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Molecular characterization of NDM-1-producing *Klebsiella pneumoniae* ST29, ST347, ST1224, and ST2558 causing sepsis in neonates in a tertiary care hospital of North-East India

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ABSTRACT

Geographical differences can manifest in different spectra of microorganisms and patterns of antibiotic resistance. Considering this, Enterobacteriacae isolated from septicemic neonates from a tertiary care centre in Agartala, India were studied with focus on carbapenem resistance. Two hundred non-duplicate Enterobacteriaceae, of which 12 NDM-1-producing *Klebsiella pneumoniae* were recovered. Antibiotic susceptibility tests and detection of ESBLs and carbapenemases were performed for all Enterobacteriaceae. For NDM-1-producing isolates, plasmid-mediated quinolone resistance genes, addiction systems, genetic environment of bla_{NDM-1} and virulence genes was investigated by PCR. Bacterial clonal relatedness was established using REP-PCR, PFGE, and multi-locus sequence typing (MLST). Transferability of bla_{NDM-1} was tested by conjugation and transconjugants were characterized.

K. pneumoniae was the primary organism causing sepsis in neonates. Resistance to different antimicrobials was high except for aminoglycosides and carbapenems. bla_{CTX-M} was present in all isolates. All carbapenem-resistant isolates harboured bla_{NDM-1} as the only carbapenemase. $bla_{CTX-M-15}$ and *qnrS1* were detected in all NDM-1-producing isolates. Plasmid analysis of transconjugants revealed that bla_{NDM-1} along with $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib*, *aac(6′)-Ib-cr* and *ccdAB* or *vagCD* addiction systems were carried on large IncFIIK conjugative plasmids of varied sizes. bla_{NDM-1} was associated with IS*Aba125* or IS*Ec33* element at its 5′-end. In addition, isolates also harboured *wabG*, *uge*, *fimH*, *mrkD*, and *entB* virulence genes. The NDM-1-producing *K. pneumoniae* belonged to four distinct clones and were distributed in 4 STs (ST347, ST29, ST2558, and ST1224), of which ST347 was predominant. The association of bla_{NDM-1} with diverse STs in *K. pneumoniae* from neonates indicates the promiscuity of the gene and its widespread dissemination.

1. Introduction

Every year around 2.6 million children die in the first month of life (neonatal period) around the globe (https://data.unicef.org/topic/child-survival/neonatal-mortality/) and around 670,000 of these deaths occur due to sepsis (Molyneux and Gest, 2015). According to UNICEF 2017 data, among the different regions, neonatal mortality was highest in South Asia, which reported 28 deaths per 1000 live births (https://data.unicef.org/topic/child-survival/neonatal-mortality/). About 0.75 million neonates die every year in India of which 30 deaths per 1000 live births are due to neonatal sepsis which is very high compared to other countries in the world (Sankar et al., 2016).

Treatment of neonatal sepsis is fraught with difficulties more so when the causative organism is carbapenem-resistant. Carbapenem-resistant Enterobacteriaceae (CRE) has become a major clinical concern worldwide particularly in neonatal units (Logan and Weinstein, 2017). As these resistance determinants are often associated with other resistance alleles, they confer resistance to almost all classes of antibiotics (Magiorakos et al., 2012) limiting treatment options severely.

Till date, different classes of carbapenemases [Ambler class A (KPC type), class B (NDM, VIM and IMP types) and class D (OXA-48-like)] have been reported in CRE (Hrabak et al., 2014). Among them, the most

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notorious is New-Delhi Metallo-β-lactamase-1 (NDM-1). Since its emergence in 2009, NDM-1 has spread rapidly to many countries worldwide and appears to be endemic in South Asia (Yong et al., 2009). The Indian sub-continent and the Balkan region are the most important reservoirs of the NDM-1-producing organisms (Dortet et al., 2014). The bla_{NDM-1} gene has been detected on different transferable plasmid scaffolds (IncA/C, IncFII, IncN or untypeable plasmids), enabling transfer and rapid dissemination of multidrug resistance (Voulgari et al., 2014).

In addition, among the members of the Enterobacteriaceae family, *Escherichia coli* and *Klebsiella pneumoniae* are known to harbour a repertoire of virulence determinants which protect them from the host immune response and simultaneously enhance their infectious potential. The incidence of hypervirulent *K. pneumoniae* (hvKP) infection was first detected in Asia (Liu et al., 1986). Since then, several hypervirulent clones have been identified in *K. pneumoniae* from different regions of the world (Shon et al., 2013) and convergence of specific virulence genes with carbapenem resistance has begun to occur. Recently, an outbreak was reported from China which showed the presence of hypervirulence determinants in a transmissible ST11 clone of *K. pneumoniae* (Gu et al., 2018). The combination of virulence and resistance is extremely worrisome and needs to be studied extensively to combat the devastating clinical crisis.

With the rapid spread of strains harbouring bla_{NDM}, accurately identifying these strains has also become important using typing methods. Multi-locus sequence typing (MLST) has an advantage over other typing methods as the data can be compared easily across the globe facilitating epidemiological surveillance. Although there are reports of bla_{NDM-1}-producing organisms from the Indian sub-continent, the epidemiology of strains harbouring bla_{NDM-1} and their comparison to strains from other parts of the world has been limited due to lack of MLST data. This study evaluated the prevalence of carbapenem resistance in a neonatal unit over a year in the North-eastern region of India, Tripura. In addition, a detailed characterization of the strains with respect to carbapenem resistance and its transmissibility, MLST, and virulence factors are central to this study. The North-East of India is geographically distinct from other regions of India and the study provides a glimpse of NDM-harbouring strains from this region.

2. Materials and method

2.1. Collection of blood specimens, culture procedure, and identification of bacterial isolates

During July 2016–December 2017, a total of 746 blood specimens were drawn from neonates suspected of sepsis, admitted to a 42-bed facility [Neonatal Intensive Care Unit (NICU)-10, Pediatric Intensive Care Unit (PICU)-6, and Neonatal Ward (NNW)-26] in a tertiary care hospital in Tripura, India. Detection of organisms in blood specimens was done by BacT/ALERT® 3D 60 automated microbial detection system (bioMe'rieux, Marcy l'E´ toile, France). For any culture that flagged positive, Gram staining was performed and subculture was done on MacConkey agar (HIMEDIA, India) and 5% sheep blood agar (HIMEDIA, India) for Gram-negative and Gram-positive organisms, respectively. For Gram-positive organisms, Catalase and Coagulase tests were done to identify the bacteria and for Gram-negative organisms, Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate, Triple Sugar Iron (TSI), Urease, and Mannitol motility tests were performed. Of the specimens cultured, 240 were positive (including gram-positive bacteria, gram-negative bacteria and fungal isolates). The clinical data was noted from the hospital registers. All gram-negative isolates were obtained as pure cultures and were cryopreserved at −80 °C in 20% glycerol. The isolates were revived on Luria-Bertani agar (LB agar) plates and in Luria-Bertani broth (LB broth) (Difco Laboratories, Detroit, MI, USA) prior to experiments. Ethical approval was also obtained for using the clinical specimens. The patient records/information were anonymized and de-identified prior to analysis.

