Isolate	Sex	Age	Source	Diagnosis	Ward	Admission time	Foreign travel	Antibiotic treatment	Outcome
0711	Male	55	Venous indwelling catheter	Lymphoma	General Surgery	Feb.21 2011	No	Yes	Cured ¹
0807	Female	63	Abdominal drainage	Gastric cancer	General Surgery	Feb.18 2011	No	Yes	Cured
1311	Female	48	Venous indwelling catheter	Colorectal cancer	General Surgery	Feb.21 2011	No	Yes	Cured

¹Cured: The *bla*_{NDM-1}-positive strains were not detected for 2 weeks.

TABLE 2. Antibiotic susceptibilities and β-lactamase detection of *bla*_{NDM-1}-positive *Enterobacter aerogenes* (mg/l)

Isolate	CZ*	GEN	AM	CTX	CIP	FEP	FOX	CRO	TZP	PIP	IPM	MEC	AMK	LVX	CAZ	ATM	PB
0711	>8 [†]	>16	>32	>16	≤1	>16	>16	>32	>64/4	>64	2	4	>32	≤2	>16	>8	<2
0807	>8	>8	>16	>16	≤1	>16	>16	>32	>64/4	>64	>8	2	>32	≤2	>16	>8	<2
1311	>8	>16	>32	>32	≤1	>32	>16	>32	>64/4	>64	4	8	>32	≤2	>16	>8	<2

*CZ, cefazolin; GEN, gentamicin; AM, Ampicillin; CTX, cefotaxime; CIP, ciprofloxacin; FEP, cefepime; FOX, ceftaxime; CRO, ceftriaxone; TZP, piperacillin/tazobactam(E-test); PIP, piperacillin; IPM, imipenem(E-test); MEC, meropenem(E-test); AMK, amikacin; LVX, levofloxacin; CAZ, ceftazidime; ATM, aztreonam; PB, polymyxin B(E-test).

[†]MICs were determined by the BD PhoenixTM-100 automated bacterial identification susceptibility analyzers system or the E-test. Where a “>” sign is present in an entry with two values, the sign applies to both values in the entry. The second MIC refers to the second drug in the compound.

Enterobacter aerogenes metalloenzyme phenotype experiments were positive.

Every isolate amplified four belts in the ERIC2 typing. The three isolates were of the same molecular weight belts in Marker 2000 belts, Marker 1000 belts, Marker 750 belts, and Marker 150 belts, respectively. So it was indicated that the three *Enterobacter aerogenes* were of the same clone (Fig. 1).

Molecular Detection of Resistance Genes

The isolates were detected for the presence of *bla*_{NDM-1} by PCR. Approximately a 1,000-bp fragment from DNA of the three *Enterobacter aerogenes* was

obtained (Fig. 2), and the three fragments were of the same DNA sequence by gene sequencing. The fragment was entirely sequenced by primer walking which revealed 99% identity to the sequence reported for *A Klebsiella pneumoniae* RJF866. The fragment entirely sequenced in our study has already been accepted by GenBank. The accession number is KJ577744.

DISCUSSION

The *bla*_{NDM-1} gene was first reported in 2009 in India. Since then, the global distribution of the *bla*_{NDM-1} gene has been extensively described (1, 7–9). However, the presence of this gene in *Enterobacter aerogenes* has not been reported. Here, in our study, we find three *bla*_{NDM-1}-positive *Enterobacter aerogenes* in clinical cancer patients in China. Based on the detailed clinical information, the surgery of patients may be more susceptible, and contact transmission was the main route of infection.

In addition, we found that the *bla*_{NDM-1}-positive *Enterobacter aerogenes* were highly resistant to all antibiotics except levofloxacin, ciprofloxacin, imipenem, and meropenem (Table 2). It was different from previous reports in antimicrobial susceptibility results. The antimicrobial susceptibility testing to *bla*_{NDM-1}-positive *Acinetobacter* and *Klebsiella pneumoniae* indicated that the *bla*_{NDM-1}-positive strains has been reported in China, which were resistant to most antibiotics, only sensitive to polymyxin and tigecycline (10, 11). In fact, the therapeutic agents prescribed to the patients were followed by empirical

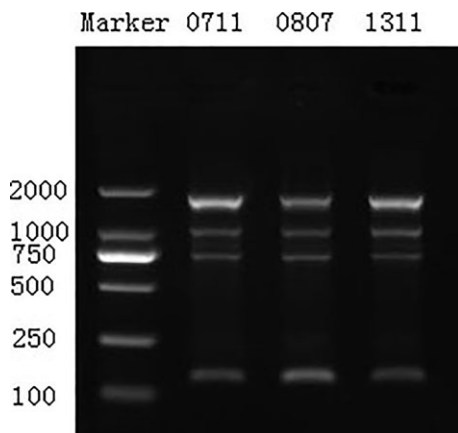


Fig. 1. ERIC2-PCR amplification products of the three *Enterobacter aerogenes*.

