Title- Concurrent carriage of *bla*$_{NDM}$ plasmids in distinct Gram-negative bacterial species in the gut microbiota of pregnant mothers and neonates

Short running title- Co-colonization and plasmid transmission of *bla*$_{NDM}$

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Abstract

Objectives

New Delhi metallo-β-lactamase (NDM), a broad-spectrum carbapenemase, can disseminate via plasmids and is a major global healthcare challenge. The gut acts as a niche for the exchange of such genes. This study investigates the transmission dynamics of *bla*$_{NDM}$-bearing plasmids among co-colonized bacterial species in pregnant mothers/neonates.

Methods

Rectal isolates from mothers and neonates underwent antimicrobial susceptibility testing, detection of *bla*$_{NDM}$ variants, molecular typing and whole-genome/plasmid sequencing. Transmissibility of *bla*$_{NDM}$ was evaluated through conjugation.

Results

Among mothers (n=86) and sick neonates (n=93) analyzed, 17 were colonized with multiple carbapenem-resistant species; with 9 patients colonized with multiple carbapenem-resistant Enterobacterales (CREs), primarily *bla*$_{NDM}$-harbouring *Escherichia coli* and *Klebsiella pneumoniae*. Isolates were distinct and belonged to diverse sequence types, including epidemic clones (ST11/15/101/147/167/648). *bla*$_{NDM}$-variants (*bla*$_{NDM-1}$>*bla*$_{NDM-5}$>*bla*$_{NDM-7}$>*bla*$_{NDM-4}$) were found to reside on large conjugative plasmids (46-271kb), primarily belonging to IncFIA-FIB-FII replicons in these isolates. Comparison of *bla*$_{NDM-}$plasmid backbones in co-colonized bacteria revealed high diversity and different *bla*$_{NDM}$-variants, while the immediate genetic environment of *bla*$_{NDM}$ was very similar. The diverse *bla*$_{NDM-}$plasmids indicated an independent acquisition of *bla*$_{NDM}$ instead of its transmission amongst co-colonized bacteria in individuals. However, in one neonate, co-colonized species (*E. coli* & *K. pneumoniae*) possessing *bla*$_{NDM-5}$ showed similarities in plasmid backbones indicating possible transmission of *bla*$_{NDM}$ among these co-

colonized species. Additionally, transmission of *bla*$_{NDM}$-plasmids was observed between different neonates.

Conclusions

The high co-colonization of *bla*$_{NDM}$-harboring bacteria, some epidemic clones, calls for targeted intestinal CRE screening. However, exchange of such genes was very low in the gut, indicating independent acquisition of *bla*$_{NDM.}$

Introduction

Antimicrobial resistance is a critical global problem, particularly in clinical settings.[1] Carbapenem resistance, emerging over the past two decades, significantly contributes to morbidity and mortality worldwide.[2] Infections caused by carbapenem-resistant Enterobacterales and carbapenem-resistant *Acinetobacter baumannii* are challenging to treat due to limited therapeutic options leading to the mortality.

Carbapenem resistance is largely driven by carbapenemase production, particularly New Delhi metallo-β-lactamase (NDM), a plasmid-borne carbapenemase, which has disseminated worldwide, with the Indian subcontinent recognized as its principal reservoir. After the initial detection of *bla*$_{NDM-1,}$ multiple variants (*bla*$_{NDM-4}$, *bla*$_{NDM-5}$ & *bla*$_{NDM-7}$) have emerged across diverse species.[3]

The spread of *bla*$_{NDM}$ is mediated by mobile genetic elements (MGEs) such as plasmids, IS and transposons.[4] Plasmid incompatibility groups including IncA/C, IncFIA, IncFIB, IncFII and IncX3 frequently disseminate *bla*$_{NDM}$ within Enterobacterales.[3] MGEs, including IS elements, transposons, and different ISCR elements, accentuate the acquisition and transmission of *bla*$_{NDM}$ in different plasmids of Enterobacterales.[5] Over the years, the genetic environment of *bla*$_{NDM}$ has

remain conserved i.e. association with IS*Aba125* and Tn*125*.[6] Although recent studies[3,6,7] have documented minute variations in the IS element i.e. truncation of IS*Aba125* with IS*26*, IS*3000*, IS*903*, etc., but the mobility of the gene remains unaltered.

Carbapenem-resistant bacteria inhabit multiple reservoirs including hospitals, the human gut, environment and animals from where they can spread to other reservoirs.[8] The gut serves as a reservoir for commensal and pathogenic bacteria (harbouring antimicrobial resistance genes, ARGs) and provides an ideal platform for the exchange of ARG-plasmids between them.[9,10] The complex gut microbiota fosters horizontal gene transmission (HGT) of ARGs including bla_{NDM} which often reside on self-transmissible promiscuous plasmids.[5,10] Studies have depicted *in vivo* HGT of carbapenemases (bla_{NDM}/bla_{KPC}/bla_{OXA-48}) between species isolated from rectal swabs[11,12,13] or stool samples[5,14,15] but none involved samples from pregnant mothers and neonates.

A multi-center study titled Burden of Antibiotic Resistance in Neonates from Developing Societies (BARNARDS), conducted across low- and middle-income countries (LMICs), reported high prevalence of bla_{NDM} in neonatal gut (42%) than maternal gut (8%) in India.[16] The study also highlighted a broader carriage of ESBL and carbapenemases within the gut of mothers and sick neonates.[16] Beyond the initial findings, the present study noted the co-colonization of bla_{NDM}-harbouring Gram-negative bacteria in mothers and neonates, providing a unique opportunity to examine *in-vivo* plasmid exchange. This study, therefore aimed to provide a holistic assessment and transmission dynamics of bla_{NDM}-harboring plasmids recovered from different carbapenem-resistant Gram-negative bacterial species in colonized mothers and neonates.

Methods

Ethical approval

The study protocol was approved by the Institutional Ethics Committee of the ICMR-National Institute of Cholera and Enteric Diseases (A-1/2016-IEC, 17.11.2016, and IPGME&R/IEC/2017/442-B). Patient consent was obtained and data were anonymized before analysis. All procedures followed relevant guidelines and regulations.

Study setting

Rectal swabs were collected from pregnant women (in labour or immediate postpartum) and sick neonates enrolled in the BARNARDS[16] study (July–December 2017) at IPGMER and SSKM Hospital, Kolkata, after written informed consent. Mother-to-neonate transmission of carbapenem-resistant Enterobacterales has been reported previously.[17] This study evaluated the transmission dynamics of *bla*$_{NDM}$-harbouring plasmids among co-colonized species from mother or neonate.

Isolation of Gram-negative bacteria

Rectal swabs were cultured on vancomycin (10 mg/L; MP Biomedicals, CA, USA) and ertapenem (2 mg/L; VE; Sigma-Aldrich, USA) supplemented chromogenic agar (BD BBL, MD, USA) plates to recover all carbapenem-resistant Gram-negative bacteria. All colonies (pink/green/white) were further screened for carbapenemases (*bla*$_{NDM}$, *bla*$_{KPC}$ and *bla*$_{OXA-48-like}$) by PCR as described previously.[16,17] When more than 1 colony of similar colour harboring carbapenemases were found, those colonies were checked for clonality using repetitive extragenic palindromic elements-PCR (rep-PCR)[18]. When indistinguishable rep-PCR patterns were noted, one representative of such colonies for a particular mother/neonate were retained for further analysis and were designated as IN-MRXY and IN-BRXY respectively, where IN stands for India, MR stands for mother rectal specimen, BR for baby rectal specimen, X stands for patient ID[16] and Y stands for abbreviated name of the organism.

Identification, antibiotic susceptibility testing (AST)

Isolates were identified by Enterosystem 18R (Liofilchem, Italy) and confirmed using VITEK2 (BioMérieux, France). AST was performed using Kirby-Bauer disk diffusion method (Enterobacterales) and VITEK2 AST-N281 (Gram-negative non-Enterobacterales). MIC for meropenem and ertapenem were determined via broth microdilution using *Escherichia coli* ATCC 25922 as quality control. Results were analyzed according to CLSI (2025) except for tigecycline following EUCAST (2023) criteria.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed for bla_{NDM}-producing isolates in a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules and CA). DNA was digested with *Xba*I[17,19] (50 U/plug) (New England Biolab, Massachusetts) and *Apa*I endonuclease (20U/µl)[20,21] (New England Biolab, Massachusetts) for Enterobacterales and Gram-negative non-Enterobacterales respectively. DNA fragments were separated by electrophoresis in 1% agarose gel and dendrogram was generated by FPQuest version 4.5 Software (Bio-Rad Laboratories, Hercules, CA, USA) using UPGMA (unweighted pair group method with arithmetic mean) method with bandwidth tolerance and optimization values set to 1.5%. The isolates with dice similarity index ≥90% were considered to be identical and placed within same PFGE cluster.

Transmissibility of carbapenem-resistant determinants

To assess the transferability of bla_{NDM} among bla_{NDM}-possessing isolates (Enterobacterales/ Gram-negative non-Enterobacterales), a solid-mating conjugation assay was performed with azide-resistant *Escherichia coli* J53 as recipient[22,20] at 37°C for 18hrs. For certain isolates in which transfer was unsuccessful, conjugation assays were conducted at 30°C, as reported in earlier studies[22]. Transconjugants were selected on LB agar plates supplemented with either cefoxitin (10

mg/L) (Sigma-Aldrich, St. Louis, MO, USA) or ertapenem (0.5 mg/L) or meropenem (0.25 or 0.5 mg/L) and sodium azide (100 mg/L) (Sigma-Aldrich, St. Louis, MO, USA) to assess the transmissibility of bla_{NDM} or $bla_{OXA-181}$. bla_{NDM} can hydrolyse all the mentioned antibiotics but $bla_{OXA-181}$ hydrolyses ertapenem and not cefoxitin. Transconjugants were screened by PCR for bla_{NDM} and other resistance genes[19], followed by determination of MIC for meropenem and ertapenem. PCR-based replicon typing (PBRT) was carried out to assess replicon types in the transconjugants.[23]

Whole genome sequencing (WGS)

All study isolates underwent short-read (paired-end) sequencing on the Illumina NovaSeq 6000 platform. For short-read sequencing, the total bacterial genomic DNA was extracted using Wizard Genomic DNA purification kit (Promega, Spain) and paired-end libraries (2x150bp) were constructed using Nextera XT Kit (Illumina, San Diego, CA, USA) for carbapenem-resistant isolates.

