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First Indian report of IncX3 plasmid carrying \( \text{bla}_{\text{NDM}-7} \) in \textit{Escherichia coli} from bloodstream infection: potential for rapid dissemination

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Abstract

\textit{Enterobacteriaceae} with \( \text{bla}_{\text{NDM}-7} \) is only infrequently observed. Self-transmissible plasmids carrying the \( \text{bla}_{\text{NDM}} \) gene increase the dissemination of carbapenem resistance in developing countries. This study investigates the whole genome sequence of a \( \text{bla}_{\text{NDM}-7} \)-positive \textit{Escherichia coli}. The isolate was an extended-spectrum \( \beta \)-lactamase producer by combined disc diffusion test and carbapenemase producer by CarbaNP method. Sequencing results revealed the isolate as \textit{E. coli} ST-167 with IncX3 plasmid carrying \( \text{bla}_{\text{NDM}-7} \) in addition to \( \text{bla}_{\text{TEM}-1} \) and \( \text{bla}_{\text{CGT-HY42}} \) genes. The identification of IncX3-\( \text{bla}_{\text{NDM}-7} \) combination is the first report in India where \( \text{bla}_{\text{NDM}-7} \) is known to cause higher resistance to carbapenems compared to its variants. © 2017 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: \( \text{bla}_{\text{NDM}-7} \), carbapenem resistance, IncX3, ST167 \textit{Escherichia coli}, whole genome sequencing

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Introduction

Extended-spectrum \( \beta \)-lactamase (ESBL)-producing organisms pose unique challenges in community and clinical settings. Carbapenems are known to be promising drugs for ESBL-producing organisms, but increasing resistance to carbapenems among \textit{Enterobacteriaceae} has been reported. The prevalence of carbapenem-resistant \textit{Enterobacteriaceae} in India has been reported to vary from 7 to 51%. Carbapenem-resistant \textit{Enterobacteriaceae} has been associated with high mortality and morbidity rates of 40 to 50% [1]. New Delhi metallo-\( \beta \)-lactamase (NDM) protein has an extensive pattern of spread as a result of its plasmid location. \( \text{bla}_{\text{NDM-1}} \) is the most prevalent variant among \textit{Enterobacteriaceae}. However, \( \text{bla}_{\text{NDM-4}}, \text{bla}_{\text{NDM-5}}, \text{bla}_{\text{NDM-7}} \) and \( \text{bla}_{\text{NDM-14}} \) were reported to have enhanced hydrolytic activity compared to \( \text{bla}_{\text{NDM-1}} \), although only a few cases were reported. Further, a wide range of Gram-negative genera containing diverse \( \text{bla}_{\text{NDM}} \)-harbouring plasmids has been reported in >15 countries worldwide [2]. The various plasmid-borne \( \text{bla}_{\text{NDM}} \) are listed in Table 1.

Several studies have reported \( \text{bla}_{\text{NDM-1}} \)-carrying IncX3 plasmid in \textit{Enterobacteriaceae}. The IncX3 plasmids carrying \( \text{bla}_{\text{NDM-1}} \) or \( \text{bla}_{\text{NDM-5}} \) were found to have identical backbones [9]. Therefore, patients infected or colonized with bacteria containing \( \text{bla}_{\text{NDM-7}} \) IncX3 plasmids will pose challenges to infection control compared to other \( \text{bla}_{\text{NDM}} \) variants [11].

Spread of antimicrobial resistance through plasmid has become common but is difficult to track. Outbreaks due to plasmid often go undetected, as this may occur among different genera or species. Thus, tracking is of greater medical importance to prevent the spread among other genera or species. Specific molecular techniques like next-generation sequencing will be handy to identify such plasmids. We report the draft genome sequence of \( \text{bla}_{\text{NDM-7}} \)-carrying, IncX3-positive \textit{Escherichia coli} isolated from clinical blood specimen.

Methods

Bacterial strains

In total 773 \textit{E. coli} isolates were obtained from blood specimen during the study period of January to December 2014. A total of 15.7% (\( n = 122 \)) of these isolates were carbapenem resistant, of which a multidrug-resistant \textit{E. coli} isolate (B37305) positive for \( \text{bla}_{\text{NDM}} \) was selected for whole genome sequencing. The selected isolate was obtained from a patient, aged 65 years, who experienced complications related to a gallbladder carcinoma, who obtained care at Christian Medical College, Vellore, India.

