International Journal of Antimicrobial Agents 53 (2019) 525–529

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Molecular characterization of novel sequence type of carbapenem-resistant New Delhi metallo-β-lactamase-1-producing *Klebsiella pneumoniae* in the neonatal intensive care unit of an Indian hospital

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ARTICLE INFO

Article history:
Received 26 September 2018
Accepted 15 December 2018

Keywords:
Klebsiella pneumoniae
MLST
OXA-48
NDM
Carbapenem-resistant
NICU

ABSTRACT

Emergence of multi-drug resistance, especially carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a major threat to public health. The aim of this study was to characterize CRKP isolates from infants admitted to the Neonatal Intensive Care Unit (NICU) to find the clonal outbreak of New Delhi metallo-β-lactamase (NDM) producers. In this study 17 CRKP isolates were analysed. Antimicrobial susceptibility of the isolates was determined by the disc diffusion and micro-dilution method. Carba-NP test and double-disk synergy test (DDST) were performed for the detection of carbapenemase and metallo-β-lactamase-producing *K. pneumoniae*. Antibiotic-resistant markers were detected by polymerase chain reaction (PCR) followed by sequencing. Clonal relatedness of the isolates was checked by multi-locus sequence typing. Conjugation experiments were performed to determine the transferability of the plasmids. All 17 CKRP isolates were found to carry bla_{NDM} (13 bla_{NDM-1}, 1 bla_{NDM-4} and 3 bla_{NDM-5}), seven isolates carried bla_{OXA-48}, 13 isolates had $bla_{CTX-M-15}$, seven isolates carried bla_{CMY-1} and five isolates were found to carry bla_{SHV-1} on conjugative plasmids of different types (IncFIA, IncFIB, IncFIIAs, IncFIC, IncA/C, IncF, IncK, IncX, IncW and IncY). Of six different sequence types (STs) identified, ST3344 was detected as a novel ST in two *K. pneumoniae* isolates. Genetic environment analysis revealed IS*Aba125* and bleomycin genes flanking to all bla_{NDM} variants. This is the first report of novel ST3344 in two NDM-1-producing *K. pneumoniae* isolates from neonates admitted to the NICU of a North Indian Hospital. This study is provides understanding of the genetic features of this newly emerged strain type.

1. Introduction

Klebsiella pneumoniae is one of the most common causative organisms of neonatal infections in hospitalized immunocompromised patients admitted to neonatal intensive-care units (NICU), where it may cause outbreaks of infections resulting in adverse outcomes, including death, in affected infants as well as higher healthcare costs [1]. Moreover, *K. pneumoniae* is the most commonly implicated pathogen responsible for neonatal sepsis. Recently, multi-drug-resistant (MDR) *K. pneumoniae* outbreaks in NICU had been reported in developing countries [2,3]. India has the highest neonatal mortality rate due to neonatal sepsis caused by bacterial resistance to first-line antibiotics. Approximately one-fifth of total neonates died with sepsis in the hospital, and the mortality rate is 50% for those with culture-proven sepsis [4]. Metallo-β-lactamase (MBL)- and carbapenemase-producing *K. pneumoniae* strains make the clinical management of these infections more challenging. According to the Centre for Disease Dynamics, Economics and Policy (CDDEP), up to 60% of the Indian *K. pneumoniae* isolates were resistant to carbapenems and 80% resistant to cephalosporins. India has witnessed an increase in carbapenem-resistance rates from 9% in 2008 to 44% in 2010 [5]. In Italy, the prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) isolates, non-existent in 2008, rose to 60% in 2013 (CDDEP). This rapid increase in carbapenem-resistance reflects a worrisome trend.

Furthermore, several carbapenemase encoding genes have been described in *K. pneumoniae* species, including class A β-lactamase KPC, class B β-lactamases New Delhi metallo-β-lactamase

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https://doi.org/10.1016/j.ijantimicag.2018.12.005
0924-8579/

(NDM-1), IMP, VIM, and class D β-lactamase OXA-48 [6]. NDM-1 emerged in 2009 and since then it has spread globally in the most important reservoir of NDM-1-producing bacteria, i.e. the Indian subcontinent and the Balkan region [7]. The Ambler class D β-lactamase OXA-48-producing *K. pneumoniae* was first isolated from a patient in Turkey in 2001 [8]. The aim of this study was to investigate CRKP from the NICU of a North Indian tertiary care hospital to understand the clonal outbreak.