Long-read sequencing on the nanopore PromethION platform using PromethION flow cell (FLO-PRO114M) (Nanopore, Oxford, UK) was additionally performed for 25 Enterobacterales based on (i) one representative from each clonal cluster of PFGE, (ii) diverse plasmid sequence types (pSTs) identified from short-read data. *A. baumannii* were excluded due to differences in bla_{NDM} plasmid contexts from Enterobacterales. For long-read sequencing, bacterial DNA from overnight cultures were extracted using DNeasy Blood and Tissue Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Quantification and purity of the samples were performed using Nanodrop and Qubit HS DNA kit (Thermo fisher scientific, USA). The Ligation Sequencing Kit (ONT, UK) or NEBNext Ultra II End Repair Kit (New England Biolabs, MA, USA) was used for preparing sequencing libraries from dsDNA. Base calling of the Nanopore

Signal Dataset was performed using Guppy (v.5.0.1) (https://timkahlke.github.io/) and long-read quality check was performed using LongQC (https://github.com/yfukasawa/LongQC). Short-reads were trimmed using fastp (v.023.2) (https://github.com/OpenGene/fastp) with default parameters and long-reads were trimmed using nanoq (v.0.10.0) (--trim-end 20 --trim-start 20 -l 200 -q 10) (https://github.com/esteinig/nanoq). For isolates with short-reads only, trimmed fastq were assembled into contigs using shovill (v.0.9.0) (https://github.com/tseemann/shovill). For isolates with both long- and short-reads, two approaches were performed to assess genome assembly metrics. Firstly, unicycler (v.0.5) (https://github.com/rrwick/Unicycler) was used with both --conservative mode and --normal mode providing two draft genomes. Secondly, flye (v.2.9.0) (https://github.com/mikolmogorov/Flye) was used to assemble reads to contigs followed by one round of medaka (v.1.11.3) polishing (https://github.com/nanoporetech/medaka) and one round of pypolca (v.0.3.1) (https://github.com/gbouras13/pypolca) polishing with default parameters. All assemblies were assessed using QUAST (v.5.0.2) (https://github.com/ablab/quast) to exclude hyper-fragmented genomes (>500 contigs) and/or genomes with >10% plus the expected genome size.

Bioinformatic analysis

Genomes were annotated using Bakta (v.1.9.3) (https://github.com/oschwengers/bakta). Different pipelines were used for analysis of whole genomes- i) MLST (v.2.0) (https://cge.food.dtu.dk/services/MLST/) for multilocus sequence typing (MLST): [Pasteur scheme (*K. pneumoniae*, *A. baumannii*, *Enterobacter hormaecheii*); warwick scheme (*E. coli*)]; ii) clermontyping (http://clermontyping.iame-research.center/) for detection of phylogroups in *E. coli* while BIGSdb-Pasteur website (https://bigsdb.pasteur.fr/cgibin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef&page=sequenceQuery) for determination of phylogroups in *K.*

pneumoniae; iii) Resfinder (v.4.5.0) (http://genepi.food.dtu.dk/resfinder) for acquired resistance genes iv) PlasmidFinder (v.2.1) (https://cge.food.dtu.dk/services/PlasmidFinder/) for plasmid replicons, v) pMLST (v.2.0) (https://cge.food.dtu.dk/services/pMLST/) for plasmid MLST (pMLST); vi) Mobile Element Finder (v.1.0.3) (https://cge.food.dtu.dk/services/MobileElementFinder/) for determination of IS elements and transposons within the study plasmids. The genetic environment of carbapenemases such as bla_{NDM} and $bla_{OXA-181}$ were evaluated through SnapGene viewer tool (v.7.1) (https://www.snapgene.com/snapgene-viewer) and were restricted to the size of the contigs harboring the carbapenemases.

Phylogenetic analysis

The genetic relationship between the isolates was illustrated by the construction of phylogenetic tree based on single nucleotide polymorphism (SNP) calling using CSI Phylogeny (v.1.4) (https://cge.food.dtu.dk/services/CSIPhylogeny/). The complete genome sequences of *Escherichia coli* K-12 strain MG1655 (GCF_904425475.1), *Klebsiella pneumoniae* strain HS11286 (GCF_000240185.1) and *Acinetobacter baumannii* ATCC17978 (GCF_902728005.1) were used as reference genomes for *E. coli*, *K. pneumoniae* and *A. baumannii* isolates, respectively. The resulting phylogenetic trees were visualized using the Interactive Tree of Life (iTOL) platform, and presence of bla_{NDM} variants were annotated onto the trees using the iTOL editor (v.1.1).

Analysis of bla_{NDM}-carrying plasmids

The extracted plasmids from hybrid assemblies were assessed using MOB-typer (v.3.1.8) (https://github.com/jrober84/mob-typer). The plasmids were annotated using RAST webserver (https://rast.nmpdr.org/). Detection of origin of transfer (*oriT)* region, other transfer-related modules, including the genes encoding relaxases, type IV coupling proteins (T4CP) and type IV

secretion system (T4SS) on *bla*$_{NDM}$ carrying plasmids were performed using oriTfinder (v.1.1) (http://bioinfo-mml.sjtu.edu.cn/oriTfinder) and ICEberg (v.3.0) (https://tool2-mml.sjtu.edu.cn/ICEberg3/). IS elements associated with *bla*$_{NDM}$ were identified using ISfinder (https://isfinder.biotoul.fr/about.php). Comparative BLAST+ analysis of *bla*$_{NDM}$ study plasmids was performed using Genome-to-Genome Distance Calculator (GGDC) (v.4.0) (https://ggdc-test.dsmz.de/ggdc.php) using the recommended formula 2 (identities/high-scoring pair lengths). For comparison of *bla*$_{NDM}$-harboring plasmids, study *bla*$_{NDM}$ plasmids were aligned with the similar plasmids having highest percentage of nucleotide identity available in the bacterial plasmid database (PLSDB) (v.2024_05_31_v2). These plasmids were then compared amongst themselves using BLAST Ring Image Generator (BRIG) (v.0.95) tool**.** Plasmids with similar genetic context, plasmid size and typing, whole plasmid nucleotide sequences were aligned for pairwise similarity with MUMmer (nucmer) (v.4.0.1). The --maxmatch, delta-filter and show-coords parameters were used to calculate summary statistics including aligned length, percent identity and coverage.

Data availability

All bacterial genomes were deposited into NCBI GenBank database under the Bioproject number: PRJNA939406. IN-MR210EC was submitted with the accession number JAYKKU000000000.

Results

Carbapenem-resistant bacteria in maternal and neonatal gut: distribution, antimicrobial susceptibility and genotypic characterization

The study comprises 86 pregnant mothers delivering 93 neonates (seven twin births) with suspected sepsis (Figure S1). Carbapenemases (NDM and/or OXA-48) were detected in maternal rectal (MR) samples (n=20; 23%) and neonatal rectal (BR) samples (n=42; 45%). Of them, 80

isolates (MR=29 & BR=51) possessing either *bla*$_{NDM}$ or *bla*$_{OXA-48}$ were recovered. Most mothers and neonates were colonized with *bla*$_{NDM}$ and/or *bla*$_{OXA-48}$-possessing *E. coli* (MR=13; BR=21), *K. pneumoniae* (MR=8; BR=15) and *A. baumannii* (MR=6; BR=7). Apart from these, other organisms producing *bla*$_{NDM}$ or *bla*$_{OXA-48}$ were also detected (Figure S1). Amongst them, only those MR (n=7) and BR (n=10) which were co-colonized with multiple carbapenem-resistant species harboring *bla*$_{NDM}$ were considered for further analysis. Altogether, 37 carbapenem-resistant Gram-negative bacteria (16 from 7 MR & 21 from 10 BR) were recovered from 17 patients which were distributed as follows: 16 & 20 *bla*$_{NDM}$-positive isolates from 7 mothers and 10 neonates respectively and one isolate carried *bla*$_{VIM-2}$ obtained from a neonate (Figure S1, Figure 1 & Table 1). Additionally, 6 isolates co-harbored *bla*$_{OXA-181}$ with *bla*$_{NDM}$ (Figure 1, Table 1 & Figure S3.f).

The 37 carbapenem-resistant isolates were distributed as follows: 29 carbapenem-resistant Enterobacterales (CREs)- *E. coli* (EC) (n=14), *K. pneumoniae* (KP) (n=14) and *Enterobacter hormaechei* (EH) (n=1), and 8 carbapenem-resistant non-fermenters (CRNFs) comprising *A. baumannii* (AB) (n=7) and *Pseudomonas mosellii* (PM) (n=1) (Figure 1, Table 1). Most isolates exhibited MDR phenotype, showing resistance to carbapenems, third and fourth-generation cephalosporins and fluoroquinolones (Table 1). Few isolates were susceptible to tigecycline and fosfomycin as depicted in Table 1. MIC values for meropenem and ertapenem were distributed between 16-128 mg/L and 8-512 mg/L, respectively (Table 1).

The most frequent carbapenemase detected in these isolates was *bla*$_{NDM,}$ followed by *bla*$_{OXA-48}$ with no *bla*$_{KPC}$ (Figure 1 & Table 1).Four *bla*$_{NDM}$ variants were detected: *bla*$_{NDM-1}$ (n=16), *bla*$_{NDM-4}$ (n=1), *bla*$_{NDM-5}$ (n=14), *bla*$_{NDM-1+NDM-5}$ (n=1) and *bla*$_{NDM-7}$ (n=4). *bla*$_{NDM-5}$ predominated in *E. coli* (12/14) whereas *bla*$_{NDM-1}$ in *K. pneumoniae* (8/14). ABs and EH possessed only *bla*$_{NDM-1}$. Few

252 samples (MR=3; BR=1) were co-colonized with different species having same *bla*$_{NDM}$-variants,
253 while others (MR=4; BR=9) harboured multiple species with different *bla*$_{NDM}$-variants (Figure 1).

254 Isolates also possessed ESBLs, AmpC β-lactamases, aminoglycoside & fluoroquinolone-
255 resistant genes, *bla*$_{OXA-23/51}$ variants and other resistance determinants in various combinations
256 (Table 1). The data mentioned in Table 1 for the isolates MR16KP, MR774KP, BR16KP &
257 BR548KP has already been presented in previous publication[17] but in a different context.

258 **PFGE, STs and phylogroups of carbapenem-resistant isolates**

259 PFGE revealed that *bla*$_{NDM}$-producing ECs, KPs and ABs were diverse (Figure 2). Six ECs formed
260 a single cluster (A), while KPs formed three clusters (B-D) composed of 2-3 identical isolates and
261 ABs with one cluster (E) comprising two isolates (Figure 2). Since for EH and PM only one
262 isolate was obtained, molecular typing for comparison of clones was not carried out for them.

263 ECs were distributed to seven distinct STs, primarily ST167 (n = 7) and ST448 (n = 2) and
264 one isolate each of other STs (Table 1; Figure 2A). Similarly, KPs were distributed into 8
265 different STs such as ST147 and ST889 (n=3) followed by ST1310 (n=2), along with other STs
266 each comprising of a single isolate (Table 1; Figure 2B). EH belonged to ST231 (Table 1).
267 Likewise, ABs belonged to 6 diverse STs (Table 1; Figure 2C). PM could not be typed due to
268 absence of a reference MLST scheme.

269 The ECs primarily belonged to phylogroup A (8/14) and KPs, on the other hand, belonged
270 to phylogroup Kp1 only (Table 1, Figure 2).

271 **Phylogenomic insights into maternal and neonatal gut bacteria**

272 The core-genome phylogenetic analysis also revealed diversity of genomes with few clusters
273 isolated. *bla*$_{NDM}$-producing ECs, KPs and ABs were found to form clusters in respective
274 phylogenetic trees based on STs. Among ECs, 2 clusters belonging to ST167 & ST448 and

amongst KPs, four different clusters- ST147, ST1310, ST15 and ST889 were observed, while other isolates were distinct (Figure 3). Similarly, in ABs, only a single cluster (ST1) was found (Figure 3). For all the trees, the clustering of STs matched with the clusters depicted in PFGE (Figure 2).

