First Indian report of IncX3 plasmid carrying *bla*$_{NDM-7}$ in *Escherichia coli* from bloodstream infection: potential for rapid dissemination

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Abstract

Enterobacteriaceae with *bla*$_{NDM-7}$ is only infrequently observed. Self-transmissible plasmids carrying the *bla*$_{NDM}$ gene increase the dissemination of carbapenem resistance in developing countries. This study investigates the whole genome sequence of a *bla*$_{NDM-7}$-positive *Escherichia coli*. The isolate was an extended-spectrum β-lactamase producer by combined disc diffusion test and carbapenemase producer by CarbaNP method. Sequencing results revealed the isolate as *E. coli* ST-167 with IncX3 plasmid carrying *bla*$_{NDM-7}$ in addition to *bla*$_{TEM-1}$ and *bla*$_{CMY-42}$ genes. The identification of IncX3-*bla*$_{NDM-7}$ combination is the first report in India where *bla*$_{NDM-7}$ is known to cause higher resistance to carbapenems compared to its variants.

Keywords: *bla*$_{NDM-7}$, carbapenem resistance, IncX3, ST167 *Escherichia coli*, whole genome sequencing

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Introduction

Extended-spectrum β-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 *Enterobacteriaceae* has been reported. The prevalence of carbapenem-resistant *Enterobacteriaceae* in India has been reported to vary from 7 to 51%. Carbapenem-resistant *Enterobacteriaceae* has been associated with high mortality and morbidity rates of 40 to 50% [1].

New Delhi metallo-β-lactamase (NDM) protein has an extensive pattern of spread as a result of its plasmid location. *bla*$_{NDM-1}$ is the most prevalent variant among *Enterobacteriaceae*. However, *bla*$_{NDM-4}$, *bla*$_{NDM-5}$, *bla*$_{NDM-7}$ and *bla*$_{NDM-14}$ were reported to have enhanced hydrolytic activity compared to *bla*$_{NDM-1}$, although only a few cases were reported. Further, a wide range of Gram-negative genera containing diverse *bla*$_{NDM}$-harbouring plasmids has been reported in >15 countries worldwide [2]. The various plasmid-borne *bla*$_{NDM}$ are listed in Table 1.

Several studies have reported *bla*$_{NDM-1}$-carrying IncX3 plasmid in *Enterobacteriaceae*. The IncX3 plasmids carrying *bla*$_{NDM-1}$ or *bla*$_{NDM-5}$ were found to have identical backbones [9]. Therefore, patients infected or colonized with bacteria containing *bla*$_{NDM-7}$ IncX3 plasmids will pose challenges to infection control compared to other *bla*$_{NDM}$ 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 *bla*$_{NDM-7}$-carrying, IncX3-positive *Escherichia coli* isolated from clinical blood specimen.

Methods

Bacterial strains

In total 773 *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 *E. coli* isolate (B37305) positive for *bla*$_{NDM}$ 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 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), piperacillin/

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TABLE 1. 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*	IncL/M, IncFII, IncA/C	NDM-1	Potron *et al.* [4]
France	*E. coli*	IncFII	NDM-1	Bonnin *et al.* [5]
Germany	*E. coli*	IncX3	NDM-7	Gottig *et al.* [6]
India	*Enterobacteriaceae*	IncF, IncA/C	NDM-1, -5, -6, -7	Rahman *et al.* [7]
India	*Klebsiella pneumoniae*	IncX3	NDM-5	Krishnaraju *et al.* [8]
China	*E. coli*	IncX3	NDM-5	Yang *et al.* [9]
China	*K. pneumoniae*/ *Raoultella planticola*	IncX3	NDM-1	Qu *et al.* [10]
Canada	*Enterobacteriaceae*	IncX3	NDM-7	Chen *et al.* [11]

NDM, New Delhi metallo-β-lactamase.

tazobactam (100/10 μg), cefoperazone/sulbactam (75/30 μg), amikacin (30 μg), netilmicin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), imipenem (10 μg) and meropenem (10 μg) according to Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines [12]. Quality control strains used were *E. coli* ATCC 35218 for ampicillin and *E. coli* ATCC 25922 for rest of the antibiotics. The combined disc method of cefotaxime with/without clavulanic acid for ESBL production was performed as recommended by CLSI 2014 [12]. Carbapenemase production was tested by the CarbaNP method as recommended by CLSI 2015 [13].