2. Material and Methods

2.1. Bacterial isolates and hospital setting

From October 2016 to April 2017, 17 non-duplicated CRKP were included in this study, isolated from neonates admitted to the NICU of Jawaharlal Nehru Medical College and Hospital (JNMCH), Aligarh Muslim University, Aligarh. This is a tertiary care unit of 1300-bed capacity, in which 90 beds were allotted for pediatric patients and 35 beds for the NICU.

2.2. Bacterial identification

Isolates were identified by using BD Phoenix^{TM-100} automated microbiology system (Becton Dickinson Company) using panel NMIC/ID-55 (Gram-negative susceptibility card) and further confirmed by 16s rRNA gene sequencing.

2.3. Antimicrobial susceptibility testing and MICs

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar plates with commercially available discs (Hi-Media, Mumbai, India) for different classes of antimicrobials such as carbapenems: imipenem (10 µg), meropenem (10 µg) and doripenem (10 µg); cephalosporins: cefotaxime (30 µg), ceftazidime (30 µg), and cefepime (30 µg); monobactam: aztreonam (30 µg); fluoroquinolones: ciprofloxacin (5 µg) and levofloxacin (5 µg), aminoglycosides: amikacin (30 µg), gentamicin (10 µg) and polymyxin-B, colistin (10 µg) by the Kirby Bauer disk diffusion method and results were interpreted according to CLSI-2016 guidelines.

The minimum inhibitory concentrations (MICs) of imipenem, meropenem and doripenem (Sigma-Aldrich) were determined by the micro-broth dilution method following CLSI-2016 guidelines.

2.4. Detection of MBL and carbapenemase phenotypes

MBL production was detected by the double-disk synergy test using two imipenem discs (10µg) (Hi-Media Laboratories Pvt. Ltd.), one containing 10 µl of 0.1 M anhydrous ethylene diamine tetra-acetic acid (EDTA). The discs were placed 25 mm apart on Mueller–Hinton agar plates. The Carba-NP test was performed for the detection of carbapenemase activity in *K. pneumoniae* isolates.

2.5. Polymerase chain reaction amplification of antibiotic-resistant genes

For the detection of the antibiotic-resistant gene, whole-cell DNA of strains was prepared by taking fresh colonies from a pure culture plate of CRKP isolates. Each colony was suspended in 100 µl of nuclease-free sterilized water and incubated at 95°C for 10 min followed by centrifugation at 8000 × g at 4°C for 10 min. The supernatant was used as a to perform polymerase chain reaction (PCR) on a Veriti-TM 96-Well Thermal Cycler (Model#9902; Applied Biosystems, Foster City, CA, USA) using the primers as described previously [9,10] for the detection of bla_{NDM} and other resistance markers (bla_{SHV}, bla_{VIM}, bla_{OXA-48}, bla_{CMY}, bla_{TEM}, $bla_{CTX-M-15}$ and bla_{KPC}).

2.6. DNA sequencing

PCR-generated fragments were purified from the gel using a Gene JET Gel Extraction Kit (Thermo Fisher Scientific) following the manufacturer's protocol. The purified DNA fragments were sequenced at AgriGenom Labs Pvt. Ltd. (Kerala, India). To ascertain the NDM variant, the deduced protein sequence was aligned with NDM variants to confirm the amino acid substitution in the query sequence with respect to known variants using Clustal omega tool (http://www.ebi.ac.uk/Tools/msa/clustalo/). Furthermore, to identify the similarity between the amplified nucleotide sequence and the deduced protein sequences, online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/) was used and confirmed as NDM variant. These sequences have been submitted in the GeneBank nucleotide database under accession numbers: MF360080, MF360082, MF360086, MF360087, MF360091, MF360094, MF360095, MF360100, MH064484, MH064486, MH064487, MH064488, MH064489, MH064490, MH064491, MH064493, and MH064497 available at the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