Assessment of transmissibility of *bla*$_{NDM}$ plasmid via conjugation

Conjugation assays of 36 *bla*$_{NDM}$-harboring CRE & CRNF isolates showed successful transfer of *bla*$_{NDM}$ in 23 CRE isolates (12 ECs, 11 KPs). The remaining 6 CRE isolates and CRNFs (7 ABs) failed to transfer *bla*$_{NDM.}$ Most of the CREs (n=20) showed successful conjugation at 37^{o}C, while three CRE isolates (IN-MR774EC, IN-BR919EC, and IN-BR1285KP) could transfer *bla*$_{NDM}$ at 30^{o}C. Several antibiotic-resistant determinants such as *bla*$_{CTX-M}$, *bla*$_{TEM}$, *rmtB*, *bla*$_{CIT}$, *oqxAB*, *aac(6')-Ib* and *aac(6')-Ib-cr* were co-transferred along with *bla*$_{NDM}$ in the transconjugants in different combinations (Table 2). Successful co-transfer of *bla*$_{OXA-181}$ and *bla*$_{NDM}$ (n=5) were noted except for one isolate i.e. IN-BR244EC (Table 2).

Among 23 NDM-positive CRE-transconjugants, 16 carried multiple replicons while others had a single replicon type. Diverse incompatibility groups were detected, predominantly IncF-type plasmids [IncFIA-IncFIB-IncFII (7/15) being most common] in the transconjugants, most of which were transferred successfully (Table 2). Further, the presence of complete conjugation modules (*oriT*, *tra*, *trb*, *vir*) noted from WGS data, validated self-transferability of IncF plasmids (Table 3). NDM-positive transconjugants exhibited increased MIC values ranging from 4-64 mg/ L (meropenem) and 16-256 mg/L (ertapenem) (Table 2).

Diversity and characterization of *bla*$_{NDM}$-positive plasmids

Based on PFGE and short-read data, 25 representative CREs underwent long-read sequencing. Hybrid assemblies precisely identified *bla*$_{NDM}$-bearing plasmids in 20 of the 25 sequenced isolates,

while four (IN-MR16EC, IN-BR26EC, IN-MR1225KP, and IN-BR1285KP) were excluded due to poor assembly quality and one (IN-MR165EH) carried chromosomal *bla*$_{NDM}$ (Table 2 & 3). The *bla*$_{NDM}$ gene was associated with six plasmid replicon types (46-271kb), predominantly IncF (83-240kb) with diverse pMLSTs, mainly multi-replicon IncFIA-FIB-FII plasmids (n=7) (Figure S2 & Table 3). Patients co-colonized with multiple CREs mostly harboured distinct *bla*$_{NDM}$-variants and plasmid types, indicating limited evidence for *in vivo* plasmid transfer, except one case (BR499) showing probable transfer of *bla*$_{NDM}$-plasmids among co-colonized isolates (Table 1 & Table 3).

Genetic context of *bla*$_{NDM}$ and *bla*$_{OXA-181}$

bla$_{NDM-1/4/5/7}$ was commonly associated with IS*Aba125* at the 5'-end and *ble*$_{MBL}$ at the 3'-end. Most *bla*$_{NDM-5}$-possessing isolates (n=11) carried a common *bla*$_{NDM-5}$ region (IS*26*-ΔIS*Aba125*-*bla*$_{NDM-5}$-*ble*$_{MBL}$-*trpF*-*dsbD*-IS*91*-like IS*CR1*-*sul1*-*qacE*-*aadA*-*DUF1010*-*dfrA12*-*intI1*-IS*26*) surrounded by two copies of IS*26* with the same orientation (Figure S3.a). The genetic context of *bla*$_{NDM-5}$ was comparable among the neonates and resembled that of *bla*$_{NDM-4}$ (Figure.S3.a-b). The immediate genetic environment of *bla*$_{NDM-1}$ was similar among CREs with a truncated IS*Aba125* upstream (Figure.S3.c). However, a variation was observed in pKP-MR210-NDM-1-plasmid, where *bla*$_{NDM-1}$ was flanked by IS*3000* embedded in a Tn*3000* composite transposon arranged in three-tandem copies on the same plasmid extracted from hybrid assembly (Figure.S3.c). The genetic background of *bla*$_{NDM-7}$ was similar to *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$, with an inclusion of IS*5* between IS*Aba125* and *bla*$_{NDM-7}$ (Figure.S3.d). In comparison to CREs, ABs had an intact IS*Aba125* upstream of *bla*$_{NDM-1}$ (Figure. S3.e).

Transmission dynamics of *bla*$_{NDM}$-bearing plasmids amongst CRE isolates

Transmission of *bla*$_{NDM}$-bearing plasmids was assessed primarily within the gut microbiota of an individual but also across samples from different individuals. Hybrid assembly revealed the

presence of *bla*$_{NDM}$ on diverse replicons. Comparison of *bla*$_{NDM}$-bearing plasmids among co-colonized isolates from the same individual revealed either the presence of different replicons, or, when replicons were similar, differences in pMLST were noted. These findings suggest that horizontal transfer of *bla*$_{NDM}$-carrying plasmids between co-colonized isolates does not appear to be taking place, except one patient (BR499) co-colonized with EC & KP. These isolates possessed *bla*$_{NDM-5}$ with plasmid backbones (IncFIA-IncFIB-IncFII; 144kb [pEC-BR499-NDM-5] and IncFII; 97kb [pKP-BR499-NDM-5]). Overall, 73kb aligned with a 97.55% identity between the two IN-BR499 plasmids. The genetic context surrounding *bla*$_{NDM-5}$ in EC was similar to that of pKP-BR499-NDM-5, indicating either a possible transposition-like exchange of *bla*$_{NDM}$-bearing gene cassette between these two plasmids within the patient (Figure 4a), or the co-presence of two plasmids within similar plasmid backbone.

Additionally, *bla*$_{NDM}$-bearing plasmids across mothers and neonates were compared. IN-BR302KP (ST147) and IN-BR707KP (ST889), despite belonging to different STs, revealed extremely high concordance of nucleotide similarity of both *bla*$_{NDM-1}$ plasmids (110kb), with 110,700bp aligned (99.98-100% coverage of reference-query pairwise) between both plasmids at 99.97% identity (Figure 4b). Likewise, two *E. coli* isolates belonging to different STs, IN-BR244EC (ST448) and IN-BR1045EC (ST692), exhibited comparable *bla*$_{NDM-5}$-bearing plasmid backbones with 63kbp of the sequences aligned at 99% identity (Figure 4b).

Apart from plasmid-mediated transmission, clonal transmission was also evaluated. *bla*$_{NDM}$-positive ECs (ST167) and KPs (ST147, ST889 and ST1310) isolated from different mothers and neonates shared genomic similarities, indicating clonal transmission of drug-resistant bacteria (Figure 5). Clonal isolates were detected mostly in neonates compared to mothers, indicating acquisition of bacteria from the hospital environment.

Discussion

This study primarily aimed to evaluate the transmission of ARGs amongst species present in the rectal specimens of mothers and neonates. The *bla*$_{NDM}$ gene is a promiscuous gene which can be easily transmitted between different species and is also endemic in the Indian subcontinent. The previous BARNARDS study[16] estimated carriage of this gene in mothers and neonates but did not evaluate co-colonized carbapenem-resistant isolates or the possibility of interspecies transmission.

When the rectal samples of mothers and neonates were analyzed, a small percentage had multiple species possessing *bla*$_{NDM}$ (mothers= 7/86, 8%; neonates= 10/93, 11%). Although this percentage was small, the presence of *bla*$_{NDM}$ in diverse communities of MDR pathogens such as *E. coli*, *K. pneumoniae* and *A. baumannii* indicates the high prevalence of *bla*$_{NDM}$-possessing species in India, including in vulnerable neonates. A previous study documented co-colonisation of multiple carbapenemase-producing Enterobacterales in *bla*$_{OXA-48}$ endemic regions.[12] Overall carriage of *bla*$_{NDM}$ in neonates was much higher than that of mothers (42% for neonates and 8% for mothers)[16] probably due to their longer hospital stay leading to acquisition of drug-resistant strains from the hospital environment. The presence of indistinguishable strains (mostly ST167, ST147 & ST889) among different neonates indicated their acquisition from the hospital environment.

The predominant species carrying *bla*$_{NDM}$ in the gut were *E. coli* and *K. pneumoniae* which corroborated with previous studies.[14,24,25,26,27] Overall, the primary *bla*$_{NDM}$-variants noted in this study was *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$, *bla*$_{NDM-5}$ was prevalent in the gut of neonates. However, no organism could be isolated from the blood culture of these 10 study neonates and correlation of the blood & gut organism was not possible. Earlier studies[28,29] have shown translocation of resistant organisms from gut to the blood probably due to pristine and immature gut of neonates,

especially those who had low-birth weight and were premature. Additionally, the genetic context of *bla*$_{NDM}$ and the replicon types associated with *bla*$_{NDM}$ was similar to earlier reports of isolates found in blood of neonates[20,30,31] indicating circulation of these strains in the population.

bla$_{NDM}$-harbouring *E. coli* and *K. pneumoniae* belonged to diverse STs, which implied that the spread of *bla*$_{NDM}$ was not linked to any particular clonal lineage as has also been observed in other studies.[22,27] Some isolates belonged to high-risk epidemic clones such as ST11, ST15 & ST147 for *K. pneumoniae*[32] and ST101, ST167 and ST648 for *E. coli.*[33] An earlier study from this unit also reported prevalence of *bla*$_{NDM}$-possessing ST167 clone causing sepsis in neonates.[30] Analysis of *E. coli* phylogroups primarily indicated predominance of phylogroup A and B1 which are known to be commensals.[34] In case of *K. pneumoniae*, Kp1 was the dominant phylogroup as also previously reported to be present in the gut.[35] Similarly, *A. baumannii* belonged to diverse STs, of which ST1 (a major international clone)[36] exhibited limited reports of its colonization in the human gut. Other STs such as ST2, ST25, ST32 and ST149 found in this study were previously reported in clinical *A. baumannii* isolates.[37]

Most *bla*$_{NDM}$-harboring plasmids were conjugative as confirmed by conjugation experiments and WGS data. *bla*$_{NDM}$-positive plasmids were diverse in terms of replicon types (six different plasmid types predominantly IncFIA-FIB-FII), pMLST, and backbones. Interspecies transmission of *bla*$_{NDM}$ in co-colonized isolates in an individual gut sample or different gut samples could not be confirmed in most cases as the sequenced plasmids were distinct with no sequence similarities suggesting independent acquisition of *bla*$_{NDM.}$ However, one case of probable intra-patient transmission, wherein co-colonized isolates (BR499EC,KP) harboured *bla*$_{NDM-5}$ in significantly similar plasmids and two probable cases of inter-patient transmission of *bla*$_{NDM}$ plasmids [(BR707KP & BR302KP; *bla*$_{NDM-1}$ and BR244EC & BR1045EC; *bla*$_{NDM-5}$)] indicated by

a high degree of overlapping regions between plasmid backbones. A previous study[5] showed trans-acquisition of *bla*$_{NDM}$ via plasmids among four patients co-colonized with multiple species possessing *bla*$_{NDM}$ but did not perform long-read sequencing of plasmids, as done in this study.