Antimicrobial susceptibility testing

The isolate was screened for antimicrobial susceptibility by the disc diffusion method using cefotaxime (30 \( \mu \)g), ceftriaxone (30 \( \mu \)g), ceftazidime (30 \( \mu \)g), cefepime (30 \( \mu \)g), piperacillin/
Diverse NDM-harbouring plasmids and their origin

| Country   | Organism                  | Plasmid   | NDM variant | Reference |
|-----------|---------------------------|-----------|-------------|-----------|
| Japan     | Escherichia coli          | IncA/C    | NDM-1       | Sekizuka et al. [3] |
| France    | Enterobacteriaceae        | IncF, IncFII | NDM-1      | Potron et al. [4] |
| France    | E. coli                   | IncFII    | NDM-1       | Bonnin et al. [5] |
| Germany   | E. coli                   | IncX3     | NDM-7       | Gortzel et al. [6] |
| India     | Klebsiella pneumoniae     | IncX3     | NDM-5       | Krishnaraj et al. [8] |
| China     | E. coli                   | IncX3     | NDM-5       | Yang et al. [9] |
| China     | K. pneumoniae             | IncX3     | NDM-1       | Qu et al. [10] |
| Canada    | Raoultella planticola     | IncX3     | NDM-7       | Chen et al. [11] |

NDM, New Delhi metallo-β-lactamase.

Isolation of genomic DNA was performed using QIAamp DNA mini kit (Qiagen, Hilden, Germany). The sequencing was performed as per the manufacturer’s instructions.

**Downstream bioinformatic analysis**

Data obtained were assembled de novo using AssemblerSPAdes 4.4.0.1 software in Torrent suite server version 4.4.3. The sequence was annotated using Rapid Annotation using Subsystem Technology (RAST) pipeline [20–22] (http://rast.nmpdr.org); PATRIC, the bacterial bioinformatics database and analysis resource [23] (http://www.patricr.org); and the National Center for Biotechnology Information Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP; http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Downstream analysis was performed using the CGE server (http://www.cbs.dtu.dk/services), RAST and PATRIC.

**Results**

**Phenotypic characterization**

The isolate B37305 was phenotypically resistant to gentamicin, piperacillin/tazobactam, cefoperazone/sulbactam, imipenem, meropenem, cefotaxime, ceftriaxone, ceftazidime and cefepime, and susceptible to amikacin, netilmicin and ciprofloxacin.

**Molecular characterization**

On the basis of these results, the multidrug-resistant E. coli (B37305) was selected for whole genome sequencing to identify the molecular mechanism behind the resistance pattern and the associated genes. The whole genome shotgun sequence has been deposited at DDBJ/ENA/GenBank under accession number LSAQ00000000. The version described here is version LSAQ01000000.

The strain was identified to be sequence type ST-167 using MLST 1.8 in the CGE server [24]. Presence of antimicrobial resistance genes such as blaoNDM-7, blaoTEM-1, and blaoCMY-22 were identified using the ResFinder 2.1 tool [25]. A self-transferable IncX3 plasmid was identified using PlasmidFinder 1.3 [26].

**FIG. 1.** Schematic representation of genetic environment of NDM-7. blaoNDM-7 is shown in black, ble, gene encoding bleomycin resistance protein; dsbD, gene encoding oxidoreductase DsbD superfamily protein, followed by resolvase; ISEc8, insertion sequence and transposase; ORF, open reading frame; parA, partitioning protein; trp1, gene encoding putative phosphoribosylanthranilate isomerase. Upstream of blaoNDM-7 has yhhI, transposase, and ISEc26, insertion sequence.
genetic environment of $\text{bla}_{\text{NDM-7}}$ is depicted in Fig. 1. In addition, the PATRIC analysis revealed the presence of two $\beta$-lactamase enzymes that were previously reported as $\text{bla}_{\text{EC}}$ family class C $\beta$-lactamases (GenBank accession no. WP_039000307).

Discussion

Carbapenem resistance among Enterobacteriaceae is becoming a major problem, as this drug is known as being the therapy of last resort for serious infections caused by Gram-negative bacteria. As reported earlier, NDM is endemic to the Indian subcontinent but has spread worldwide. The prevalence rates of NDM-producing Enterobacteriaceae in India were found to be 5 to 18.5% [14]. E. coli–producing NDM carbapenemase have also been reported in Canada and Cameroon, as well as other Asian and European countries [27].

Further, the spread of ESBL producers is an important driving force for usage of carbapenems, which enhances the selection of carbapenemase producers [14]. The present study provides evidence that carbapenemase producers are often multidrug resistant and also coexpresses other antibiotic resistance genes which might be carried by the same plasmid.

In addition, Dortet et al. [14] reported the presence of an $\text{bla}_{\text{NDM-7}}$ variant in E. coli. Similarly, a previous study from the literature investigated the first identification of $\text{bla}_{\text{NDM-7}}$ in E. coli ST-167 [27]. There was a previous report of IncX3 plasmid carrying $\text{bla}_{\text{NDM-5}}$ from India [8]. However, the identification of IncX3 carrying $\text{bla}_{\text{NDM-7}}$ is the first to be so described in India.