2.7. Conjugation experiment and plasmid analysis

The transfer of resistant markers (bla_{NDM}, bla_{CMY}, bla_{OXA}, $bla_{CTX-M-15}$ and bla_{SHV}) was carried out by conjugation, using an azide-resistant *Escherichia coli* J53 strain as the recipient. Transconjugants were screened on Luria–Bertani agar supplemented with ceftazidime (10 µg/mL) (Sigma-Aldrich) and sodium azide (100 µg/mL) (Hi-Media Laboratories, India). The PCR amplification data confirmed the presence of resistant markers in transconjugants. Kieser method [11] was used to extract plasmids for determination of their size. Plasmid incompatibility group was determined by the PCR-based replicon typing (PBRT) method. Plasmid DNA was amplified by five multiplex and three simplex PCRs using 18 pairs of primers as previously described [12]. These were recognized as Inc replicon types: IncFIA, IncFIB, IncFIC, IncHI1, IncHI2, IncI1, IncL/M, IncN, IncP, IncW, IncT, IncA/C, IncK, IncB/O, IncX, IncY, IncF, and IncFIIA.

2.8. Genetic Environment Analysis

The genetic analysis was performed to ascertain the genes present upstream and downstream of $bla_{NDM,}$ using methods described earlier [9].

2.9. Multi-locus sequence typing

Multi-locus sequence typing (MLST) of the *K. pneumoniae* isolates was carried out to amplify seven conserved housekeeping genes (*gapA, infB, mdh,pgi, phoE, rpoB* and *tonB*) using adequate primers (http://bigsdb.pasteur.fr/klebsiella/primers_used.html) and protocols available at the MLST Pasteur website (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). Alleles and sequence types (STs) are available on the MLST online database. Novel alleles and STs were submitted to the administrator of the database and assigned new ST. Minimum spanning tree was constructed using PHYLOViZ (https://online.phyloviz.net/index) online software.

3. Results

3.1. Clinical characteristics of the CRKP isolates

Microbiological characteristics of the seventeen CRKP isolates, including the date of isolation, neonate age, sex, birth weight as shown in table 1. The isolates were collected from 17 newborns of low birth weight (07 males and 10 females) with an average age of 5 days (01-11 days).

Table 1
Demographics and phenotypes characteristics of 17 carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates.

Isolate ID	Date of isolation	Age (days)/sex	Ward	Birth weight	Carba-NP	MBL	MIC (μg/mL)			Antimicrobial-resistant phenotype
							MRP	IMP	DOR	
AK-119	13 October 2016	1/M	NICU	1.910	Positive	Present	1024	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-121	13 October 2016	8/M	NICU	1.720	Positive	Present	512	512	512	IMP, MRP, DOR, CTX, CAZ, CPM, CIP, LE, AK, GEN
AK-125	07 November 2016	3/F	NICU	2.440	Positive	Present	512	1024	512	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-126	07 November 2016	4/F	NICU	2.310	Positive	Present	1024	2048	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-130	25 January 2017	6/F	NICU	2.050	Positive	Present	256	512	512	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-133	31 January 2017	5/F	NICU	1.420	Positive	Present	1024	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-134	31 January 2017	4/F	NICU	1.620	Positive	Present	1024	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, CIP, LE, AK, GEN
AK-139	31 January 2017	6/M	NICU	1.810	Positive	Present	512	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-140	01 April 2017	2/F	NICU	1.300	Positive	Present	1024	2048	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-142	01 April 2017	2/M	NICU	1.270	Positive	Present	512	1024	512	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-143	01 April 2017	11/M	NICU	1.195	Positive	Present	512	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-144	01 April 2017	7/F	NICU	1.590	Positive	Present	1024	1024	512	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-145	01 April 2017	7/M	NICU	1.470	Positive	Present	512	1024	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-146	01 April 2017	2/M	NICU	2.475	Positive	Present	256	512	512	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-147	01 April 2017	3/F	NICU	2.100	Positive	Present	1024	2048	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-149	01 April 2017	11/F	NICU	1.220	Positive	Present	1024	2048	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN
AK-158	01 April 2017	5/F	NICU	1.510	Positive	Present	1024	2048	1024	IMP, MRP, DOR, CTX, CAZ, CPM, AT, CIP, LE, AK, GEN

AK, amikacin; AT, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CPM, cefepime; CTX, cefotaxime; DOR, doripenem; GEN, gentamicin; IMP, imipenem; LE, levofloxacin; MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; MRP, meropenem; NICU, neonatal intensive care unit.