CarbaNP test for detection of carbapenemase

The isolate was subjected to classical CarbaNP test using BPERII lysis; protocol was followed as previously described by Dortet *et al.* [14].

Multiplex PCR for screening of β-lactamase genes

Multiplex PCR for detection of ESBL genes bla_{TEM}, bla_{SHV}, bla_{PER} and bla_{VEB} were performed as mentioned previously [15]. AmpC (bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{EBC}, bla_{FOX}) and $bla_{CTX\text{-}M\text{-}1}$ were tested using primers described earlier [16,17]. Class A, B and D carbapenemase genes were screened by testing (bla_{KPC}, bla_{GES}, bla_{SPM}, bla_{IMP}, bla_{VIM}, bla_{NDM} and $bla_{OXA\text{-}48}$-like) [14,15,17–19].

Whole genome sequencing

The genome was sequenced using Ion torrent (PGM; Life Technologies, Carlsbad, CA, USA) with 200 bp chemistry. 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 bioinformatics 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.patricbrc.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. The carbapenem resistance was confirmed by the CarbaNP test, and the isolate was phenotypically positive for ESBL production.

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 $bla_{NDM\text{-}7}$, $bla_{TEM\text{-}1B}$ and $bla_{CMY\text{-}42}$ were identified using the ResFinder 2.1 tool [25]. A self-transferable IncX3 plasmid was identified using PlasmidFinder 1.3 [26]. The

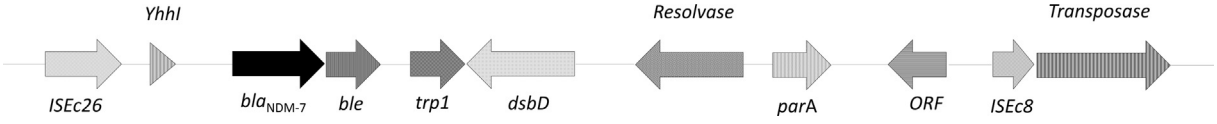

FIG. 1. Schematic representation of genetic environment of NDM-7. $bla_{NDM\text{-}7}$ is shown in black. *ble*, gene encoding bleomycin resistance protein; *dsb*D, gene encoding oxidoreductase DsbD superfamily protein, followed by resolvase; *ISEc8*, insertion sequence and transposase; ORF, open reading frame; *par*A, partitioning protein; *trp1*, gene encoding putative phosphoribosylanthranilate isomerase. Upstream of $bla_{NDM\text{-}7}$ has *yhhI*, transposase, and *ISEc26*, insertion sequence.

genetic environment of bla_{NDM-7} is depicted in Fig. 1. In addition, the PATRIC analysis revealed the presence of two β-lactamase enzymes that were previously reported as bla_{EC} family class C β-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 bla_{NDM-7} variant in *E. coli*. Similarly, a previous study from the literature investigated the first identification of bla_{NDM-7} in *E. coli* ST-167 [27]. There was a previous report of IncX3 plasmid carrying bla_{NDM-5} from India [8]. However, the identification of IncX3 carrying bla_{NDM-7} is the first to be so described in India.

This study emphasizes the importance of screening for bla_{NDM-7} and its associated plasmids. Because the IncX3 plasmid is known for its self-transmissible properties, association of bla_{NDM-7} with IncX3 enhances the dissemination potential. Further studies on expression levels of bla_{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.

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