2.2. Laboratory procedure

All Enterobacteriaceae were identified by standard biochemical tests (as stated above) and in case of any discrepancy, identification of the organism was confirmed by the VITEK2 compact system (bioMe'rieux, Marcy l'E´ toile, France). Antibiotic susceptibility profiles followed by the phenotypic detection of metallo-β-lactamases (MBLs), genotypic detection of extended spectrum β-lactamases (ESBLs) and carbapenemase determinants were carried out for all Enterobacteriaceae. Whereas, minimum inhibitory concentration (MIC), detailed molecular characterization, conjugation experiment followed by plasmid analysis, and molecular typing were performed only for the carbapenem-resistant isolates.

2.3. Antibiotic susceptibility, MIC, and detection of carbapenemase phenotypes

Antimicrobial susceptibility of all Enterobacteriaceae were determined by the Kirby-Bauer standard disk diffusion method (Bauer et al., 1966) for different antimicrobial agents such as: piperacillin (100 μg), ceftazidime (30 μg), aztreonam (30 μg), cefoxitin (30 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), tigecycline (15 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg) (BD Diagnostics, Franklin Lakes, NJ, USA) and interpretation was done in accordance with the criteria recommended by Clinical and Laboratory Standards Institute as modified in 2017 (Clinical and Laboratory Standards Institute, 2017) and for tigecycline, interpretation was done by the European Committee on Antimicrobial Susceptibility Testing guidelines as modified in 2016 (The European Committee on Antimicrobial Susceptibility Testing, 2016). Production of metallo-β-lactamase (MBL) was phenotypically assessed with an in-house combination disc diffusion test using meropenem (10 μg) and meropenem (10 μg)/dipicolinic acid (1000 μg). Isolates exhibiting the zone difference of ≥ 5 mm in the combination disc diffusion test were categorized as MBL positive.

Minimum Inhibitory Concentrations (MIC) of different β-lactam antimicrobials such as ceftazidime, cefoxitin, aztreonam, ertapenem, meropenem and non β-lactam antibiotics such as amikacin, gentamicin, ciprofloxacin, and tigecycline against only the carbapenem-resistant isolates were evaluated by *E*-test method (AB Biodisk, Solna, Sweden) and were interpreted according to CLSI guidelines as modified in 2017.

2.4. Molecular characterization of NDM-1-harbouring K. pneumoniae in terms of antibiotic resistance determinants

Genotypic detection of different extended spectrum β-lactamases (ESBLs) ($bla_{CTX-M,\ TEM,\ SHV,\ OXA-1}$) (Colom et al., 2003; Saladin et al., 2002) and carbapenemases (Class A- $bla_{KPC,\ IMI,\ SME,\ SPM,\ GES}$; Class B- $bla_{VIM,\ IMP,\ NDM,\ SPM,\ GIM,\ SIM}$; Class D- bla_{OXA-48}) were performed for all Enterobacteriaceae. In addition, for bla_{NDM-1}-carrying isolates, plasmid-mediated AmpC β-lactamases ($bla_{MOX,\ CMY,\ DHA,\ ACC,\ MIR/ACT,\ FOX}$), 16S rRNA methylase-encoding genes (*rmtA, rmtB, rmtC, rmtD* & *armA*), and plasmid-mediated quinolone resistance (PMQR) genes (*qnrA, qnrB, qnrC, qnrD, qnrS, qepA,* and *aac(6′)-Ib/Ib-cr*) were determined using PCR (Datta et al., 2014; Kim et al., 2009). For all bla_{NDM-1}-harbouring isolates, PCR amplicons were further sequenced using Applied Biosystems 3730 DNA Analyzer (Perkin Elmer, USA). Moreover, PCR mapping and sequencing was carried out to evaluate the genetic environment of bla_{NDM-1}. Primers were selected on the basis of previous reports (Datta et al., 2017).

Table 1
Antimicrobial activity of different β-lactam and non β-lactam antibiotics and occurrence of cephalosporin and carbapenem resistance determinants among Enterobacteriaceae (n = 200) during the study period (July 2016–December 2017).

Antimicrobial agents	% susceptible	ESBLs and MBL determinants Total (%)					
		*bla*TEM	*bla*SHV	*bla*OXA	*bla*CTXM	*bla*NDM	Negative for all determinants
Piperacillin	0						
Ceftazidime	0						
Aztreonam	0						
Cefoxitin	93.5						
Meropenem	87						
Amikacin	86.5	131 (65.5)	22 (11)	131 (65.5)	200 (100)	12 (6)	0
Gentamicin	99.5						
Ciprofloxacin	5.5						
Tigecycline	76						
Trimethoprim/sulfamethoxazole	0						

2.5. Transfer of bla_{NDM-1}, characterization of transconjugants, and plasmid analysis

Conjugal transfer of bla_{NDM-1} to the azide-resistant *E. coli* J53 recipient (lactose fermenting and plasmid-free) was tested by solid mating assay at 37 °C. Briefly, both the donor and recipient were cultured on MacConkey agar plate and a single colony obtained after overnight growth was inoculated in 3 mL LB broth at 37 °C for 3 h under weak agitation. Conjugation assay was performed on LB agar plates using 1:2, 1:5, and 1:10 donor to recipient ratio. After 18 h of incubation, cells were streaked on the selective LB agar plates containing ertapenem (1 mg/L) and 100 mg/L of sodium azide (SIGMA-ALDRICH, USA). Putative transconjugants obtained after overnight culture were confirmed by biochemical profiling and PCR-based detection of bla_{NDM-1}. MIC of bla_{NDM-1}-carrying transconjugants for different antimicrobials was detected by *E*-test and co-transfer of other resistance determinants [ESBLs and PMQRs] was confirmed by respective PCRs. Plasmid DNA was isolated from both parental (bla_{NDM-1}-carrying) isolates and transconjugants by modified Kado–Liu method (Kado and Liu, 1981). Plasmid sizes were determined using *Escherichia coli* V517 and *Shigella flexneri* YSH6000 as megaplasmid marker. The isolated plasmid DNA was subjected to 0.8% agarose gel (SIGMA-ALDRICH, USA) electrophoresis under constant 50 V in 1XTAE (Tris-Acetic acid-EDTA) buffer, and visualized after being stained with ethidium bromide (0.5 μg/mL). Quantity One 1-D Analysis Software (BIO-RAD) was used for size determination. Plasmid incompatibility was determined by PCR-based replicon-typing (PBRT) (DIATHEVA, Italy). Presence of different plasmid addiction systems (*pemKI, ccdAB, relBE, parDE, vagCD, hok–sok, pndCA,* and *srnBC*) were evaluated by PCR (Datta et al., 2017) for the parental isolates and transconjugants.