3.2. Antibacterial susceptibility, MICs and MBL production

The antimicrobial susceptibility profiles of the CRKP isolates are listed in (Table 1). All 17 isolates were resistant to at least three classes of antibiotics and these were considered as MDR. The MICs of carbapenem agents for all isolates are shown in table 1. MBL activity was found in all 17 CRKP isolates (Table 1).

3.3. Carbapenemase Production

All 17 *K. pnemoniae* isolates were found to be positive for the Carba-NP test, indicating the production of a carbapenemase as shown in Table 1.

3.4. Detection of antibiotic resistance genes

PCR amplification and sequencing confirmed that all CRKP isolates harbored bla_{NDM} (13 bla_{NDM-1}, 1 bla_{NDM-4}, and 3 bla_{NDM-5}). These NDM sequences were submitted to the NCBI database. Of the 17 bla_{NDM} producing isolates, 76.5 % (13/17) $bla_{CTX-M-15}$, 41.2 % (7/17) bla_{OXA-48}, 41.2 % (7/17) bla_{CMY-1} and 29.4 % (5/17) bla_{SHV-1}, respectively, were co-associated with bla_{NDM} as shown in Table 2.

3.5. Plasmid analysis

These carbapenem-resistant *K.pnemoniae* (CRKP) were found to carry detectable plasmids of size (38 kb, 6 kb and 4 kb) as shown in Table 2. PBRT detected 10 plasmid replicon types in the 17 CRKP isolates (IncFIA, IncFIB, IncFIIAs, IncFIC, IncA/C, IncF, IncK, IncX, IncW and IncY). Sixteen isolates carried one or more plasmid replicons, whereas AK-139 isolate was found to be untypable. Replicon types (IncA/C (n=12), IncX (n=09), IncF(n=07), IncFIIAs (n=05), IncFIA (n=05), IncK (n=04), IncFIB (n=03), IncFIC (n=01), IncW (n=01), and IncY (n=01)) were found to be predominant in this study. Most frequent type replicons were IncA/C, IncX and IncF.

3.6. Genetic environment of the bla_{NDM}

PCR-based genetic environment analysis of the bla_{NDM} gene was performed, bleomycin resistance gene (ble_{MBL}) was found downstream of bla_{NDM} in all isolates (Table 2). The complete IS*Aba125* sequence was found upstream of bla_{NDM-1} in 11 strains (AK-121, AK-125, AK-126, AK-130, AK-133, AK-139, AK-140, AK-144, AK-145, AK-147 and AK-149), one NDM-4- (AK-119) and two NDM-5-producing strains (AK-134 and AK-146). Furthermore, two bla_{NDM-1} (AK-142 and AK-143) and one bla_{NDM-5} (AK-158) carrying *K. pneumoniae* had truncated IS*Aba125*, upstream of bla_{NDM} (Table 2).

3.7. Molecular typing

MLST analysis revealed two suspected outbreak isolates which shared a new allelic profile (2-1-2-2-4-31-4), assigned as novel ST3344 as a first report. Among the 17 CRKP isolates, six STs were identified, including ST15 (seven isolates), ST16 (five isolates), ST11 (one isolate), ST657 (one isolate), ST873 (one isolate), and ST3344 (two isolates). Our study revealed that NDM producers were detected on ST15, ST657 and ST3344. Whereas, the strains carrying NDM in association with OXA-48 belonged to ST11, ST16 and ST873.

4. Discussion

In this study, we identified novel ST3344 in two *K. pneumoniae* strains (AK-142 and AK-143), carrying NDM-, whereas all the CRKP isolates were susceptible to colistin and polymyxin-B. However, because of their nephrotoxic effect, they are not recommended for treatment [13]. In the absence of effective antibiotic treatment, early monitoring of CRKP infection or colonization on admission may play a more important role for timely control of the increase in CRKP. Infectious diseases caused by NDM-1-producing isolates were known to be associated with significant morbidity and mortality, and it is even worse among the pediatric population due to limited therapeutic options.