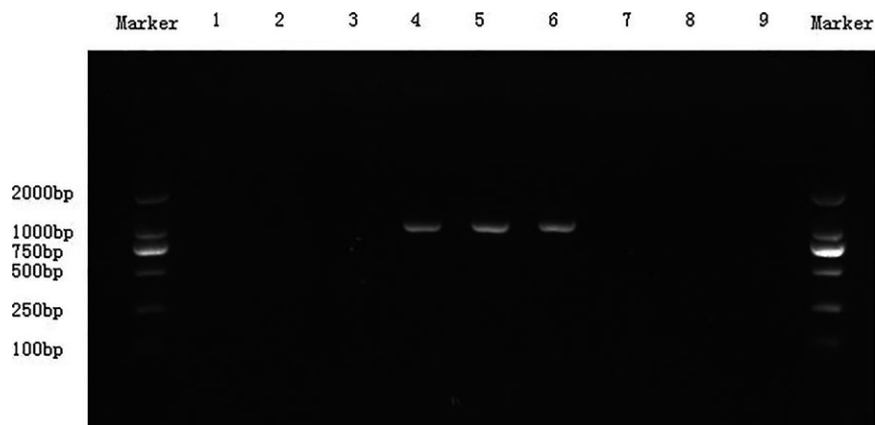


Fig. 2. *bla*_{NDM-1}-PCR products of the three *Enterobacter aerogenes*; 1–9 isolates are all *Enterobacter aerogenes*: 1, 0125; 2, 0478; 3, 0684; 4, 0711; 5, 0807; 6, 1311; 7, 1987; 8, 2063; 9, 2794. Approximately a 1000 bp fragment from DNA of 4(0711), 5(0807), and 6(1311) was obtained, respectively. Others were negative.

antibiotic treatment paradigms rather than the results of antimicrobial susceptibility. Thus, the antibiotics could have been no effective for the control of infections produced by *bla*_{NDM-1}-positive *Enterobacter aerogenes*.

As a gene resistant to the metallo-beta-lactamase, the *bla*_{NDM-1} gene can decompose most antibiotics, including cephalosporins, penicillins, carbapenems, and other beta-lactam antibiotics. It was reported that the *bla*_{NDM-1} gene was in the coding sequence of plasmid. There were often some other resistance genes inserted around the coding sequence (12). Thus, it was a complex resistant phenotype to *bla*_{NDM-1}-positive isolates.

We detected three *bla*_{NDM-1}-positive *Enterobacter aerogenes* which had the same molecular weight belt by ERIC2-PCR and *bla*_{NDM-1}-PCR. So we can realize that the *bla*_{NDM-1} gene was broken out in small scope. The three patients had not gone abroad, and they were the first to be hospitalized. All of them were infected after surgery in hospital. Coincidentally, they almost lived in the same ward of a department in the meantime. Thus, there was the existence of cross time and space to infect.

The isolates, *bla*_{NDM-1}-producing metalloenzymes, had strong ability to spread by previous studies which had been reported the *bla*_{NDM-1}-positive isolates could cause widespread popularity in the world. There were some small outbreaks reported (8,13). It was found that four of the same ST-typing *Klebsiella pneumoniae* isolates producing *bla*_{NDM-1} prevailed in South Korea (14). Same type of ST-type *Acinetobacter* isolates producing *bla*_{NDM-1} were reported in French (15). Further studies had shown that *bla*_{NDM-1} gene is typically encoded on the plasmid. There were many movable original copies around the gene (e.g., ISAb125), which could make the *bla*_{NDM-1} gene had strong

spread ability. If the patients had underlying disease, it was easy to cause small range of popularity through crossing infection, even large-scale outbreak of the epidemic. Thus, it is important to mandate prudent usage of antibiotics and implement infection control measures to control the spread of these resistant *bla*_{NDM-1}-positive strains.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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