The plasmid sequence in co-colonized isolates mostly did not match but it was observed that the genetic context of *bla*$_{NDM-5}$ was similar i.e., bracketed between IS*26* pseudo-compound transposon across several isolates from different patients. IS*26*, a composite transposon, plays a crucial role in the evolution of *bla*$_{NDM}$-harboring plasmids by co-integrating different resistance cassettes.[38,39] In this study, presence of different resistance genes such as *bla*$_{TEM}$, *rmtB* have been noted on *bla*$_{NDM}$ plasmids which might have been due to cointegration of MDR cassette with the study plasmids with aid from IS*26*.[38] Previous studies[40,41] have reported the association of Tn*3000* with *bla*$_{NDM-1}$, but this study for the first time reports presence of 3 tandem repeats of Tn*3000* in a single *bla*$_{NDM-1}$-bearing plasmid. The immediate genetic environment of *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ was found to be similar, despite having variations in their plasmid backbones, indicating the circulation of IS*Aba125* in this population.

BARNARDS study primarily focused on the carriage of carbapenem-resistant bacteria and gut samples were selected on ertapenem-supplemented media. This led to the selection of only carbapenem-resistant isolates and do not reflect the true heterogeneity of the gut microbiome. Transmission of other endemic genes such as *bla*$_{CTX-M}$ could not be studied. In addition, samples were collected at one point of time and subsequent sampling was not conducted. This being the limitation of the study, probably led to inadequate cases of ARG transmission. The study utilized long-read sequencing to assess the similarity of *bla*$_{NDM}$-harboring plasmids which enhanced the understanding of transmission of the plasmids. Previous studies have shown transmission of ARGs among co-colonized bacteria in the gut involving either *bla*$_{NDM}$[14,15], or *bla*$_{OXA-48}$[11,12] or

413 bla_{KPC}[14] but not in pregnant mothers and neonates. This study reports a probable case of *in vivo*
414 transfer of bla_{NDM} based on sequence similarities of plasmids and two cases of interpatient
415 transmission of bla_{NDM}, further supported by conjugation assay results. The presence of multiple
416 CREs co-colonised in otherwise healthy mothers and hospitalized neonates is worrisome. Such
417 CREs carrying different bla_{NDM} variants and other resistance genes in gut of mothers reflects
418 circulation of such strains in the community. The circulation of such bla_{NDM} plasmids can also be
419 driven by the antibiotics other than the carbapenems because of presence of other resistance genes
420 such as *qnrB*, *qnrS*, *rmtB*, bla_{CTX-M} on bla_{NDM} plasmids[42]. The co-colonization of multiple species
421 in neonates with prolonged hospital stay suggests independent nosocomial acquisitions of bla_{NDM}.
422 The presence of such isolates in septicemic neonates, some of which are epidemic clones also
423 increases the risk of translocation of such isolates.

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433 **Transparency declarations**

434 None to declare

435 **References**

1. Aslam B, Wang W, Arshad MI, *et al.* Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 2018; **11**: 1645–58.

2. Codjoe F, Donkor E. Carbapenem Resistance: A Review. *Med Sci* 2017; **6**: 1.

3. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM Metallo-β-Lactamases and Their Bacterial Producers in Health Care Settings. *Clin Microbiol Rev* 2019; **32**: e00115-18.

4. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clin Microbiol Rev* 2018; **31**: e00088-17.

5. Wailan AM, Sartor AL, Zowawi HM, Perry JD, Paterson DL, Sidjabat HE. Genetic Contexts of $bla_{\text{NDM-1}}$ in Patients Carrying Multiple NDM-Producing Strains. *Antimicrob Agents Chemother* 2015; **59**: 7405–10.

6. Mitra S, Naha S, Chakraborty J, *et al.* Diversity of mobile genetic elements in carbapenem-resistant Enterobacterales isolated from the intensive care units of a tertiary care hospital in Northeast India. *Front Microbiol* 2025; **16**: 1543427.

7. Acman M, Wang R, Van Dorp L, *et al.* Role of mobile genetic elements in the global dissemination of the carbapenem resistance gene blaNDM. *Nat Commun* 2022; **13**: 1131.

8. Tilahun M, Kassa Y, Gedefie A, Belete MA. Emerging Carbapenem-Resistant Enterobacteriaceae Infection, Its Epidemiology and Novel Treatment Options: A Review. *Infect Drug Resist* 2021; **14**: 4363–74.

9. van Schaik W. The human gut resistome. *Philos Trans R Soc B Biol Sci* 2015; **370**: 20140087.

10. McInnes RS, McCallum GE, Lamberte LE, van Schaik W. Horizontal transfer of antibiotic resistance genes in the human gut microbiome. *Curr Opin Microbiol* 2020; **53**: 35–43.

11. Kocer K, Boutin S, Dalpke AH, Heeg K, Mutters NT, Nurjadi D. Comparative genomic analysis reveals a high prevalence of inter-species *in vivo* transfer of carbapenem-resistance plasmids in patients with haematological malignancies. *Clin Microbiol Infect* 2020; **26**: 780.e1-780.e8.

12. Hernández-García M, Pérez-Viso B, Navarro-San Francisco C, *et al.* Intestinal co-colonization with different carbapenemase-producing Enterobacterales isolates is not a rare event in an OXA-48 endemic area. *EClinicalMedicine* 2019; **15**: 72–9.

13. Marí-Almirall M, Ferrando N, Fernández MJ, *et al.* Clonal Spread and Intra- and Inter-Species Plasmid Dissemination Associated With *Klebsiella pneumoniae* Carbapenemase-Producing Enterobacterales During a Hospital Outbreak in Barcelona, Spain. *Front Microbiol* 2021; **12**: 781127.

14. Ji J, Zhu Y, Zhao F, *et al.* Co-colonization of different species harboring KPC or NDM carbapenemase in the same host gut: insight of resistance evolution by horizontal gene transfer. *Front Microbiol* 2024; **15**: 1416454.

15. Li X, He J, Yu Y, Zhou H, Tu Y, Hua X. Dynamic evolution and inter-species transfer of *bla*$_{NDM-5}$ plasmid *in vivo* in a single patient. *Clin Microbiol Infect* 2023; **29**: 265–8.

16. Carvalho MJ, Sands K, Thomson K, *et al.* Antibiotic resistance genes in the gut microbiota of mothers and linked neonates with or without sepsis from low- and middle-income countries. *Nat Microbiol* 2022; **7**: 1337–47.

17. Basak P, Naha S, Sands K, *et al.* Genomic similarity of carbapenem-resistant Enterobacterales collected from mothers and their neonates. *J Glob Antimicrob Resist* 2025; **44**: 166–79.

18. Versalovic J, Koeuth T, Lupski R. Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial genomes. *Nucleic Acids Res* 1991; **19**: 6823–31.

19. Mukherjee S, Bhattacharjee A, Naha S, *et al.* 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. *Infect Genet Evol* 2019; **69**: 166–75.

20. Chatterjee S, Datta S, Roy S, *et al.* Carbapenem Resistance in *Acinetobacter baumannii* and Other *Acinetobacter spp.* Causing Neonatal Sepsis: Focus on NDM-1 and Its Linkage to IS*Aba125*. *Front Microbiol* 2016; **7**. Available at: http://journal.frontiersin.org/Article/10.3389/fmicb.2016.01126/abstract. Accessed December 31, 2022.

21. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y. Multidrug-Resistant *Acinetobacter baumannii*. *Emerg Infect Dis* 2005; **11**: 22–9.

22. Datta S, Mitra S, Chattopadhyay P, Som T, Mukherjee S, Basu S. Spread and exchange of *bla* $_{NDM-1}$ in hospitalized neonates: role of mobilizable genetic elements. *Eur J Clin Microbiol Infect Dis* 2017; **36**: 255–65.

23. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

24. Kumar A, Mohapatra S, Bir R, *et al.* Intestinal Colonization Due to Carbapenem-Resistant Enterobacteriaceae Among Hematological Malignancy Patients in India: Prevalence and Molecular Charecterisation. *Indian J Hematol Blood Transfus* 2022; **38**: 1–7.

25. Deng X, Wang S, Hou P, *et al.* Fecal carriage and molecular characterization of carbapenem-resistant Enterobacteriaceae from hospitalized children in a tertiary hospital of Shandong, China. *Front Microbiol* 2025; **16**: 1542207.

26. Xu Q, Pan F, Sun Y, *et al.* Fecal Carriage and Molecular Epidemiology of Carbapenem-Resistant Enterobacteriaceae from Inpatient Children in a Pediatric Hospital of Shanghai. *Infect Drug Resist* 2020; **13**: 4405–15.

27. Pan F, Tian D, Wang B, *et al.* Fecal carriage and molecular epidemiology of carbapenem-resistant Enterobacteriaceae from outpatient children in Shanghai. *BMC Infect Dis* 2019; **19**: 678.

28. Carl MA, Ndao IM, Springman AC, *et al.* Sepsis From the Gut: The Enteric Habitat of Bacteria That Cause Late-Onset Neonatal Bloodstream Infections. *Clin Infect Dis* 2014; **58**: 1211–8.

29. Das P, Singh AK, Pal T, Dasgupta S, Ramamurthy T, Basu S. Colonization of the gut with Gram-negative bacilli, its association with neonatal sepsis and its clinical relevance in a developing country. *J Med Microbiol* 2011; **60**: 1651–60.

30. Bhattacharjee A, Sands K, Mitra S, *et al.* A Decade-Long Evaluation of Neonatal Septicaemic *Escherichia coli*: Clonal Lineages, Genomes, and New Delhi Metallo-Beta-Lactamase Variants Papp-Wallace KM, ed. *Microbiol Spectr* 2023; **11**: e05215-22.

31. Datta S, Roy S, Chatterjee S, *et al.* A Five-Year Experience of Carbapenem Resistance in Enterobacteriaceae Causing Neonatal Septicaemia: Predominance of NDM-1 Vadivelu J, ed. *PLoS ONE* 2014; **9**: e112101.

32. Arcari G, Carattoli A. Global spread and evolutionary convergence of multidrug-resistant and hypervirulent *Klebsiella pneumoniae* high-risk clones. *Pathog Glob Health* 2023; **117**: 328–41.

33. Kocsis B, Gulyás D, Szabó D. Emergence and Dissemination of Extraintestinal Pathogenic High-Risk International Clones of *Escherichia coli*. *Life* 2022; **12**: 2077.

34. Das P, Singh AK, Mukherjee S, *et al.* Composition of *Escherichia coli* population in the neonatal gut: phylogroups and virulence determinants. *J Med Microbiol* 2013; **62**: 1680–7.

35. Raffelsberger N, Hetland MAK, Svendsen K, *et al.* Gastrointestinal carriage of *Klebsiella pneumoniae* in a general adult population: a cross-sectional study of risk factors and bacterial genomic diversity. *Gut Microbes* 2021; **13**: 1939599.

36. Shelenkov A, Akimkin V, Mikhaylova Y. International Clones of High Risk of *Acinetobacter Baumannii*—Definitions, History, Properties and Perspectives. *Microorganisms* 2023; **11**: 2115.

37. Roy S, Chatterjee S, Bhattacharjee A, *et al.* Overexpression of Efflux Pumps, Mutations in the Pumps' Regulators, Chromosomal Mutations, and *aac(6')-Ib-cr* Are Associated With Fluoroquinolone Resistance in Diverse Sequence Types of Neonatal Septicaemic A*cinetobacter baumannii*: A 7-Year Single Center Study. *Front Microbiol* 2021; **12**: 602724.