This study revealed 17 CRKP isolates with four diverse carbapenemase genes (bla_{NDM}, bla_{OXA-48}, bla_{CMY-1}, and bla_{SHV-1}). This study further highlights the prevalence of CRKP with bla_{NDM} and bla_{OXA-48} genes together or alone, in an NICU of a North Indian tertiary care hospital. The bla_{NDM} and bla_{OXA-48} co-producing strains exhibited high MIC values against carbapenems. The dissemination of *K. pneumoniae* isolates harboring carbapenem-resistant genes, in particular, bla_{NDM} and bla_{OXA-48} continues to be reported around the world, especially in European and Asian countries [14,15].

The PHYLOViZ-generated minimum-spanning tree is based on the allele number matrix of the gene loci included in the *K. pneumoniae* MLST scheme, and is shown in Fig. 1. Black numbers in the

Table 2
Genetic characterization of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates.

Strain	Carbapenemase gene	Sequence type	Inc group	Associated resistant marker	Size of plasmid (kb)	Genetic environment of bla_{NDM}	
						IS*Aba125*	ble_{MBL}
AK-119	NDM-4, OXA-48	ST-16	X	CTX-M-15, SHV-1	6	Complete	Present
AK-121	NDM-1	ST-15	A/C, X	CTX-M-15, CMY-1	6,38	Complete	Present
AK-125	NDM-1	ST-15	FIA, A/C, X	CTX-M-15	4, 6	Complete	Present
AK-126	NDM-1, OXA-48	ST-16	FIA, A/C	CTX-M-15	4, 6	Complete	Present
AK-130	NDM-1	ST-15	A/C, Y	CTX-M-15	6, 38	Complete	Present
AK-133	NDM-1	ST-657	FIIAs, A/C, X	CTX-M-15	4, 6, 38	Complete	Present
AK-134	NDM-5	ST-15	FIIAs	CTX-M-15	38	Complete	Present
AK-139	NDM-1, OXA-48	ST-16	UT	CTX-M-15	6	Complete	Present
AK-140	NDM-1, OXA-48	ST-16	FIC	CTX-M-15	6, 38	Complete	Present
AK-142	NDM-1	ST-3344*	FIIAs, A/C, F, K, X	CTX-M-15, SHV-1, CMY-1	4, 6, 38	Truncated	Present
AK-143	NDM-1	ST-3344*	FIIAs, A/C, F, K, X	CTX-M-15, CMY-1	4, 6, 38	Truncated	Present
AK-144	NDM-1	ST-15	FIA, FIB, A/C, F, X	SHV, CMY-1	4, 38	Complete	Present
AK-145	NDM-1	ST-15	FIA, FIB, A/C, F, X	CTX-M-15, SHV-1 CMY-1	4, 38	Complete	Present
AK-146	NDM-5	ST-15	A/C	CMY-1	38	Complete	Present
AK-147	NDM-1, OXA-48	ST-11	FIIAs, A/C, F, X, W	-	4, 6, 38	Complete	Present
AK-149	NDM-1, OXA-48	ST-16	F, K	SHV-1	38	Complete	Present
AK-158	NDM-5, OXA-48	ST-873	FIA, FIB, A/C, F, K	CTX-M-15, CMY-1	4, 6, 38	Truncated	Present

UT, untypable.
* Novel sequence type.