2.6. Determination of capsular serotypes, virulence determinants and hypermucoviscosity (HV) phenotype

Assessment of different capsular serotypes (K1, K2, K5, K20, K54, and K57) and various virulence factor-encoding genes of bla_{NDM-1}-carrying *K. pneumoniae* such as *magA*, *rmpA* and *rmpA2* (wzy-like polymerase and transcriptional regulators, respectively for hyper-capsule synthesis); *wcaJ* (UDP-phosphate galactose phosphotransferase for capsular polysaccharide synthesis); *uge* and *wabG* (UDP galacturonate 4-epimerase and GalA transferase, respectively for LPS production); *fimH* and *mrkD* (Type I and Type III fimbrial adhesin, respectively); *kfuBC* (an ABC iron transporter); *entB*, *iucA, iroN, ybtS,* and *alls* (iron scavenging siderophore- enterobactin, aerobactin, transporter of salmochelin, yersiniabactin, and activator of allantoin regulon) was carried out by PCR (Brisse et al., 2009; Lee et al., 2016).

In addition, the hypermucoviscous (HV) phenotype was determined by evaluating the formation of a viscous string ≥ 5 mm in length when bacterial colonies on an agar plate are stretched by an inoculation loop (Catalán-Nájera et al., 2017).

2.7. Molecular typing of bla_{NDM-1}-carrying *K. pneumoniae* isolates by REP-PCR, PFGE, and MLST

Clonal relatedness was analyzed for all NDM-1-producing *K. pneumoniae* isolates by repetitive extragenic palindromic elements-polymerase chain reaction (REP-PCR) using the primers REP1R (5P-III ICG ICG ICA TCI GGC-3P) and REP2I (5P-ICG ICT TAT CIG GCC TAC−3P). DNA amplification was performed as previously described (Versalovic et al., 1991). The PCR amplification programme involved an initial denaturation at 95 °C for 7 min followed by 30 cycles of denaturation at 90 °C for 30 s, annealing at 40 °C for 1 min, and extension at 65 °C for 8 min and with a final extension at 65 °C for 16 min. 10 μL of PCR amplification products underwent 2% agarose gel (SIGMA-ALDRICH, USA) electrophoresis for 3 h under constant 70 V in 1XTBE (Tris-Boric acid-EDTA) buffer, and visualized after being stained with ethidium bromide (0.5 μg/mL). Two known clonally related *K. pneumoniae* clinical isolates were used for the standardization of the REP-PCR, to assess reproducibility and as a positive control. PyElph version 1.4 Software was used to generate a dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) clustering (PyElph) (Pavel and Vasile, 2012).

Pulse field gel electrophoresis (PFGE) was carried out for twelve NDM-1-producing *K. pneumoniae* by following the PulseNet standardized procedure (http://www.cdc.gov/pulsenet/protocols.htm) in a CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules and CA). DNA digestion was carried out overnight at 37 °C using *Xba*I enzyme (New England Biolab, Massachusetts) followed by electrophoresis in 1% pulse field certified agarose gel (Bio-Rad Laboratories) for 18 h at 14 °C, 120^0 included angle, linear ramp factor with switch times of 2.16 s and 54.17 s at 6 V/cm. *Salmonella* serotype Braenderup H9812 was used as the marker. The dendrogram was generated by FPQuest version 4.5 Software (Bio-Rad Laboratories, Hercules, CA, USA).

Strain genotyping was performed by multi-locus sequence typing (MLST) to determine the sequence type (ST) of the isolate and to establish a comparison with previously reported NDM-1–producing isolates. Allelic numbers were obtained on the basis of sequences of seven conserved housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB,* and *tonB*) (www.pasteur.fr/recherche/genopole/PF8/mlst/K.pneumoniae.html).

3. Results

3.1. Bacterial Isolates

During July 2016 – December 2017, 83.33% of the 240 culture positive isolates yielded Enterobacteriaceae. The 200 non-duplicate clinical isolates of Enterobacteriaceae including *Escherichia coli* (n = 4, 2%), *Klebsiella pneumoniae* (n = 184, 92%), *Enterobacter cloacae* (n = 6, 3%) and *Pantoea agglomerans* (n = 6, 3%) were analyzed. *Klebsiella pneumoniae* was found to be the predominant enterobacteria causing

Table 2
Minimum Inhibitory Concentration (mg/L) of different antimicrobial agents and genotypic characterization of 12 NDM-1-producing *K. pneumoniae* isolates and their transconjugants.