38. Zhao Q-Y, Zhu J-H, Cai R-M, *et al.* IS*26* Is Responsible for the Evolution and Transmission of bla_{NDM}-Harboring Plasmids in *Escherichia coli* of Poultry Origin in China Yang Y-L, ed. *mSystems* 2021; **6**: 10.1128/msystems.00646-21.

39. Weber RE, Pietsch M, Frühauf A, *et al.* IS*26*-Mediated Transfer of bla_{NDM-1} as the Main Route of Resistance Transmission During a Polyclonal, Multispecies Outbreak in a German hospital. *Front Microbiol* 2019; **10**: 2817.

40. Campos JC, Da Silva MJF, Dos Santos PRN, *et al.* Characterization of Tn*3000*, a Transposon Responsible for bla_{NDM-1} Dissemination among Enterobacteriaceae in Brazil, Nepal, Morocco, and India. *Antimicrob Agents Chemother* 2015; **59**: 7387–95.

41. Berdichevski MKH, Guerra RR, Pereira DC, *et al.* Plasmidome analyses of *Klebsiella pneumoniae* co-producing *bla*$_{KPC-2}$ and *bla*$_{NDM-1}$ in Southern Brazil: characterisation of mobile genetic elements. *J Glob Antimicrob Resist* 2025; **42**: 214–21.

42. Mitra S, Mukherjee S, Naha S, Chattopadhyay P, Dutta S, Basu S. Evaluation of co-transfer of plasmid-mediated fluoroquinolone resistance genes and *bla*$_{NDM}$ gene in Enterobacteriaceae causing neonatal septicaemia. *Antimicrob Resist Infect Control* 2019; **8**: 46.

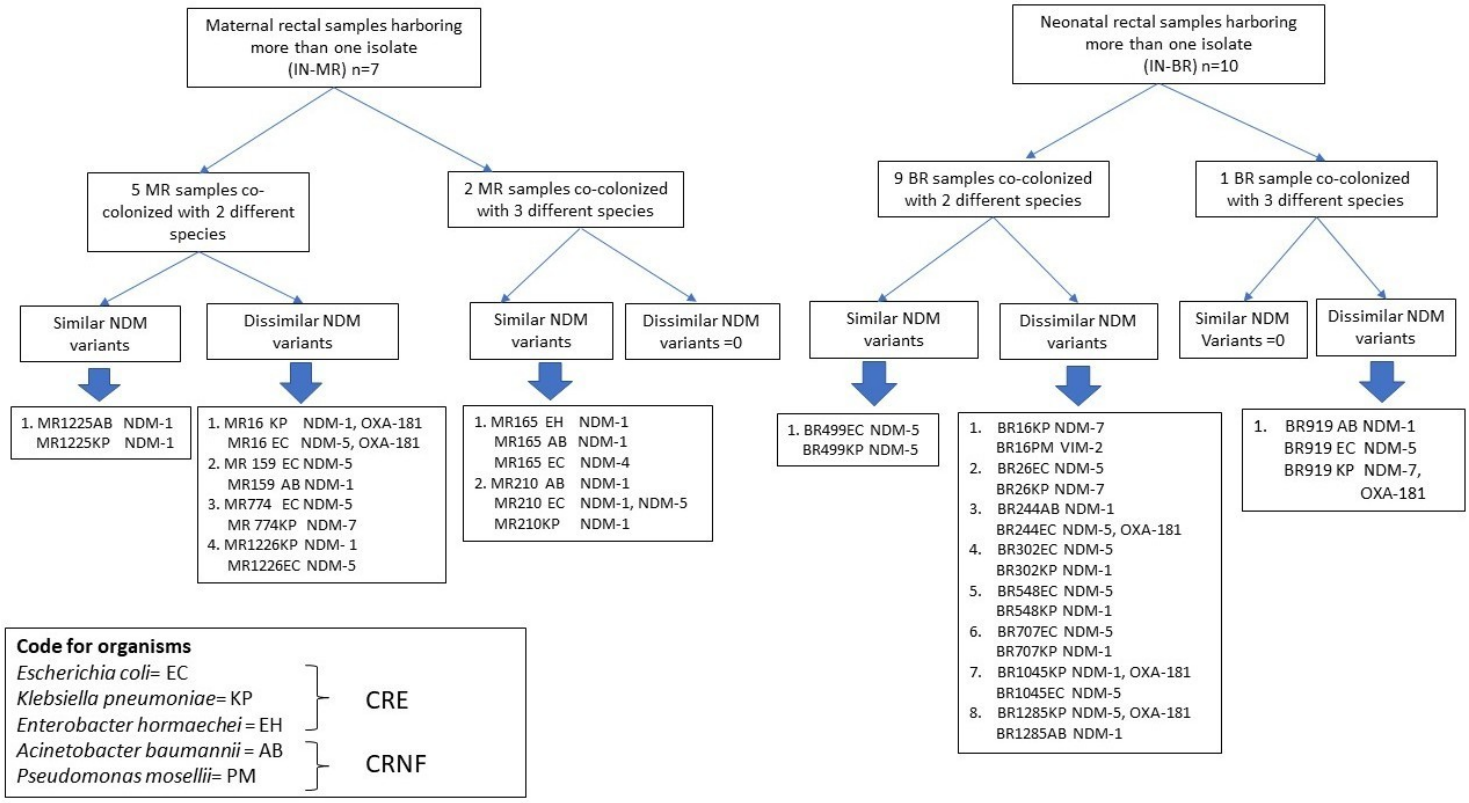
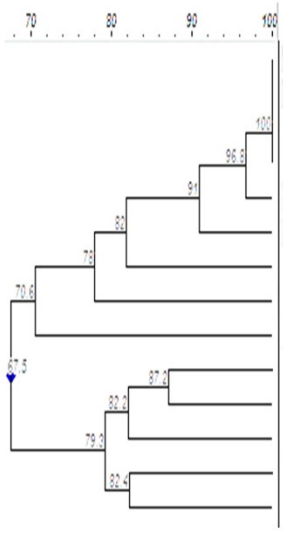


Figure 1. Overview of co-colonized patients with multiple carbapenem-resistant Gram-negative bacteria (CRE & CRNF) possessing similar or different *bla*$_{NDM}$-variants.

Abbreviations: CRE: carbapenem-resistant Enterobacterales, CRNF: carbapenem-resistant non-fermenters, IN-MR: Indian maternal rectal sample, IN-BR: Indian neonatal rectal sample

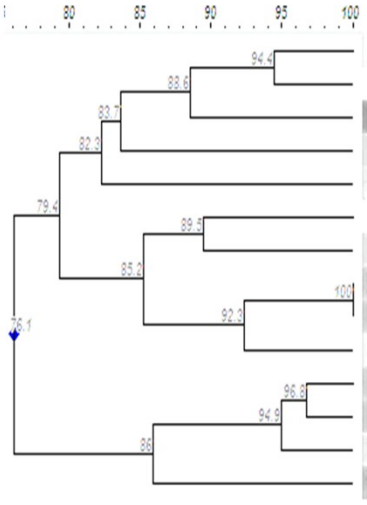
a



Escherichia coli isolates

Isolate Id	Cluster	Phylogroup	ST	*bla*$_{NDM}$ variants	*bla*$_{OXA-48}$ variants
IN-BR302EC	A	A	167	*bla* $_{NDM-5}$	0
IN-BR499EC		A	167	*bla* $_{NDM-5}$	0
IN-BR707EC		A	167	*bla* $_{NDM-5}$	0
IN-MR1226EC		A	167	*bla* $_{NDM-5}$	0
IN-BR548EC		A	167	*bla* $_{NDM-5}$	0
IN-BR919EC		A	167	*bla* $_{NDM-5}$	0
IN-BR26EC		A	167	*bla* $_{NDM-5}$	0
IN-MR165EC		A	11021	*bla* $_{NDM-4}$	0
IN-MR774EC		F	648	*bla* $_{NDM-5}$	0
IN-BR1045EC		C	692	*bla* $_{NDM-5}$	0
IN-MR16EC		C	2851	*bla* $_{NDM-5}$	*bla* $_{OXA-181}$
IN-MR210EC		B1	101	*bla* $_{NDM-1 \& NDM-5}$	0
IN-BR244EC		B1	448	*bla* $_{NDM-5}$	*bla* $_{OXA-181}$
IN-MR159EC		B1	448	*bla* $_{NDM-5}$	0

b



Klebsiella pneumoniae isolates

Isolate Id	Cluster	Phylogroup	ST	*bla*$_{NDM}$ variants	*bla*$_{OXA-48}$ variants
IN-BR16KP	B	Kp1	1310	*bla* $_{NDM-7}$	0
IN-BR26KP		Kp1	1310	*bla* $_{NDM-7}$	0
IN-MR16KP		Kp1	11	*bla* $_{NDM-1}$	*bla* $_{OXA-181}$
IN-BR1285KP		Kp1	16	*bla* $_{NDM-5}$	*bla* $_{OXA-181}$
IN-BR499KP		Kp1	15	*bla* $_{NDM-5}$	0
IN-BR1045KP		Kp1	1	*bla* $_{NDM-1}$	*bla* $_{OXA-181}$
IN-MR1225KP		Kp1	15	*bla* $_{NDM-1}$	0
IN-BR548KP	C	Kp1	889	*bla* $_{NDM-1}$	0
IN-BR707KP		Kp1	889	*bla* $_{NDM-1}$	0
IN-MR1226KP		Kp1	889	*bla* $_{NDM-1}$	0
IN-BR919KP	D	Kp1	147	*bla* $_{NDM-7}$	*bla* $_{OXA-181}$
IN-MR774KP		Kp1	147	*bla* $_{NDM-7}$	0
IN-BR302KP		Kp1	147	*bla* $_{NDM-1}$	0
IN-MR210KP		Kp1	395	*bla* $_{NDM-1}$	0

c

Acinetobacter baumannii isolates

Isolate Id	Cluster	ST	*bla*$_{NDM}$ variants
IN-BR919AB		2	*bla* $_{NDM-1}$
IN-MR1225AB		32	*bla* $_{NDM-1}$
IN-MR159AB		25	*bla* $_{NDM-1}$
IN-BR244AB		149	*bla* $_{NDM-1}$
IN-MR210AB		2244	*bla* $_{NDM-1}$
IN-BR1285AB	E	1	*bla* $_{NDM-1}$
IN-MR165AB		1	*bla* $_{NDM-1}$

559

Figure 2. Genetic relatedness of *bla*$_{NDM}$-producing bacteria isolated from maternal and neonatal gut. a) *E. coli* b) *K. pneumoniae* c) *A. baumannii* isolates. The isolates whose PFGE band patterns had a similarity of >90% and an optimization of 1.5 with a position tolerance of 1.5% were interpreted as clonal.