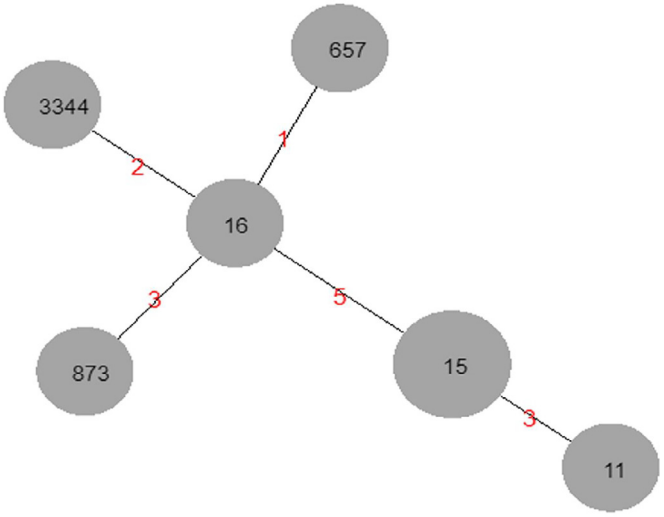

Fig. 1. Multi-locus sequence typing (MLST)-based minimum-spanning tree. The PHYLOViZ-generated minimum-spanning tree is based on the allele number matrix of the gene loci included in the *Klebsiella pneumoniae* MLST scheme. Black numbers in the circles indicate the MLST sequence type. Red numbers along the linking lines indicate the absolute distance.

circles indicate the MLST sequence type. Red numbers along the linking lines indicate the absolute distance. MLST analysis revealed the occurrence of seven ST15 *K. pneumoniae* strains in which five were NDM-1 and two were NDM-5 producers. ST16 was the second most common ST carrying bla_{NDM} with bla_{OXA-48} producing *K. pneumoniae*. The co-production of these two markers in ST16 accounted for its high resistance to carbapenems (Table 1). Over 50% of NDM-producing *K. pneumoniae* isolates in India belonged to either ST11 or ST147 [16]. In the previous studies NDM-1-producing *K. pneumoniae* clinical isolates from India, Sweden and the UK showed that the most frequently detected STs were ST14, ST11, ST149, ST231, and ST147 [17]. Moreover, ST14, ST15, ST101, ST147, and ST405 clones in OXA-48-producing *K. pneumoniae* have been reported in many countries, such as India [16], Spain [18], USA [16], and Germany [19]. Our study represents the first detection of NDM-1-producing ST3344 *K. pneumoniae* isolates in an NICU.

In the previous study, bla_{NDM-1} was identified on various transferable plasmids (IncFII, IncA/C, IncN or untypeable plasmids) [20]. Moreover, the bla_{OXA-48} was also reported to transfer through different plasmid types, IncL/M, IncN, IncA/C or untypeable [15]. This study explored varying replicon types (IncFIA, IncFIB, IncFIIAs, IncFIC, IncA/C, IncF, IncK, IncX, IncW and IncY), in these NDM-producing *K. pneumoniae* strains.

The complete IS*Aba125* upstream of bla_{NDM} in the majority of the isolates implies its function in horizontal gene transfer of the bla_{NDM}-producing Enterobacteriaceae family [9]. Moreover, in the present study, all 17 CRKP isolates carried the bleomycin resistance gene (ble_{MBL}) downstream of bla_{NDM}. The high prevalence of association of the ble_{MBL} and bla_{NDM} genes suggests that they may have mobilized together from a common progenitor, which many thought to protect bla_{NDM} [12].

5. Conclusion

This is the first report of novel ST3344 in two NDM-1-producing *K. pneumoniae* isolates from neonates admitted to an NICU of one of the North Indian hospitals. Moreover, these strains were also found to carry $bla_{CTX-M-15}$, bla_{CMY-1} and bla_{SHV-1}. The genetic feature of these two novel ST3344 clones and their resistance profile may help in patient management in the hospital setting. The vulnerability to colonization and infection with CRKP isolates among neonates highlights the necessity of intervention with strict infection-control measures, including proper hand sanitation, contact precautions, and cohort nursing care to decrease the cross-infection and avoid the quick spread or clonal dissemination of CRKP strains in an NICU setting.

Acknowledgments

The authors acknowledge the team of the curators of the Institute Pasteur MLST system (Paris, France) for importing novel alleles, profiles and isolates at http://bigsdb.web.pasteur.fr.

Funding

The internal funds of the Biotechnology unit are highly acknowledged for funding this work.

Competing Interests

None to declare.

Ethical Approval

Ethical approval was obtained (no. 151/201517/PDFWM-2015-2017-UTT-31140 (SAH))

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.12.005.

References

[1] Stone PW, Gupta A, Loughrey M, Della-Latta P, Cimiotti J, Larson E, et al. Attributable costs and length of stay of an extended-spectrum beta-lactamase-producing Klebsiella pneumoniae outbreak in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2003;24:601–6.