Isolate	Organism	Sequence types (ST)	MIC values (mg/L)									Genetic determinants	Insertion sequences (IS)	Association of ble_{MBL}	Addiction system	Plasmid sizes (~kb)	Replicon type associated with bla_{NDM-1}
			TZ	FX	AT	ETP	MP	AK	GM	CI	TGC						
KP1	*Klebsiella pneumoniae*	ST347	> 256	> 256	> 256	> 32	24	6	0.75	8	0.38	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*, *ccdAB*	153, 4.8, 3.4, 1.9	L/M, Y, FIIK
KP1·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	2	0.75	6	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*ccdAB*	153	FIIK
KP2	*Klebsiella pneumoniae*	ST347	> 256	> 256	> 256	> 32	24	6	0.75	8	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*, *ccdAB*	153, 4.8, 3.4, 1.9	L/M, Y, FIIK
KP2·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	2	0.75	6	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*ccdAB*	153	FIIK
KP3	*Klebsiella pneumoniae*	ST29	> 256	> 256	> 256	> 32	24	0.75	0.75	6	0.5	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *oqxAB*	IS*Ec33*	Yes	*vagCD*	150, 5.1, 3.7, 2.3	FIIK, Y, X2, FIC, K
KP3·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	0.75	0.75	4	0.5	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*	IS*Ec33*	Yes	*vagCD*	115	FIIK
KP4	*Klebsiella pneumoniae*	ST347	> 256	> 256	> 256	> 32	24	6	0.75	8	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*, *ccdAB*	153, 4.8, 3.4, 1.9	L/M, Y, FIIK
KP4·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	4	0.75	6	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*ccdAB*	153	FIIK
KP5	*Klebsiella pneumoniae*	ST2558	> 256	> 256	32	24	16	0.5	1.5	6	0.75	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *aac (6′)-Ib*	IS*Ec33*	Yes	*vagCD*, *ccdAB*	173	FIIK, R
KP5·Tc	*Escherichia coli* J53	ND	> 256	> 256	32	24	4	0.5	1.5	6	0.75	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *aac (6′)-Ib*	IS*Ec33*	Yes	*vagCD*	173	FIIK
KP6	*Klebsiella pneumoniae*	ST347	> 256	> 256	> 256	> 32	24	6	0.75	8	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*, *ccdAB*	153, 4.8, 3.4, 1.9	L/M, Y, FIIK
KP6·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	2	0.75	6	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*ccdAB*	153	FIIK
KP7	*Klebsiella pneumoniae*	ST347	> 256	> 256	> 256	> 32	24	6	0.75	8	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*, *ccdAB*	153, 4.8, 3.4, 1.9	L/M, Y, FIIK
KP7·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	2	0.75	6	0.38	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*ccdAB*	153	FIIK
KP8	*Klebsiella pneumoniae*	ST29	> 256	> 256	> 256	> 32	24	0.75	0.75	6	0.5	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *oqxAB*	IS*Ec33*	Yes	*vagCD*	150, 5.1, 3.7, 2.3	FIIK, Y, X2, FIC, K
KP8·Tc	*Escherichia coli* J53	ND	> 256	> 256	> 256	> 32	6	0.75	0.75	4	0.5	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*	IS*Ec33*	Yes	*vagCD*	115	FIIK
KP9	*Klebsiella pneumoniae*	ST1224	> 256	> 256	> 256	16	8	0.75	0.25	6	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib*, *aac (6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD. ccdAB*	270, 111	HI1, FIIK
KP9·Tc	*Escherichia coli* J53	ND	> 256	> 256	128	6	4	0.75	0.25	4	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*	270	FIIK
KP10	*Klebsiella pneumoniae*	ST1224	> 256	> 256	> 256	16	8	0.75	0.25	6	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib*, *aac (6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD. ccdAB*	270,111	HI1, FIIK
KP10·Tc	*Escherichia coli* J53	ND	> 256	> 256	128	6	4	0.75	0.25	4	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*	270	FIIK
KP11	*Klebsiella pneumoniae*	ST2558	> 256	> 256	32	24	16	0.5	1.5	6	0.75	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *aac (6′)-Ib*	IS*Ec33*	Yes	*vagCD*, *ccdAB*	173	FIIK, R
KP11·Tc	*Escherichia coli* J53	ND	> 256	> 256	32	24	4	0.5	1.5	6	0.75	bla_{NDM-1}, $bla_{CTX-M15}$, *qnrS1*, *aac (6′)-Ib*	IS*Ec33*	Yes	*vagCD*	173	FIIK
KP12	*Klebsiella pneumoniae*	ST1224	> 256	> 256	> 256	16	8	0.75	0.25	6	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *oqxAB*, *aac(6′)-Ib*, *aac (6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD. ccdAB*	270,111	HI1, FIIK
KP12·Tc	*Escherichia coli* J53	ND	> 256	> 256	128	6	4	0.75	0.25	4	0.5	bla_{NDM-1}, $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib*, *aac(6′)-Ib-cr*	IS*Aba125*	Yes	*vagCD*	270	FIIK

TZ: Ceftazidime; FX: Cefoxitin; AT: Aztreonam; ETP: Ertapenem; MP: Meropenem; AK: Amikacin; GM: Gentamicin; CI: Ciprofloxacin; TGC: Tigecycline; ND: Not determined.

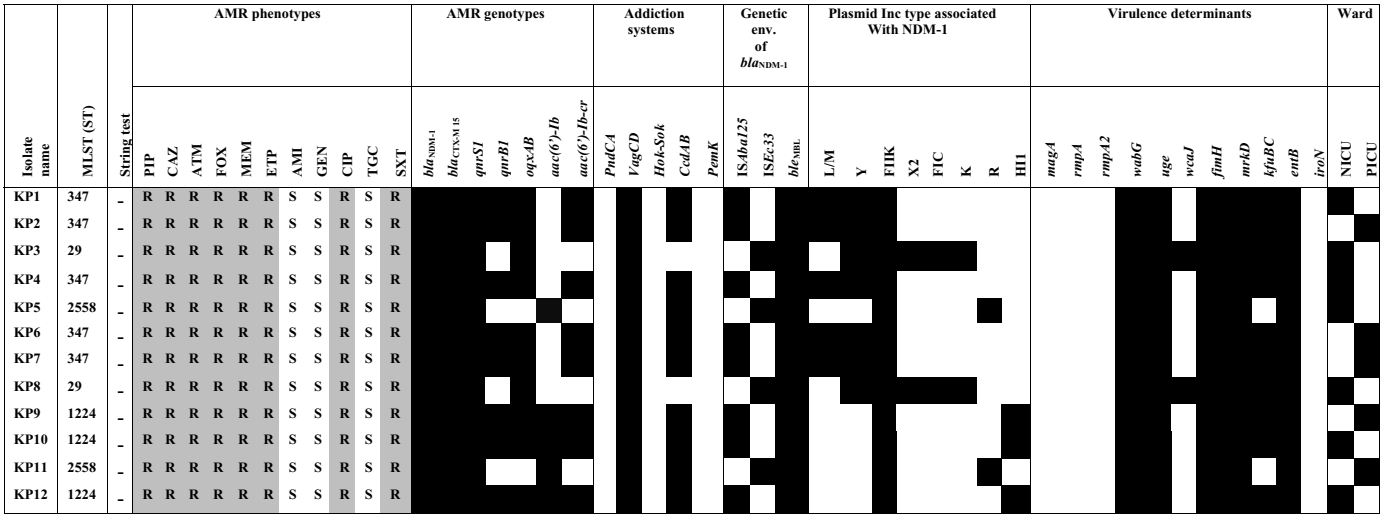


Fig. 1. Pictorial presentation of sequence types (STs), antibiotic susceptibility profile, and molecular characterization of 12 NDM-1-producing *K. pneumoniae* isolates in terms of resistance determinants, plasmid types, addiction systems and different virulence factors. Filled-up boxes indicate the presence of the respective characters. In case of susceptibility profile, resistant (R) and susceptible (S) are designated in different shades.

sepsis in neonates during the study period.