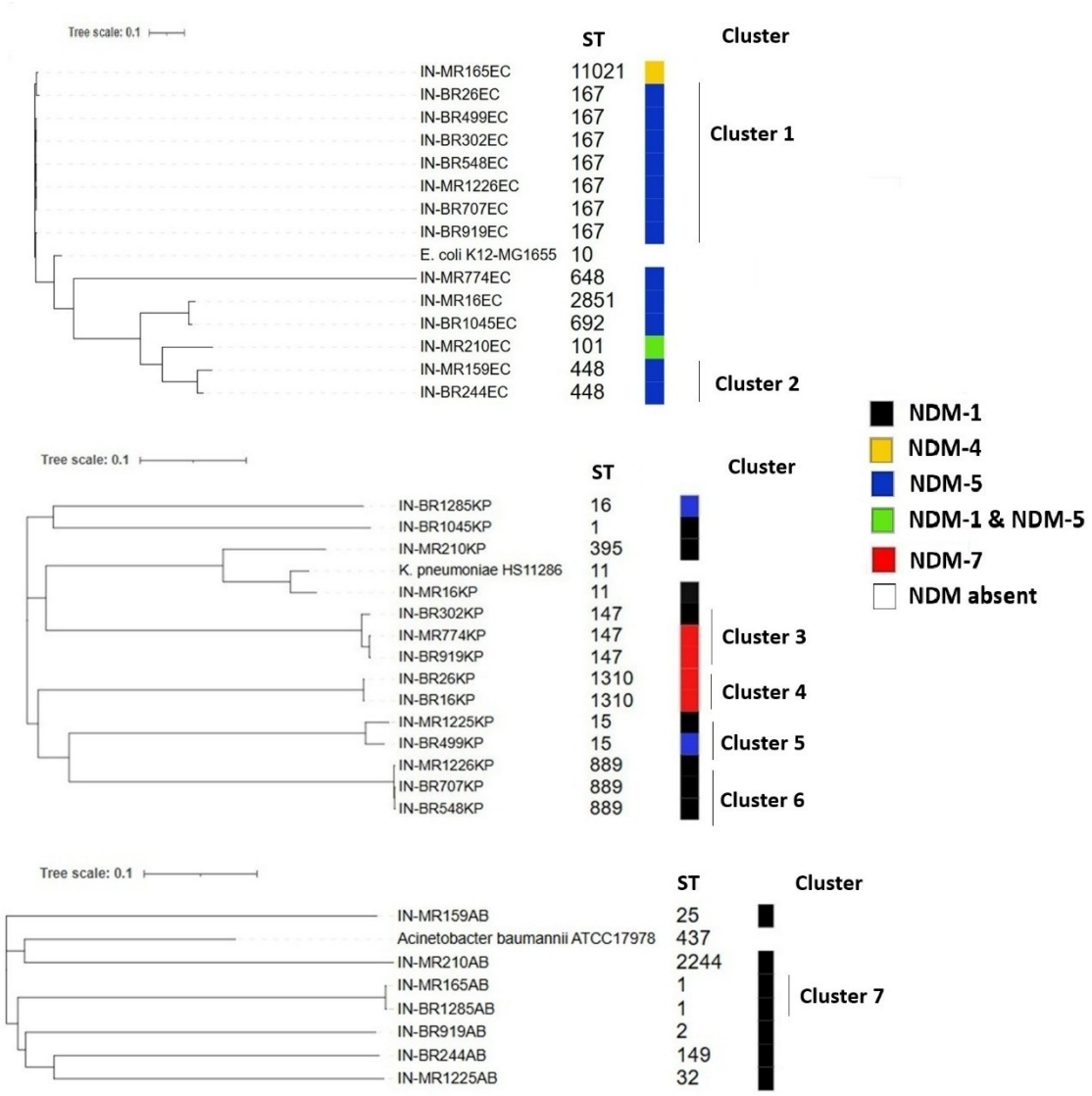


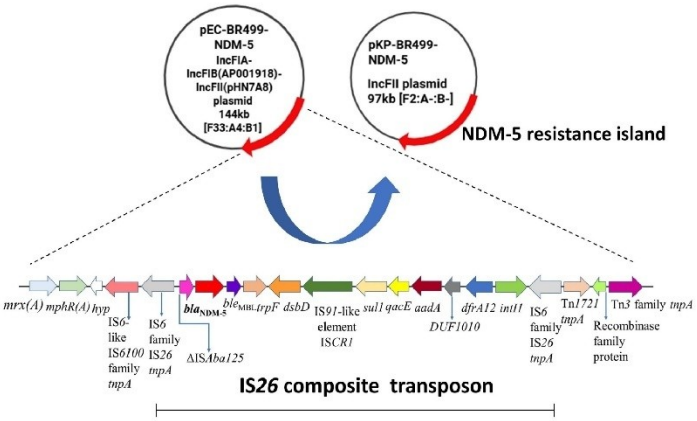
Figure 3. Core genome phylogenetic tree of *bla*$_{NDM}$-harboring *E. coli* (EC), *K. pneumoniae* (KP) and *A. baumannii* (AB) isolated from maternal and neonatal rectal swabs. The presence of *bla*$_{NDM}$ variants are indicated by different color strips. Black strips represent the

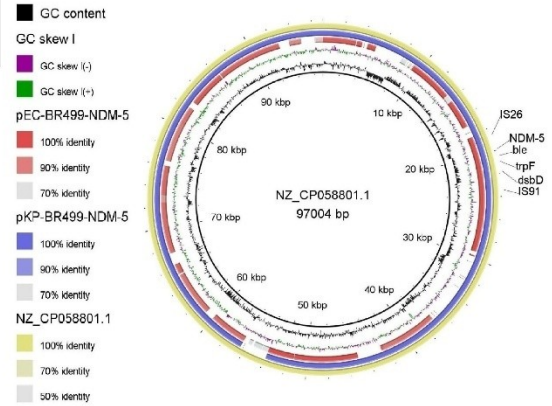
presence of *bla*$_{NDM-1}$, yellow represents the presence of *bla*$_{NDM-4}$, blue indicates the presence of *bla*$_{NDM-5}$, green indicates presence of both *bla*$_{NDM-1}$ & *bla*$_{NDM-5}$, red represents the presence of *bla*$_{NDM-7}$, and no colour represents the absence of *bla*$_{NDM}$. *E. coli* K12-MG1655, *K. pneumoniae* HS11286 and *A. baumannii* K09-14 are used as references for the construction of phylogenetic trees.

Plasmid-mediated transmission of *bla*$_{NDM}$

a) Intrapatient transmission of *bla*$_{NDM}$-harboring plasmids

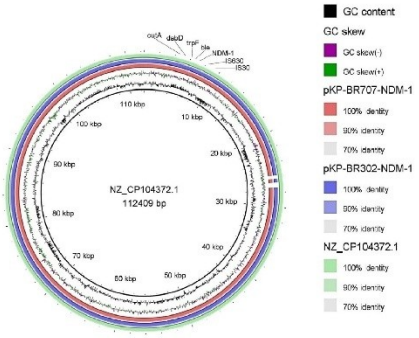
	ST	*bla*$_{NDM}$ variant	Plasmid replicon type	Size	pMLST	Genome-to-Genome Distance Calculator (GGDC) output		G+C difference
						DDH* values (%)	Prob. dDDH>= 70%	
IN-BR499EC	ST167	*bla*$_{NDM-5}$	IncFIA-IncFIB-IncFII	~144kb	[F33:A4:B1]	70.9	79.95	0.67
IN-BR499KP	ST15	*bla*$_{NDM-5}$	IncFII	~97kb	[F2:A-:B-]			





b) Interpatient transmission of *bla*$_{NDM}$-harboring plasmids

	ST	*bla*$_{NDM}$ variant	Plasmid replicon type	Size	pMLST	Genome-to-Genome Distance Calculator (GGDC) output		G+C difference
						DDH* values (%)	Prob. dDDH>= 70%	
IN-BR302KP	ST147	*bla*$_{NDM-1}$	IncFIB (pQil)-IncFIIK	~110kb	[K2:A-:B-]	99.9	98.27	0.01
IN-BR707KP	ST889	*bla*$_{NDM-1}$	IncFIB (pQil)-IncFIIK	~110kb	[K2:A-:B-]			



	ST	*bla*$_{NDM}$ variant	Plasmid replicon type	Size	pMLST	Genome-to-Genome Distance Calculator (GGDC) output		G+C difference
						DDH* values (%)	Prob. dDDH>= 70%	
IN-BR244EC	ST448	*bla*$_{NDM-5}$	IncFIA-IncFIB-IncFII	~98kb	[F1:A1:B49]	92.3	96.5	0.43
IN-BR1045EC	ST692	*bla*$_{NDM-5}$	IncFIA-IncFIB-IncFII	~114kb	[F2:A1:B49]			

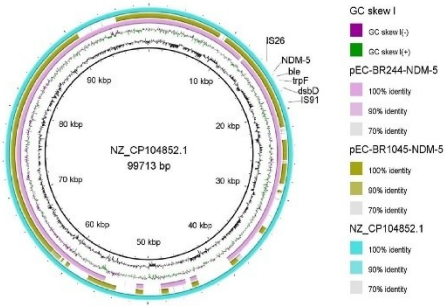
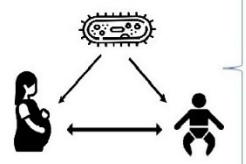


Figure 4. Diagrammatic representation of plasmid-mediated transmission of *bla*$_{NDM}$ among neonatal gut isolates. a) & b) Intrapatient and interpatient plasmid-mediated transmission of *bla*$_{NDM}$, respectively, among neonatal gut isolates, along with comparison of their respective plasmids using BRIG. Genome-to-genome distance calculation was performed by using BLAST+ comparison of study *bla*$_{NDM}$ plasmids. For comparison of study *bla*$_{NDM}$ plasmids, the following plasmids have been used as reference- b. intrapatient- NDM-5; 97kb plasmid (NZ_CP058801.1) India (blood) c. i) interpatient- NDM-1; 112kb plasmid (NZ_CP104372.1) isolated from rectal swab of a patient in *E. coli* from USA ii) NDM-5; 99kb plasmid (NZ_CP104852.1) from China. DNA-DNA hybridisation (DDH) values was calculated using genome-to-genome distance calculator (GGDC) using recommended formula 2.



	Isolate ID	ST	*bla*$_{NDM}$-variant	Plasmid replicon type	Size	pMLST
K. pneumoniae	IN-BR548KP	ST889	*bla*$_{NDM-1}$	IncFIB(pQil)-IncFII(K)	~111kb	[K2:A-:B-]
	IN-BR707KP	ST889	*bla*$_{NDM-1}$	IncFIB (pQil)-IncFIIK	~110kb	[K2:A-:B-]
	IN-MR1226KP	ST889	*bla*$_{NDM-1}$	IncFIB (pQil)-IncFIIK	~110kb	[K2:A-:B-]
	IN-BR16KP	ST1310	*bla*$_{NDM-7}$	IncX3	~52kb	NA
	IN-BR26KP	ST1310	*bla*$_{NDM-7}$	IncX3	~52kb	NA
	IN-MR774KP	ST147	*bla*$_{NDM-7}$	IncC	~271kb	pMLST1
	IN-BR919KP	ST147	*bla*$_{NDM-7}$	IncC	~271kb	pMLST1
E. coli	IN-BR302EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	~144kb	[F33:A4:B1]
	IN-BR499EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	~144kb	[F33:A4:B1]
	IN-BR548EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	~144kb	[F33:A4:B1]
	IN-BR707EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	~144kb	[F33:A4:B58]
	IN-BR919EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII	~146kb	[F36:A1:B20]
	IN-MR1226EC	ST167	*bla*$_{NDM-5}$	IncFIA, IncFIB(AP001918), IncFII(pHN7A8)	~145kb	[F33:A4:B58]
A. baumannii	IN-MR165AB	ST1	*bla*$_{NDM-1}$	Nanopore sequencing was not carried out		NA
	IN-BR1285AB	ST1	*bla*$_{NDM-1}$	Nanopore sequencing was not carried out		NA