[2] Jin Y, Shao C, Li J, Fan H, Bai Y, Wang Y. Outbreak of multidrug resistant NDM-1-producing Klebsiella pneumoniae from a neonatal unit in Shandong Province, China. PLoS One 2015;10:e0119571.

[3] Gray J, Arvelo W, McCracken J, Lopez B, Lessa FC, Kitchel B, et al. An outbreak of Klebsiella pneumoniae late-onset sepsis in a neonatal intensive care unit in Guatemala. Am J Infect Control 2012;40:516–20.

[4] PHFI, AIIMS, SC. State of India's Newborns (SOIN) 2014-a report. State of India's Newborns (SOIN) 2014-a report. Zodpey S, Paul VK, editors. New Delhi, India: Public Health Foundation of India, All India Institute of Medical Sciences and Save the Children; 2014.

[5] Parveen RM, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of Klebsiella pneumoniae in South India. Int J Pharma Bio Sci 2010;1:BS33.

[6] Berrazeg M, Diene SM, Drissi M, Kempf M, Richet H, Landraud L, et al. Biotyping of multidrug-resistant Klebsiella pneumoniae clinical isolates from France and Algeria using MALDI-TOF MS. PloS one 2013;8:e61428.

[7] Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int 2014;2014:249856.

[8] Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediatedresistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 2004;48:15–22.

[9] Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother 2011;22:AAC–00585.

[10] Ali SZ, Ali SM, Khan AU. Prevalence of IncI1-Iγ and IncFIA-FIB type plasmids in extended-spectrum β-lactamase-producing Klebsiella pneumoniae strains isolated from the NICU of a North Indian Hospital. Microbiology 2014;160:1153–61.

[11] Kieser T. Factors affecting the isolation of CCC DNA from Streptomyces lividans and Escherichia coli. Plasmid 1984;12:19–36.

[12] Ahmad N, Khalid S, Ali SM, Khan AU. Occurrence of blaNDM variants among enterobacteriaceae from a neonatal intensive care unit in a Northern India hospital. Front Microbiol 2018;9:407.

[13] Hamzan NI, Yean CY, Rahman RA, Hasan H, Rahman ZA. Detection of blaIMP-4 and blaNDM-1 harboring Klebsiella pneumoniae isolates in a university hospital in Malaysia. Emerg Health Threats J 2015;8:26011.

[14] Sidjabat HE, Townell N, Nimmo GR, George NM, Robson J, Vohra R, et al. Dominance of IMP-4-producing E. cloacae amongst carbapenemase-producing Enterobacteriaceae in Australia. Antimicrob Agents Chemother 2015:AAC–04378.

[15] Guo L, An J, Ma Y, Ye L, Luo Y, Tao C, et al. Nosocomial outbreak of OXA-48-producing Klebsiella pneumoniae in a Chinese hospital: clonal transmission of ST147 and ST383. PLoS One 2016;11:e0160754.

[16] Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JD. Surveillance and molecular epidemiology of Klebsiella pneumoniae isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. Antimicrob Agents Chemother 2013;57:130–6.

[17] Giske CG, Fröding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore DM, et al. Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of blaNDM-1 in India, Sweden and the UK. Antimicrob Agents Chemother 2012;21:AAC–06142.

[18] Cubero M, Cuervo G, Dominguez MÁ, Tubau F, Martí S, Sevillano E, et al. Carbapenem-resistant and carbapenem-susceptible isogenic isolates of Klebsiella pneumoniae ST101 causing infection in a tertiary hospital. BMC microbial 2015;15:1.

[19] Göttig S, Gruber TM, Stecher B, Wichelhaus TA, Kempf VA. In vivo horizontal gene transfer of the carbapenemase OXA-48 during a nosocomial outbreak. Clin Infect Dis 2015;60:1808–15.

[20] Voulgari E, Gartzonika C, Vrioni G, Politi L, Priavali E, Levidiotnzlou-Stefanou S, et al. The Balkan region: NDM-1-producing Klebsiella pneumoniae ST11 clonal strain causing outbreaks in Greece. J Antimicrob Chemother 2014;69:2091–2097.