3.2. Antimicrobial Susceptibility and result of the phenotypic assay

Aminoglycosides (gentamicin and amikacin) and second-generation cephamycin (cefoxitin) were found to be the most active antimicrobial agents when tested against the studied Enterobacteriaceae isolates, with 99.5% ($n = 199$) for gentamicin, 86.5% ($n = 173$) for amikacin, and 93.5% ($n = 187$) susceptibility for cefoxitin followed by carbapenem (87%, $n = 174$ for meropenem) and tigecycline (76%, $n = 152$). Alarmingly, all studied isolates were completely resistant to third-generation cephalosporin (100% resistant for ceftazidime) and 94.5% ($n = 189$) isolates were resistant to ciprofloxacin (Table 1). Out of 26 meropenem non-susceptible isolates, 53.85% ($n = 14$) isolates were intermediate and 46.15% ($n = 12$) *K. pneumoniae* isolates were resistant. Moreover, all 12 meropenem-resistant isolates showed the zone difference of ≥ 5 mm in the meropenem and meropenem/dipicolinic acid combination test, clearly suggesting the presence of MBLs in these isolates. Furthermore, the 14 isolates which showed intermediate sensitivity to meropenem were not positive for MBL production (augmentation of the zone between meropenem and meropenem with dipicolinic acid was ≤ 5 mm in all cases).

3.3. Detection of β-lactamases and carbapenemases

Based on the antibiotic susceptibility test, all Enterobacteriaceae were further analyzed for cephalosporin- (ESBLs) and carbapenem-resistant genes. Isolates harboured different combinations of any of the three ESBL types (CTX-M, TEM, and OXA). The predominant β-lactamase was CTX-M, present in 100% of the isolates ($n = 200$), followed by TEM and OXA both were present in 65.5% of the isolates ($n = 131$) (Table 1).

Out of 200 Enterobacteriaceae, only 6% ($n = 12$) isolates (*K. pneumoniae)* harboured $bla_{\text{NDM-1}}$ and NDM-1 was the only carbapenemase identified. No other carbapenemases were detected. In addition, isolates which exhibited intermediate sensitivity towards meropenem ($n = 14$) were negative for all the carbapenemase genes tested. The intermediate sensitivity may thus be attributed to the presence of ESBLs and loss of porins (Datta et al., 2014).

3.4. Characterization of NDM-1-producing K. pneumoniae isolates

3.4.1. Analysis of minimum inhibitory concentration (MIC)

The MICs of different antimicrobial agents tested against all twelve NDM-1-positive *K. pneumoniae* isolates are shown in Table 2. All 12 $bla_{\text{NDM-1}}$-harbouring isolates exhibited high MIC values for β-lactam antibiotics tested. Furthermore, MIC values for ciprofloxacin for the same isolates showed that they were quinolone-resistant. All 12 isolates were susceptible to aminoglycosides (amikacin and gentamicin) and tigecycline.

3.4.2. Genotypic detection of different antibiotic resistance determinants and the genetic environment of bla_{NDM-1}

The molecular characterization of NDM-1-producing *K. pneumoniae* isolates ($n = 12$) is represented in Fig. 1. All NDM-1-producing isolates possess $bla_{\text{CTX-M-15}}$ as the only ESBL. Genes coding for plasmid-mediated AmpC β-lactamases and 16S rRNA methylases were not detected in any isolate. Moreover, different PMQRs were identified in tested isolates among them *qnrS1* was the predominant, detected in all isolates (n = 12) followed by *qnrB1* ($n = 8$), *aac(6′)-Ib* ($n = 5$), and *aac(6′)-Ib-cr* (n = 8).

In this study, an entire IS*Aba125* element was identified at the 5′-end of $bla_{\text{NDM-1}}$ in most of the isolates (n = 8). However, in the case of 4 isolates (KP3, KP5, KP8, and KP11), IS*Ec33* was present at 5′-end instead of IS*Aba125*. In all isolates, $bla_{\text{NDM-1}}$ was found to be associated with the ble_{MBL} gene at its 3′-end (Table 2).

3.4.3. Transfer of bla_{NDM-1} and characterization of transconjugants

Conjugative transfer of $bla_{\text{NDM-1}}$ to *E. coli* J53 was successful in all cases. All $bla_{\text{NDM-1}}$-harbouring transconjugants possessed a single megaplasmid. All transconjugants displayed high MIC values for antimicrobials tested indicating the co-existence of a highly active carbapenemase, ESBL, and fluoroquinolone-resistant determinants in the same conjugative megaplasmid. $bla_{\text{CTX-M-15}}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib,* and/or *aac(6′)-Ib-cr* were co-transferred with $bla_{\text{NDM-1}}$ in various combinations (Table 2).

3.4.4. Characterization of the plasmids

Analysis of plasmids from the parental isolates and transconjugants showed the plasmids were varied in size (~1.9 to 270 kb) as well as in types. In most of the cases, parental isolates harboured multiple plasmids. A variety of plasmid incompatibility (Inc) groups were detected in