587

588**Figure 5. Schematic representation of clonal transmission of *bla*$_{NDM}$ among maternal and**
589**neonatal gut isolates. NA- not applicable.**

590 **Table 1. Antimicrobial susceptibility profile and genomic analysis of *bla*$_{NDM}$-possessing carbapenem-resistant Gram-negative bacteria**

591 **(CRE & CRNF) isolated from individual rectal samples of mothers and neonates.**

592

Patient Id	Isolate Id	Organism	ST	Phylogroup	AMR Phenotypes MIC (mg/L)				Plasmid replicon types via PlasmidFinder (Inc)			PBRT (Inc) of transconjugants	β-lactamases (*bla*)					Aminoglycoside resistance determinants	Quinolone resistance determinants	Additional resistance determinants
															Carbapenemases					
					R	S	MEM	ETP	A/C or X3	F types	Others		AmpCs	ESBLs	NDM variants	OXA variants	Others			
IN-MR16	IN-MR16 KP	*Klebsiella pneumoniae*	11	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS, SXT	NF	64	64	A/C	FIB(K), FII(K)	ColKP3, ColRNAI	A/C, FIB(K)N, FII(K), ColKP3	CMY-6	CTX-M-15, SHV-11	NDM-1	OXA-181	Absent	*rmtC*, *aph(3')-VI*	*aac(6')-Ib*, *oqxA*, *oqxB*, *qnrB1*	*dfrA14*, *fosA6*, *sul1*, *tet(A)*
	IN-MR16 EC	*Escherichia coli*	2851	C	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	32	512	A/C	FIA, FII	ColKP3, IncY	FIA, FII, ColKP3	Absent	CTX-M-15, OXA-10, TEM-1B	NDM-5	OXA-181	Absent	*rmtB*, *ant(3'')-Ia*, *aadA2*	*qnrS1*	*dfrA12*, *mph(A)*, *mph(E)*, *msr(E)*, *sul1*
IN-MR159	IN-MR159 EC	*Escherichia coli*	448	B1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	32	128	Absent	FII	Col (BS512), IncX5	FII	Absent	CTX-M-15, TEM-1B	NDM-5	Absent	Absent	*rmtB*, *aadA2*	*qnrS1*	*dfrA12*, *erm(B)*, *mph(A)*, *sul1*
	IN-MR159 AB	*Acinetobacter baumannii*	25	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, AK, CIP, SXT	TGC, MIN	32	512	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	PER-7	NDM-1	OXA-23, OXA-64	Absent	*armA*, *aph(6)-Id*, *aph(3'')-Ib*, *aac(6')-Ian*	Absent	*ARR-2*, *cmlA1*, *mph(E)*, *msr(E)*, *sul1*, *sul2*, *tet(B)*
IN-MR165	IN-MR165 EH	*Enterobacter hormaechei*	231	N/A	TZP, CAZ, CTX, FEP, ATM,	SXT	64	64	Absent	FIA(HI1)	Col440I, Col440II, IncR	Conjugation unsuccessful	ACT-16	CTX-M-15, OXA-1, OXA-9, TEM-1B	NDM-1	Absent	LAP-2	*armA*, *ant(3'')-Ia*	*aac(6')-Ib-cr*, *aac(6')-Ib*, *oqxA*, *oqxB*, *qnrS1*	*fosA*, *mph(E)*, *msr(E)*

					ETP, IMP, MEM, CN, AK, CIP, TGC, FOS														
	IN-MR165 AB	*Acinetobacter baumannii*	1	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, CIP, TGC, SXT	AK, MIN	32	128	N/A	N/A	N/A	N/A	ADC-25	Absent	NDM-1	OXA-23, OXA-69	Absent	*aph(3')-Ia, aph(6)-Id, aph(3'')-Ib, aph(3')-VIa, aadA1*	Absent	*dfrA1, sul1, sul2, tet(B), tet(39)*
	IN-MR165 EC	*Escherichia coli*	11021	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	32	512	Absent	FIA, FII	Col (BS512), IncI1-I (Gamma), IncI2 (Delta)	FIA	CMY-42	TEM-1B	NDM-4	Absent	Absent	*rmtB, aadA2, aadA5, aac(3)-IIa*	Absent	*dfrA12, dfrA17, mph(A), sul1, tet(A)*
IN-MR210	IN-MR210 AB	*Acinetobacter baumannii*	2244	N/A	TZP, CAZ, FEP, IMP, MEM, CN, CIP, SXT	CFP/SUL, AK, TGC, MIN	32	256	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	Absent	NDM-1	OXA-91	Absent	*aph(3'')-Ib, aph(6)-Id, aac(3)-IId*	Absent	*mph(E), msr(E)*
	IN-MR210 KP	*Klebsiella pneumoniae*	395	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, FOS, SXT	TGC	64	64	Absent	FIB(K), FIB(pQil)-FII(K)	Absent	Conjugation unsuccessful	Absent	CTX-M-15, SHV-36, TEM-1B	NDM-1	Absent	Absent	*rmtF, aadA2, aac(6')-Ib-Hangzhou*	*oqxA, oqxB, qnrB1, qnrS1*	*ARR-2, catA1, fosA, mph(A), sul1*
	IN-MR210 EC	*Escherichia coli*	101	B1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT	FOS	16	64	A/C	N/A	HI1A-HI1B(R27)	Conjugation unsuccessful	CMY-6	OXA-2, OXA-9, TEM-1A, TEM-1B	NDM-1, NDM-5	Absent	Absent	*armA, rmtB, aadA1, aadA2, aph(6)-Id, aph(3'')-Ib, aac(3)-IIa*	*aac(6')-Ib, aac(6')-Ib-cr*	*catA1, dfrA12, dfrA29, mph(E), msr(E), sul1*
IN-MR774	IN-MR774 EC	*Escherichia coli*	648	F	TZP, CAZ, CTX, FEP, ATM, ETP, IMP,	TGC, FOS	64	128	X3	FIA, FIB, FIB(pNDM-Mar), FII, FII (pRSB107	Col (BS512), ColpEC648	X3		CTX-M-15, OXA-1, TEM-1B, TEM-35	NDM-5	Absent	Absent	*aac(3)-IIa, aadA5*	*aac(6')-Ib-cr*	*catA1, dfrA17, erm(B), mph(A), sul1, tet(B)*

					MEM, CN, AK, CIP, SXT					), HI1B(pNDM-MAR)									
	IN-MR774 KP	*Klebsiella pneumoniae*	147	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, FOS, SXT	TGC	64	128	A/C	FIB(K), FII(K)	Absent	A/C	CMY-6	CTX-M-15, SHV-11, TEM-1A	NDM-7	Absent	Absent	*armA, rmtC, aph(6)-Id, aph(3'')-Ib, aac(6')-Ib3, aph(3')-Ia*	*aac(6')-Ib-cr, oqxA, oqxB, qnrB1*	*catA2, dfrA12, fosA, mph(A), mph(E), msr(E), sul1, sul2, tet (A)*
IN-MR1225	IN-MR1225 AB	*Acinetobacter baumannii*	32	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, AK, CIP, SXT	TGC, MIN	64	512	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	Absent	NDM-1	OXA-100, OXA-420	CARB-2	*armA, aph(3')-VI, aph(6)-Id, aph(3'')-Ib*	Absent	*floR, mph(E), msr(E), sul1, sul2*
	IN-MR1225 KP	*Klebsiella pneumoniae*	15	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS, SXT	NF	8	8	Absent	FIB(K), FII(K)	Col440I, ColpVC, IncHI1A, IncHI1B(R27)	FII(K)	DHA-1	CTX-M-15, OXA-9, TEM-1A	NDM-1	Absent	Absent	*armA, aph(6)-Id, aph(3'')-Ib, aac(3)-IIa, aac(6')-Ib3, aadA1*	*aac(6')-Ib, aac(6')-Ib-cr, oqxA, oqxB, qnrB1*	*dfrA14, mph(E), msr(E), sul1, sul2, tet(A)*
IN-MR1226	IN-MR1226 KP	*Klebsiella pneumoniae*	889	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS, SXT	NF	16	16	Absent	FIB(K), FIB(pQil), FII(K)	N/A	Conjugation unsuccessful	Absent	CTX-M-15, OXA-9, SHV-108	NDM-1	Absent	Absent	*aph(3')-VI, aadA1*	*aac(6')-Ib, aac(6')-Ib-cr, oqxA, oqxB, qnrS1*	*fosA*
	IN-MR1226 EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	128	64	Absent	FIA-FIB-FII (pHN7A8)	I1-I (Alpha)	FIA, FIB, FII	Absent	CTX-M-55, TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	Absent	*dfrA12, mph(A), sul1*
IN-BR16	IN-BR16 KP	*Klebsiella pneumoniae*	1310	Kp1	TZP, CAZ, CTX, FEP,	NF	128	64	X3	FIB(K) (pCAV1099-114),	Col440I	X3	Absent	CTX-M-15, OXA-1, SHV-36,	NDM-7	Absent	Absent	*aph(3'')-Ib, aph(6)-Id, aac(3)-IId*	*aac(6')-Ib-cr, oqxA, oqxB, qnrB1*	*catB3, dfrA30, fosA5, fosA7, sul2, tet(B)*

					ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS, SXT					FIB (pNDM-Mar), HI1B (pNDM-MAR)				TEM-1B					
	IN-BR16PM	*Pseudomonas mosselii*	N/A	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, CIP, TGC, SXT	AK, MIN	32	128	N/A	N/A	N/A	Conjugation unsuccessful	Absent	Absent	Absent	Absent	VIM-2	*aadA10*, *ant(2'')-Ia*, *aac(6')-Il*	Absent	*cmlA1*, *sul1*
IN-BR26	IN-BR26EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	32	64		FIA, FII (pCoo)	I1-I(Alpha)	FIA, FII	CMY-42	TEM-1B	NDM-5	Absent	Absent	*rmtB*, *aadA2*	Absent	*erm(B)*, *dfrA12*, *mph(A)*, *sul1*, *tet (A)*
	IN-BR26KP	*Klebsiella pneumoniae*	1310	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS SXT	NF	64	32	X3	FIB(K) (pCAV1099-114), FIB (pNDM-Mar), HI1B(pNDM-MAR)	Absent	Conjugation unsuccessful	Absent	CTX-M-15, OXA-1, SHV-12, SHV-145, TEM-1B	NDM-7	Absent	Absent	*aph(6)-Id*, *aac(3)-IId*, *aph(3'')-Ib*	*aac(6')-Ib-cr*, *oqxA*, *oqxB*, *qnrB1*	*catB3*, *dfrA30*, *fosA5*, *fosA7*, *sul2*
IN-BR244	IN-BR244AB	*Acinetobacter baumannii*	149	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, CIP, SXT	TGC, MIN	32	512	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	PER-7	NDM-1*	OXA-23, OXA-203	Absent	*armA*, *aph(6)-Id*, *aph(3'')-Ib*, *aph(3')-VI*	Absent	*ARR-2*, *cmlA1*, *mph(E)*, *msr(E)*, *sul1*, *tet(B)*, *tet(39)*
	IN-BR244EC	*Escherichia coli*	448	B1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	128	512	X3	FIA-FIB-FII (pAMA1167-NDM-5), FIB(H89-PhagePlasmid)	ColKP3, ColpEC648, I(Gamma)	Conjugation unsuccessful	CMY-42	TEM-1B	NDM-5	OXA-181	Absent	*rmtB*, *aadA2*, *aadA5*, *aac(3)-IId*	*qnrS1*	*dfrA17*, *mph(A)*, *sul1*, *tet(B)*
IN-BR302	IN-BR302EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP,	TGC, FOS	64	128	Absent	FIA- FIB-FII (pHN7A8)	I1-I(Alpha)	FIA, FIB, FII, I-1Alpha	Absent	CTX-M-55, TEM-141	NDM-5	Absent	Absent	*rmtB*, *aadA2*	Absent	*dfrA12*, *mph(A)*, *sul1*, *sitABCD*

