Journal of Infection and Public Health 12 (2019) 741–743

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Emergence of ST147 *Klebsiella pneumoniae* carrying bla_{NDM-7} on IncA/C2 with *ompK35* and *ompK36* mutations in India

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ARTICLE INFO

Article history:
Received 26 November 2018
Received in revised form 27 March 2019
Accepted 31 March 2019

Keywords:
K. pneumoniae
NDM-7
ST147
ompK35
ompK36

ABSTRACT

India is known to be endemic to NDM carbapenemases. However, NDM-7 among *Klebsiella pneumoniae* has not been described from India. Apart from carbapenemases, *ompK35* and *ompK36* also contribute to carbapenem resistance in *K. pneumoniae*. This study describes molecular mechanisms of antimicrobial resistance in an isolate from bacteraemia investigated through whole genome sequencing. bla_{NDM-7} was found on IncA/C2 plasmid which also carried *sul-1*, *aadA2*, *rmtC*, bla_{CMY-6} and *ARR-2*. *ompK35* had mutations and changes from 39th amino acid. *ompK36* was truncated to 248 amino acids. The isolate belonged to ST147. The patient was a known case systemic lupus erythematosus (SLE) and blood culture grew carbapenem resistant *K. pneumoniae*. Meropenem, colistin and tiecoplanin were administered and the patient was discharged on improvement. Emergence of new resistance variants and porin mutations among clones such as ST147 which has been prevalent has potential for rapid spread and thus challenges infection control.

Carbapenemase producing *Klebsiella pneumoniae* are prevalent in India with NDM-1 and OXA-48-like being common [1]. Currently, 16 variants of NDM have been described (http://www.lahey.org/studies/other.asp#table1). NDM-7 was first reported in 2013 from *E. coli* in Germany [2]. Till date, NDM-7 has been described among *E. coli* but not among *K. pneumoniae* in India [3–5]. In *K. pneumoniae*, outer membrane porins such as *ompK35* and *ompK36* have been reported to contribute to carbapenem resistance [6,7]. There is lack of data from Indian subcontinent regarding the prevalence of mutations in *ompK35* and *ompK36* and also the deletion of these porins in *K. pneumoniae*. Since the rate of extensively drug resistant isolates is very high in India, it is necessary to determine all the resistance mechanisms. Diverse clones are present in India with varying susceptibilities and plasmid profiles. Here we report the first case of NDM-7 *K. pneumoniae* from bacteremia in India belonging to ST147. In the present study, in addition to the production of NDM-7, the isolate was found to contain abnormal *ompK35* and *ompK36* contributing to carbapenem resistance.

The isolate was identified by standard biochemical methods [8]. Antimicrobial susceptibility testing for different classes of antimicrobials such as cephalosporins — cefotaxime(30 μg), ceftazidime(30 μg); β-lactam/β-lactamase inhibitors — piperacillin/tazobactam(100/10 μg); carbapenems — imipenem (10 μg), meropenem(10 μg); fluoroquinolones — ciprofloxacin (5 μg), levofloxacin(5 μg); aminoglycosides — amikacin(30 μg), gentamicin(10 μg); tetracycline-minocycline(30 μg) and glycylcycline–tigecycline (15 μg) was performed for the isolate by Kirby Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) and interpreted according to CLSI 2016 guidelines [9] and tigecycline interpreted as per FDA breakpoints (http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021821s016lbl.pdf). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the control strains for susceptibility testing. String test was performed to screen for hypermucoviscous phenotype [10].

DNA was extracted from 18 to 24 h old culture using QiaSymphony (Qiagen) as per manufacturer's instructions for molecular studies. It was stored at −20 °C for further use. The isolate was subjected to whole genome sequencing using Ion Torrent PGM platform with 400bp chemistry. Raw reads were assembled using Assembler SPAdes v.5.0 software in Torrent suite server version 4.4.3. The genome was annotated using RAST (Rapid Annotation using Subsystems Technology— http://rast.nmpdr.org/), Patric (Pathosystems Resource Integration Centre — https://www.patricbrc.org/) and the National Centre for Biotechnology Information Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) softwares. The MLST (https://cge.cbs.dtu.