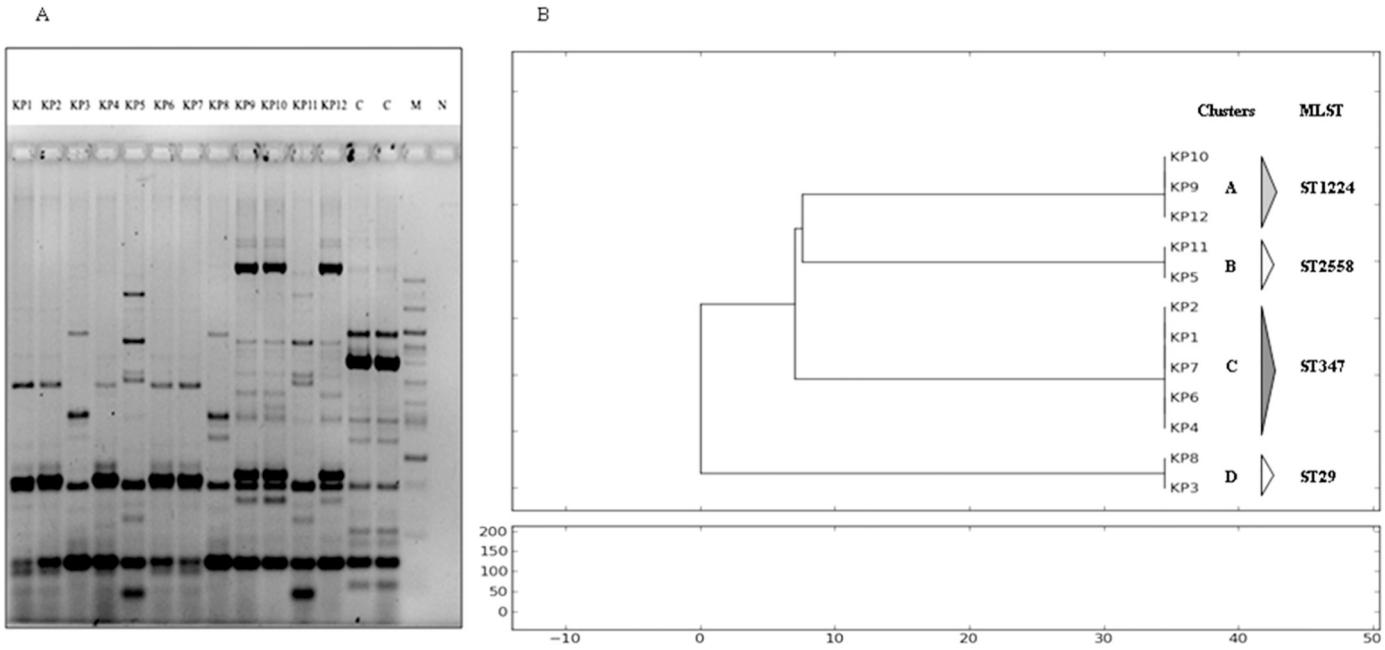


Fig. 2. A: Fingerprint pattern for the REP-PCR of 12 NDM-1-producing *K. pneumoniae* blood isolates. Lane M: 100 bp DNA ladder; C: clonally related known *K. pneumoniae* clinical isolates; N: negative control. B: Dendrogram showing the genetic relationship among NDM-1-producing isolates using PyElph version 1.4 software.

the NDM-1 producing parental isolates such as L/M, Y, FIIK, R, Y, X2, FIC, HI1, and K. Of them, only Inc-FIIK like megaplasmids of varied sizes (~115 kb, 153 kb, 173 kb, and 270 kb) were found to be associated with the transfer of bla_{NDM-1} in all cases (Table 2 and Fig. S1). In this study, the low copy number and narrow host range bla_{NDM-1}-harbouring IncF plasmid (Inc-FIIK) was found to be associated with $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib,* and/or *aac(6′)-Ib-cr*. Plasmid protein antitoxin-regulated systems *ccdAB/vagCD* was found to be co-transferred during conjugation (Table 2).

3.4.5. Clonal relatedness of the isolates

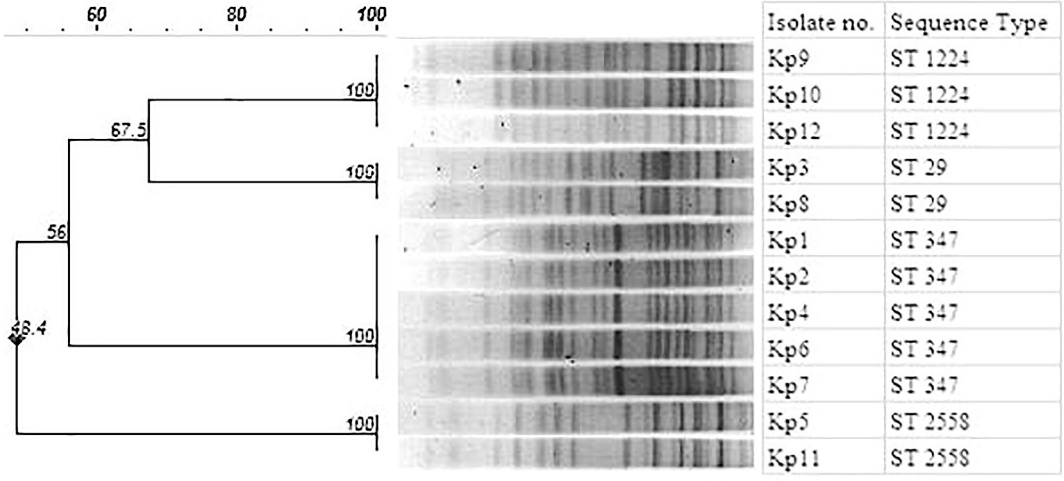
The number of bands obtained via REP-PCR ranged between 6 and 14. The size of visible bands varied between 0.15 and 2 kb. The majority of bands were observed between 300 and 1000 bp. Bands at position 150 bp and 300 bp were common to all 12 isolates. The dendrogram analysis revealed the presence of four different clonal clusters: A (KP9, KP10, and KP12), B (KP5 and KP11), C (KP1, KP2, KP4, KP6, and KP7), and D (KP3 and KP8) (Fig. 2A and B).

PFGE revealed that 12 NDM-1-producing *K. pneumoniae* isolates could be categorized into four different clones validating the REP-PCR result. The four clonal groups consisted of i) KP1, KP2, KP4, KP6, KP7; ii) KP3, KP8; iii) KP5, KP11; iv) KP9, KP10, KP12 (Fig. 3).

MLST demonstrated that all 12 isolates belonged to four different sequence types (ST) confirming the result of PFGE. KP1, KP2, KP4, KP6, and KP7 were found to be clonally related and belonged to ST347. KP3 and KP8 belonged to ST29. KP5 and KP11 belonged to ST2558. Whereas, KP9, KP10, and KP12 belonged to ST1224 (Fig. 3).

3.4.6. Virulence potential of NDM-1-producing K. pneumoniae

Type I and type III fimbrial gene (*fimH* and *mrkD*), UDP galacturonate 4-epimerase gene (*uge*), GalA transferase (*wabG*), and gene coding for enterobactin (*entB*) were detected in all 12 NDM-1-producing



Isolate no.	Sequence Type
Kp9	ST 1224
Kp10	ST 1224
Kp12	ST 1224
Kp3	ST 29
Kp8	ST 29
Kp1	ST 347
Kp2	ST 347
Kp4	ST 347
Kp6	ST 347
Kp7	ST 347
Kp5	ST 2558
Kp11	ST 2558

Fig. 3. Analysis of the genetic relationship according to Dice's similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA) (the position tolerance and optimization were set at 1.0% and 1.0% respectively) of the *Xba*I-digested patterns of 12 NDM-1-producing *K. pneumoniae* isolates.

K. pneumoniae isolates. The other genes such as ABC iron transporter (*kfuBC*) and UDP-phosphate galactose phosphotransferase (*wcaJ*) were present in some isolates (Fig. 1). All isolates were negative for *magA*, *rmpA*, *rmpA2*, *iucA*, *iroN, ybtS, allS,* and for all tested serotypes (K1, K2, K5, K20, K54, and K57).

Furthermore, none of the isolates showed the formation of a viscous string ≥5 mm in length when they were stretched by an inoculation loop. Hence, the hypermucoviscous (HV) phenotype by string test was not detected in any isolate.