This study emphasizes the importance of screening for $\text{bla}_{\text{NDM-7}}$ and its associated plasmids. Because the IncX3 plasmid is known for its self-transmissible properties, association of $\text{bla}_{\text{NDM-7}}$ with IncX3 enhances the dissemination potential. Further studies on expression levels of $\text{bla}_{\text{NDM-7}}$ relating to severity of infection and patient outcome need to be conducted in order to better understand infection control.

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Conflict of Interest

None declared.

References

[1] Nair PK, Vaz MS. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. J Microbiol Infect Dis 2013;3:207–10.
[2] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010;10:597–602.
[3] Sekizuka T, Matsui M, Yamane K, Takeuchi F, Ohnishi M, Hishinuma A, et al. Complete sequencing of the $\text{bla}_{\text{NDM-1}}$-positive IncAC Plasmid from Escherichia coli ST38 isolate suggests a possible origin from plant pathogens. PLoS One 2011;6:e25334.
[4] Potron A, Poirel L, Nordmann P. Plasmid-mediated transfer of the $\text{bla}_{\text{NDM-1}}$ gene in Gram-negative rods. FEMS Microbiol Lett 2011;324:111–6.
[5] Bonnin RA, Poirel L, Carattoli A, Nordmann P. Characterization of an IncF plasmid encoding NDM-1 from Escherichia coli ST1131. PLoS One 2012;7:e34752.
[6] Gottig S, Hamprecht AG, Christ S, Kempf VA, Wichelhaus TA. Detection of NDM-7 in Germany, a new variant of the New Delhi metallo-$\beta$-lactamase with increased carbapenemase activity. J Antimicrob Chemother 2013;68:1737–40.
[7] Rahman M, Shukla SK, Prasad KN, Ovejero CM, Pati BK, Tripathi A, et al. Prevalence and molecular characterization of New Delhi metallo-$\beta$-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant Enterobacteriaceae from India. Int J Antimicrob Agents 2014;44:30–7.
[8] Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, Sumathi G, et al. Complete sequencing of an IncX3 plasmid carrying $\text{bla}_{\text{NDM-5}}$ allele reveals an early stage in the dissemination of the $\text{bla}_{\text{NDM}}$ gene. Indian J Med Microbiol 2015;33:30–8.
[9] Yang P, Xie Y, Feng P, Zong Z. $\text{bla}_{\text{NDM-5}}$ carried by an IncX3 plasmid in Escherichia coli sequence type 167. Antimicrob Agents Chemother 2015;59:7548–52.
[10] Qui H, Wang X, Ni Y, Liu J, Tan R, Huang J, et al. NDM-1-producing Enterobacteriaceae in a teaching hospital in Shanghai, China: IncX3-type plasmids may contribute to the dissemination of $\text{bla}_{\text{NDM-1}}$. Int J Infect Dis 2015;34:8–13.
[11] Chen L, Peirano G, Lynch T, Chavda KD, Gregson DB, Church DL, et al. Molecular characterization using next generation sequencing of plasmids containing $\text{bla}_{\text{NDM-1}}$ in Enterobacteriaceae from Calgary, Canada. Antimicrob Agents Chemother 2015;60:1258–63.
[12] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
[13] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
[14] Dortet L, Cuzon G, Nordmann P. Dissemination of carbapenemase producing Enterobacteriaceae in France. J Antimicrob Chemother 2014;69:623–7.
[15] Dallené 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;65:490–5.
[16] Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40:2153–62.
[17] Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan JE, James D, et al. Community and hospital spread of Escherichia coli producing
CTX-M extended-spectrum β-lactamases in the UK. J Antimicrob Chemother 2004;54:735–43.

[18] Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006–2007. Antimicrob Agents Chemother 2011;55:1274–8.

[19] Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 2007;59:321–2.

[20] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.

[21] Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 2014;42(Database issue):D206–14.

[22] Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, et al. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 2015;5:8365.

[23] Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, et al. PATRIC, the bacterial bioinformatics database and analysis resource. Nucl Acids Res 2014;42(D1):D581–91.

[24] Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total genome sequenced bacteria. J Clin Microbiol 2012;50:1355–61.

[25] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4.

[26] Carattoli A, Zankari E, Garcia-Fernandez A, Volby Larsen M, Lund O, Villa L, et al. Plasmid Finder and pMLST: in silico detection and typing of plasmids. Antimicrob Agents Chemother 2014;58:3895–903.

[27] Cuzon G, Bonnin RA, Nordmann P. First identification of novel NDM carbapenemase, NDM-7, in Escherichia coli in France. PLoS One 2013;8:e61322.