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https://doi.org/10.1016/j.jiph.2019.03.023

dk//services/MLST/), ResFinder (https://cge.cbs.dtu.dk//services/ResFinder/) and PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) were used to find the sequence type, antibiotic resistance genes and the plasmid types present in the isolate. Virulence genes were defined with the help of database available at http://bigsdb.pasteur.fr/klebsiella/. Mutations in *ompK35* and *ompK36* were determined using KX528043.1 and JX291114.1 as references respectively.

The clinical details of the patient were collected from electronic medical records. The patient was a child with systemic lupus erythematosus with class IV lupus nephritis on prednisolone and mycophenolate mofetil for 6 months and presented with history of fever, worsening of edema with decreased urine output for 7 days. The patient was treated with cefotaxime before being brought to our tertiary centre.

The patient was febrile with generalized edema. Alopecia and bilateral conjunctival haemorrhage was present. There was no pallor, icterus, cyanosis, clubbing or significant lymphadenopathy. Heart rate was 96/min, and blood pressure was 130/82 mm of Hg. No abnormalities were found in cardiovascular system. The patient had bilateral equal air entry with crepitation present on subscapular and inter-scapular region. Abdominal examination revealed presence of gross ascites. Liver and spleen were not palpable. On admission, the patient was started on parenteral piperacillin and tazobactam. Investigations revealed elevated C-reactive proteins (CRP) and procalcitonin. There was no evidence of lupus flare. As fever was persisting, respiratory distress worsened and blood culture grew carbapenem resistant *K. pneumoniae*, antibiotics were upgraded to meropenem, teicoplanin and colistin. The pateint gradually improved and was discharged.

The isolate was resistant to all the antimicrobials except for minocycline and tigecycline as tested by disc diffusion.

The whole-genome sequence of the NDM-7 *K. pneumoniae* has been deposited at GenBank under the accession number NRHU00000000. The isolate belonged to ST147 by whole genome MLST. Resistance genes as identified by ResFinder and the mutations in *ompK35* and *ompK36* are listed in Table1.

The isolate harboured five plasmids such as IncA/C2, ColKP3, IncFIB (*pKPHS1*), ColpVC and IncFII (*pKPX1*). Resistance genes such as $bla_{\text{NDM-7}}$, $bla_{\text{CMY-6}}$, *sul1*, *aadA2*, *rmtC*, *aac(6′)-Ib* and *ARR-2* were present on IncA/C2 plasmid. IncR carried $bla_{\text{SHV-11}}$, *strA*, *strB* and *dfrA14* genes. *rmtF*, *dfrA12* and *mphA* were present on IncFII (*pKPX1*). ColKP3, ColpVC and IncFIB (*pKPHS1*) did not harbour any resistance genes. Genetic background of $bla_{\text{NDM-7}}$ on IncA/C2 plasmid is shown in Fig. 1.

The isolate was negative for string test. It lacked *rmpA* and *rmpA2* genes, the molecular markers for hypervirulence. Virulence genes present in the isolates included *mrkABCDFHI* operon coding for type3 fimbriae and yersinibactin encoding genes such as *ybtA*, *ybtS*, *ybtT* and *ybtX*. The isolate belonged to K64 capsular type as determined by wzi sequence obtained through whole genome analysis.

$bla_{\text{NDM-7}}$ has been frequently reported among *E. coli* than *K. pneumoniae* [2–4]. This is the first report of $bla_{\text{NDM-7}}$ harboured on IncA/C2 plasmid. Most of the earlier studies, report the presence of $bla_{\text{NDM-7}}$ on IncX group of plasmids [4,11,12]. However, the present study isolate lacked IncX plasmid although it contained five different plasmids. Presence of multiple plasmids indicates its high potential to acquire multiple resistance genes and so does the presence of plasmids without any resistance genes which have the potential to acquire resistance traits and spread. ST147 has been commonly reported among Indian isolates and has been frequently associated with NDM-1 and OXA48-like carbapenemases as observed from the present study centre [13].