3.4.7. Clinical outcome of neonates infected with NDM-1-producing K. pneumoniae

Table 3 shows the demographics and clinical characteristics of twelve neonates who were found to be affected with NDM-1-producing *K. pneumoniae*. Most of the neonates (except two cases – P6 and P8) developed the symptoms of early-onset sepsis. Out of twelve neonates, seven were inborn, the majority were delivered pre-term (n = 7), and were of low birth weight (n = 7) or very low birth weight (n = 2, 16.67%). Most of the neonates (n = 7) were born by low uterine cesarean delivery and the majority of mothers had either fever or premature rupture of membrane (PROM) considered to be risk factors for sepsis. Four neonates (P3, P4, P5, and P9) had been subjected to mechanical ventilation. Three patients expired due to very low birth weight (P3 and P9), severe birth asphyxia and gastrointestinal bleeding (P5) while others had a favourable clinical outcome. Amikacin along with cefotaxime was used in the NICU as a pre-emptive antimicrobial therapy for clinically suspected cases of sepsis.

4. Discussion

The emergence of carbapenem resistance particularly in Enterobacteriaceae is a considerable burden on the neonatal healthcare system in developing countries. This study describes the incidence of $bla_{\text{NDM-1}}$-carrying *K. pneumoniae* belonging to different STs, collected from a tertiary care hospital of Tripura in North-East India. Although, many authors reported the occurrence of $bla_{\text{NDM-1}}$ from across the globe as well as from the Indian sub-continent (Dortet et al., 2014), the detailed characterization of NDM-1-producing isolates and its consequences in neonates were not determined earlier from this part of India. Hence, an attempt was made to characterize the isolates in terms of their resistance and virulence and also to investigate the sequence types of *K. pneumoniae* associated with $bla_{\text{NDM-1}}$ from a neonatal setup.

K. pneumoniae was the predominant enterobacteria (n = 184, 92%) causing sepsis in neonates during the study period. Susceptibility to aminoglycosides in the isolates was high unlike *K. pneumoniae* isolated from neonatal septicemic cases from other parts of India and from across the globe (Datta et al., 2014; Ma et al., 2009). However, isolates were highly resistant, particularly to β-lactam antibiotics and ciprofloxacin. Studies from other developing countries also showed that neonatal sepsis is primarily caused by multidrug-resistant Enterobacteriaceae both in community and hospital settings (Bates et al., 2014). Assessment of $bla_{\text{CTX-M}}$ revealed that all isolates possessed this gene. This portrays a worrisome situation but is comparable to neonatal septicemic *K. pneumoniae* isolated from other regions of India and from across the globe (Datta et al., 2014; Bevan et al., 2017).

Resistance to carbapenem was 6% and twelve isolates were identified as possessing NDM-1. All twelve *K. pneumoniae* isolates harboured $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$ as the only carbapenemase and ESBL type, respectively. Co-production of NDM-1 and CTX-M-15 by the same isolates results in resistance to nearly all clinically available β-lactams. Though a number of variants of the bla_{NDM} have been reported till date, no other variant of this gene was identified in this study. Furthermore, all NDM-1-positive isolates possessed *qnrS1*. Though, the prevalence of plasmid-mediated quinolone resistance gene *qnrS1* has been reported in Enterobacteriaceae from China (Hu et al., 2014), this resistant determinant in association with $bla_{\text{NDM-1}}$ was generally not detected in the Indian sub-continent except for one case from Bangladesh (Islam et al., 2012).

All isolates were able to transfer $bla_{\text{NDM-1}}$ via conjugation. Analysis of transconjugants revealed that the $bla_{\text{NDM-1}}$ gene in the isolates, together with $bla_{\text{CTX-M-15}}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib,* and/or *aac(6′)-Ib-cr,* were carried on large conjugative plasmids belonging to the IncFIIK incompatibility group suggesting the epidemiological success of these IncFII plasmids. Transferable IncFII plasmids not only have an ability to disseminate the antibiotic resistance determinants among *K. pneumoniae*, but they also have the potential to transfer resistance genes between other enterobacterial species (Voulgari et al., 2014). The association of this plasmid with $bla_{\text{NDM-1}}$ has been noted in several studies (Datta et al., 2017; Datta et al., 2014). Presence of *vagCD/ccdAB* plasmid addiction system in $bla_{\text{NDM-1}}$-carrying conjugative plasmid suggests the stable association of carbapenemase in the studied isolates. All parental isolates carried multiple plasmids which in turn may favour the genetic rearrangement and recombination events. These events altogether can contribute to the plasmid diversity by recruiting new resistance genes into the plasmid scaffold (Carattoli, 2009).

Analysis of the immediate genetic environment of $bla_{\text{NDM-1}}$ revealed that in most of the isolates (KP1, KP2, KP4, KP6, KP7, KP9, KP10, and KP12), a full-length IS*Aba125* element was detected at 5′-end of $bla_{\text{NDM-1}}$ which correspond to other reports (Dortet et al., 2014). Whereas, in four cases (KP3, KP5, KP8, and KP11) IS*Ec33* was found at the upstream of $bla_{\text{NDM-1}}$. The association of IS*Ec33* element with $bla_{\text{NDM-1}}$ was reported in a multidrug-resistant *E. coli* strain from Australia that was associated with a patient who had been hospitalized in Bangladesh prior to medical transfer in Australia (Poirel et al., 2010). Our study revealed the occurrence of this insertion sequence in an approximately ~115 kb and ~173 kb IncFIIK conjugative plasmid in the *K. pneumoniae* isolates.

The virulence potential of NDM-1-producing *K. pneumoniae* revealed that all isolates harboured *fimH*, *mrkD*, *wabG*, *uge*, and *entB*, which code for virulence factors associated with binding, invasion, biofilm formation, and the potential to colonize and escape phagocytosis. Furthermore, other virulent determinants (*wcaJ* and *kfuBC*) were also detected only in some isolates. However, the genes coding for hypercapsule phenotype (*magA*, *rmpA*, and *rmpA2*) were not found in any isolate. These factors provide the pathogen an ability to persist not only in the patients but also in the healthcare system (Paczosa and Mecsas, 2016). Although, several virulence determinants were detected in KPC-2-producing *K. pneumoniae* (Andrade et al., 2014), very little is known about the virulence potential of NDM-1 producers. This study suggests that all NDM-1-producing *K. pneumoniae* isolates were potentially virulent as they harbour a repertoire of virulence factors. This intrinsic virulence potential in coexistence with the $bla_{\text{NDM-1}}$ might partly explain its global epidemiological success. Although, virulent and antimicrobial-resistant populations of *K. pneumoniae* were largely non-overlapping (Bialek-Davenet et al., 2014), some isolates with both hypervirulence and carbapenem resistance attributes were detected in clinical settings (Gu et al., 2018). Taken together, the convergence of multidrug resistance and enhanced virulence could lead to untreatable *K. pneumoniae* infections.

In this study, the presence of $bla_{\text{NDM-1}}$ was detected in 4 different sequence types (STs) of *K. pneumoniae* (i.e., ST29, ST347, ST1224, and ST2558). ST29 *K. pneumoniae* with hypervirulent potential was detected in Taiwan (Chuang et al., 2013) causing mycotic aneurysm. However, in this study, ST29 *K. pneumoniae* was not associated with the hypermucoviscous phenotype. Furthermore, the prevalence of OXA-48-producing ST29 *K. pneumoniae* was detected in Saudi Arabia (UzZaman et al., 2014). NDM-1, CTX-M-15, QnrB1, and AAC(6′)-Ib co-producing ST29 *K. pneumoniae* was isolated from Yemen (Gharout-Sait et al., 2014). This study suggests that $bla_{\text{NDM-1}}$ in association with IS*Ec33* element at its 5′-end, $bla_{\text{CTX-M-15}}$, *qnrS1*, and *vagCD* addiction system were carried on a large ~115 kb IncFIIK plasmid of ST29 *K. pneumoniae*. In spite of being an uncommon sequence type, one report shows

Table 3
Demographic data and clinical features of the septicemic neonates (n = 12) infected by the NDM-1-producing *K. pneumoniae*.

Patient No./ Organism (Isolate No.)	Date of birth	Date of admission to NICU/PICU	Date of sampling	Stay in NICU/PICU (days) before sampling	Sex	Inborn /Outborn	Birth weight (gm)	Mode of delivery	Gestational age (wk)	Primary cause of admission to NICU/PICU	Maternal risk factor	Onset of sepsis	Ventilation	Prescribed antibiotics	Outcome
P1/*Klebsiella pneumoniae*/ KP1	11.07.16	11.07.16	14.07.16	3	M	Inborn	2600	LUCS	37	Breathing problem	High fever	EOS	No	Cefotaxime	Discharged
P2/*Klebsiella pneumoniae*/ KP2	11.07.16	13.07.16	15.07.16	2	M	Outborn	1900	LUCS	39	Poor cry, LBW	No	EOS	No	Amikacin	Discharged
P3/*Klebsiella pneumoniae*/ KP3	24.07.16	24.07.16	29.07.16	5	F	Inborn	1300	LUCS	29	Respiratory distress, poor cry, poor reflex, pre-term, VLBW	UTI, PPROM, Prolonged labour	EOS	Yes	Amikacin	Death (due to VLBW)
P4/*Klebsiella pneumoniae*/ KP4	18.09.16	18.09.16	21.09.16	3	F	Inborn	1800	NVD	33	Pre-term, LBW	No	EOS	Yes	Ceftazidime	Discharged
P5/*Klebsiella pneumoniae*/ KP5	22.11.16	22.11.16	26.11.16	4	M	Inborn	2100	LUCS	37	Birth asphyxia, LBW	Fever	EOS	Yes	Cefotaxime	Death (due to Birth Asphyxia and gastrointestinal bleeding)
P6/*Klebsiella pneumoniae*/ KP6	30.11.16	07.12.16	09.12.16	2	M	Outborn	1800	LUCS	32	Pre-term, LBW	PROM	LOS	No	Amikacin	Discharged
P7/*Klebsiella pneumoniae*/ KP7	05.02.17	08.02.17	11.02.17	3	F	Outborn	2400	NVD	35	Breathing problem, poor reflex	PROM	EOS	No	Amikacin	Discharged
P8/*Klebsiella pneumoniae*/ KP8	28.03.17	28.03.17	31.03.17	3	M	Inborn	3100	LUCS	37	High fever, breathing problem	No	LOS	No	Cefotaxime	Discharged
P9/*Klebsiella pneumoniae*/ KP9	16.06.17	17.06.17	21.06.17	4	M	Outborn	1500	LUCS	31	Respiratory distress, vomiting, pre-term, VLBW	PROM, Prolonged labour	EOS	Yes	Amikacin	Death (due to VLBW)
P10/*Klebsiella pneumoniae*/ KP10	14.09.17	14.09.17	16.09.17	2	F	Inborn	2200	NVD	32	LBW, Breathing problem, pre-term	PROM	EOS	No	Amikacin	Discharged
P11/*Klebsiella pneumoniae*/ KP11	23.10.17	25.10.17	28.10.17	3	M	Outborn	2300	NVD	34	Pre-term, LBW	PROM	EOS	No	Amikacin	Discharged
P12/*Klebsiella pneumoniae*/ KP12	22.12.17	22.12.17	24.12.17	2	F	Inborn	2700	NVD	37	Breathing problem, poor reflex	No	EOS	No	Cefotaxime	Discharged

M: male; F: female; NICU: Neonatal Intensive Care Unit; PICU: Pediatric Intensive Care Unit; NVD: normal vaginal delivery; LUCS: low uterine cesarean delivery; Gestational age: term (≥ 37 weeks), pre-term (< 37 weeks); LBW: low birth weight (< 2500 g); VLBW: very low birth weight (< 1500 g); UTI: urinary tract infection; PPROM: pre-term premature rupture of membrane; PROM: premature rupture of membrane; EOS: early onset sepsis; LOS: late onset sepsis.

the presence of NDM-1-producing ST1224 *K. pneumoniae* from the bloodstream of a leukemic patient in China (Zhang et al., 2017). Our data revealed that neonatal septicemic ST1224 *K. pneumoniae* harboured bla_{NDM-1} along with $bla_{CTX-M-15}$, *qnrS1*, *qnrB1*, *aac(6′)-Ib*, and *aac(6′)-Ib-cr* on a large ~270 kb IncFIIK plasmid. Till date, there are no reports of NDM-1-producing *K. pneumoniae* isolates that belong to sequence type 347 (ST347). So to the best of our knowledge, this is the first report of the occurrence of NDM-1, CTX-M-15, QnrS1, QnrB1, and AAC(6′)-Ib-cr co-producing *K. pneumoniae* ST347 clone. Moreover, this is the first report of bla_{NDM-1}-carrying *K. pneumoniae* belonging to ST29, ST2558, and ST1224 from India. The presence of bla_{NDM-1} in diverse STs indicate that dissemination of carbapenemases is occurring among the new STs. The individual isolates belonging to any one of the four STs (29, 347, 1224, 2558) were found to be genetically indistinguishable by PFGE when compared with other isolates of the same sequence type. It could thus be concluded that, epidemiologically, only one isolate of the predominant ST347 clone had disseminated among the neonates in the unit.

As Tripura shares borders directly with Bangladesh, Assam, and Mizoram, infiltration of several different kinds of strains may occur in this site. The similarity with strains from China also indicates the propagation of these sequence types (STs) in these regions. The association of bla_{NDM-1} with diverse STs of *K. pneumoniae* indicates the promiscuous nature of the gene. The emergence and establishment of new strains with antibiotic resistance in the health care system are worrisome. Strict infection control measures and continuous surveillance are needed to check the spread of this gene.

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Conflicts of interest

None to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.01.024.

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