The outer membrane porins, *ompK35* and *ompK36*, are known to contribute to carbapenem resistance even in the absence of carbapenemases due to mutations or loss of porin [14,15]. Shen et al.,

Table 1
Resistance genes encoded by NDM-7 *Klebsiella pneumoniae*.

β-Lactamases	*ompK35*	*ompK36*	Aminoglycoside	Fluoroquinolone	Macrolides	Trimethoprim	Fosfomycin	Rifampicin	Sulphonamide
$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-11}}$, $bla_{\text{NDM-7}}$ and $bla_{\text{CMY-6}}$	• Truncated to 68 amino acids	• G 136 T	*rmtf*, *strA*, *strB*, *rmtC*, *aac(6′)Ib-cr*, *aadA2*	*oqxA*, *oqxB*, *aac (6′) Ib-cr*	*mphA*, *msrE*, *mphE*	*dfrA12*, *dfrA14*	*fosA*	*ARR-2*	*sul1*
	• Deletion of adenosine at 113 leading to change from 39th amino acid	• G 194 T							
		• V 180 P							
		• T 185 A							
		• F 102 Y							
		• H 223 N							
		• N 227 L							
		• Truncated to 248 amino acids							

G: glycine; T: threonine; P: proline; A: alanine; V: valine; F: phenylalanine; Y: tyrosine; H: histidine; N: asparagine; L: leucine.

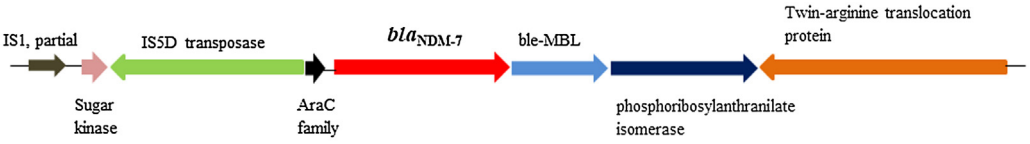

Fig. 1. Genetic background of bla_{NDM-7} in IncA/C2 plasmid.
IS5D of IS5 family was present upstream to bla_{NDM-7} on the plasmid. ble-MBL was present downstream to bla_{NDM-7} as expected.

have reported up to 81% mortality due to resistance from porins [16]. However, there is limited data on the coexistence of porin mutations and carbapenemases such as bla_{NDM}. The isolates with mutations or loss of porins are known to show variable MIC values for imipenem and meropenem as demonstrated [7]. In the present study, *ompK35* was truncated to 68 amino acids which has not been previously reported but truncation to 62 and 89 amino acids has been reported [7,17]. In *ompK36*, we observed substitution of alanine in place of threonine at position 185. Similar result was reported among OXA48 producing *K. pneumoniae* [17]. Substitutions of threonine in place of glycine at position 194 and leucine in place of asparagine at 227 has also been reported [14]. However, they belonged to diverse clones and none were among ST147 as seen in the present study.

Previous reports of NDM-7 *K. pneumoniae* have been from clones such as ST138, ST273, ST278, ST437 and ST654 [18–20]. Though NDM-7 has been rarely reported among *K. pneumoniae*, it is seen among diverse clones. Similar to the present study, a single case NDM-7 *K. pneumoniae* belonging to ST147 and carrying *rmtF* has been reported from Minnesota [21].

This study describes bla_{NDM-7} in a non-hypervirulent *K. pneumoniae* being carried on IncA/C2 plasmid. Contribution of *ompK35* and *ompK36* in addition to bla_{NDM-7} is seen aiding carbapenem resistance. Emergence of new resistance variants and porin mutations among clones such as ST147 which has been prevalent in the study setting has the potential for spread and thus challenges infection control.

Informed consent

Since the study was retrospective and does not reveal patient identity, informed consent was not obtained.

Funding

No funding Sources

Competing interests

None declared.

Ethical approval

Ethical approval was obtained by the Ethical Committee of Christian Medical College (IRB min. no. 9616 dated 01.09.2015).

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