					ATM, ETP, IMP, MEM, CN, AK, CIP, SXT														
	IN-BR302 KP	*Klebsiella pneumoniae*	147	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS	SXT	128	128	Absent	FIB (pQil)-FII(K)	R	FII(K), FIB(K)Q, R	Absent	CTX-M-15, OXA-9, SHV-11, TEM-1A	NDM-1	Absent	Absent	*aph(3')-VI, ant(3'')-Ia*	*aac(6')-Ib, oqxA, oqxB, qnrS1*	*fos(A)*
IN-BR499	IN-BR499 EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	64	128	Absent	FIA-FIB-FII (pHN7A8)	I1-I(Alpha)	FIA, FIB, FII	Absent	CTX-M-55, TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	Absent	*dfrA12, mph(A), sul1*
	IN-BR499 KP	*Klebsiella pneumoniae*	15	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT	FOS	32	64	Absent	FIA(HI1), FII, FII(K)	Col (BS512), Col440I, ColpVC, R	FII	Absent	CTX-M-15, OXA-1, SHV-106, TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	*aac(6')-Ib-cr, oqxA, oqxB*	*dfrA12, dfrA14, erm(B), fosA6, mph(A), sul1, tet(D)*
IN-BR548	IN-BR548 EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	128	512	Absent	FIA-FIB-FII (pHN7A8)	I1-I(Alpha)	FIA, FIB, FII, I-1Alpha	Absent	CTX-M-55, TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	Absent	*dfrA12, mph(A), sul1*
	IN-BR548 KP	*Klebsiella pneumoniae*	889	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, FOS	TGC, SXT	64	32	Absent	FIB(K), FIB(pQil)-FII(K), FII(pKP91)	Absent	FIIK	Absent	CTX-M-15, SHV-108	NDM-1	Absent	Absent	*aph(3')-VI, ant(3'')-Ia*	*aac(6')-Ib, oqxA, oqxB, qnrS1*	*fosA6*
IN-BR707	IN-BR707 EC	*Escherichia coli*	167	A	TZP, CAZ,	TGC	128	128	Absent	FIA- FIB-FII	I1-I(Alpha)	FIA, FIB, FII, I-1Alpha	Absent	CTX-M-55, TEM-1B	NDM-5	Absent	Absent	*aadA2*	Absent	*dfrA12, mph(A),*

				CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, LEV, FOS, SXT					(pHN7A8)								*sitABCD, sul1*
	IN-BR707 KP	*Klebsiella pneumoniae*	889	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, FOS	TGC, SXT	16	16	Absent	FIB(K), FIB (pQil)-FII(K), FII (pKP91)	Absent	FIB(K)Q, FII(K)	Absent	CTX-M-15, OXA-9, SHV-108	NDM-1	Absent	Absent	*aph(3')-VI, aadA1*	*aac(6')-Ib, aac(6')-Ib-cr, oqxA, oqxB, qnrS1*	*fosA*
IN-BR919	IN-BR919 AB	*Acinetobacter baumannii*	2	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, CIP, TGC, SXT, MIN	NF	128	512	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	Absent	NDM-1*	OXA-23, OXA-66	Absent	*armA, aph(3'')-Ib, aph(6)-Id, aph(3')-VIa*	Absent	*mph(E), msr(E), sul2, tet(B)*
	IN-BR919 EC	*Escherichia coli*	167	A	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, LEV, FOS, SXT	TGC	64	128	Absent	FIA- FIB-FII	I(Gamma)	FIA, FIB, FII, I-1Alpha	CMY-42	TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	Absent	*erm(B), dfrA12, mph(A), sul1*
	IN-BR919 KP	*Klebsiella pneumoniae*	147	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, SXT	TGC, FOS	128	32	A/C	FIB(K), FII(K)	ColKP3	A/C, ColKP3	CMY-6	CTX-M-15, SHV-11, TEM-1A	NDM-7	OXA-181*	Absent	*armA, rmtC, aph(6)-Id, aph(3')-Ia, aph(3'')-Ib aadA2*	*aac(6')-Ib3, oqxA, oqxB, qnrB1*	*catA2, dfrA12, dfrA14, fosA, mph(A), mph(E), msr(E), sul1, sul2, tet(A)*
IN-BR1045	IN-BR1045KP	*Klebsiella pneumoniae*	1	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, FOS, SXT	NF	32	32	Absent	FIA(HI1)-FII(K), FII(K), FIB(K)	ColKP3, X1	FII(K)	Absent	CTX-M-15, OXA-1, OXA-9, SHV-187, TEM-1, TEM-1A, TEM-1B	NDM-1	OXA-181	Absent	*aph(3')-VI, aadA1, aac(3)-IIa*	*aac(6')-Ib, aac(6')-Ib-cr, oqxA, oqxB, qnrS1*	*bleo, catA1, catB3, dfrA1, dfrA14, fosA, sul1, sul3, tet(A)*

	IN-BR1045EC	*Escherichia coli*	692	C	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, LEV, SXT	TGC, FOS	32	64	Absent	FIA-FIB-FII	Col (BS512)	FIA, FIB, FII	CMY-2	TEM-1B	NDM-5	Absent	Absent	*rmtB, aadA2*	Absent	*catA1, dfrA12, sul1, tet(B)*
IN-BR1285	IN-BR1285KP	*Klebsiella pneumoniae*	16	Kp1	TZP, CAZ, CTX, FEP, ATM, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT	FOS	128	256	Absent	FIA (pBK30683), FIB(K), FII(K)	Col440II, ColKP3	FIA, FIB(K)N, FII(K), ColKP3	Absent	CTX-M-15, OXA-1, SHV-1, TEM-1B	NDM-5	OXA-181	Absent	*rmtB, aac(3)-IIa, aph(6)-Id, aph(3'')-Ib, aadA2*	*aac(6')-Ib-cr, oqxA, oqxB, qnrS1*	*catB3, dfrA12, dfrA14, fosA5, mph(A), sul1, sul2*
	IN-BR1285AB	*Acinetobacter baumannii*	1	N/A	TZP, CAZ, CFP/SUL, FEP, IMP, MEM, CN, AK, CIP, SXT, MIN	TGC	64	256	N/A	N/A	N/A	Conjugation unsuccessful	ADC-25	Absent	NDM-1	OXA-23, OXA-69	Absent	*aac(3)-Ia, aph(3'')-Ib, aph(3')-Ia, aadA1, aph(6)-Id*	Absent	*dfrA1, sul1, sul2, tet(B)*

Footnotes:

Abbreviations: Antimicrobial resistance (AMR), susceptible (S), resistant (R), pipericillin-tazobactum (TZP), ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP), aztreonam (ATM), ertapenem (ETP), imipenem (IMP), meropenem (MEM), gentamicin (CN), amikacin (AK), ciprofloxacin (CIP), levofloxacin (LEV), tigecycline (TGC), fosfomycin (FOS), trimethoprim-sulfamethoxazole (SXT), minimum inhibitory concentration (MIC), Indian maternal rectal sample (IN-MR), Indian neonatal rectal sample (IN-BR), incompatibility (Inc), N/A: not applicable, NF: not found, carbapenem-resistant Enterobacterales (CRE), carbapenem-resistant non-fermenters (CRNF). Plasmid replicon typing scheme for

Acinetobacter baumannii and *P. mosellii* not available. Sequence typing (ST) schemes: Warwick scheme for *E. coli*, Pasteur scheme for *K. pneumoniae*, Pasteur scheme for *A. baumannii*, no MLST schemes available for *P. mosellii* and the schemes for the determination of phylogroups of *A. baumannii* and *E. hormacheii* are not available. * indicates that the carbapenem-resistant gene remained undetected via WGS but PCR & Sanger sequencing confirmed the presence of the gene in the isolates. Some data included in the current manuscript has already been presented in previous publications (eg. Clonal and plasmid characterization of isolates, IN-BR548KP, IN-MR16KP, IN-BR16KP, IN-MR774KP were already reported in Basak et al., 2025).

Table 2. Genotypic characterization of *bla*$_{NDM}$-positive isolates and its transconjugants from patients colonized with multiple carbapenem-resistant Gram-negative bacteria [carbapenem-resistant Enterobacterales (CRE) & carbapenem-resistant non-fermenters (CRNF)]

Patient ID	Isolate Id/ Transconjugants Id	Organism	bla$_{NDM}$ & bla$_{OXA-48}$ variants	Conjugation	Other resistance genes (present/transferred) [PCR]	MIC (mg/L)		Plasmid Inc types present/transferred (PBRT & WGS)	Long-read sequencing	*bla*$_{NDM}$-harbouring plasmid-Plasmid Id; size [Kb]; replicon type [pMLST]
						MEM	ETP			
IN-MR16	IN-MR16KP	*Klebsiella pneumoniae*	*bla*$_{NDM-1}$, *bla*$_{OXA-181}$	Yes	*bla*$_{CTX-M}$, *bla*$_{CIT}$, *rmtC, aac(6')-Ib-cr, oqxAB, qnrB*	64	64	ColKP3, ColRNAI, C, FIB(K), FII(K)	Yes	pKP-MR16-NDM-1; 142kb; IncC [ST1]
	IN-MR 16KP.TC1	*E. coli* J53	*bla*$_{NDM-1}$, *bla*$_{OXA-181}$		*bla*$_{CTX-M}$, *bla*$_{CIT}$, *rmtC, aac(6')-Ib-cr, oqxAB*	8	16	ColKP3, A/C, FIB(K)N, FII(K)		
	IN-MR16EC	*Escherichia coli*	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$	Yes	*bla*$_{CTX-M}$, *bla*$_{TEM}$, *rmtB, qnrS*	32	512	ColKP3, FIA, FII, X3, Y	Yes	Poor assembly
	IN-MR16EC.TC1	*E. coli* J53	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$		*bla*$_{TEM}$, *rmtB*	8	16	ColKP3, FIA, FII		
IN-MR159	IN-MR159EC	*Escherichia*	*bla*$_{NDM-5}$	Yes	*bla*$_{CTX-M}$, *bla*$_{TEM}$, *rmtB,*	32	128	Col(BS512), FII, X5	Yes	pEC-MR159-NDM-5;

		coli			*qnrS*					103kb; IncFII [F2:A-:B-]
	IN-MR159EC.TC2	*E. coli* J53	bla_{NDM-5}		bla_{TEM}, *rmtB*	16	16	FII		
	IN-MR159AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	*armA*	32	512	N/A	No	
	IN-MR159AB.TC1	Conjugation unsuccessful								
IN-MR165	IN-MR165EH	*Enterobacter hormaechei*	bla_{NDM-1}	No	bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, *armA, aac(6')-Ib-cr, qnrS*	64	64	Col440II, IncFIA(HI1), IncR	Yes	bla_{NDM-1} is present in chromosome (4,834,863bp)
	IN-MR165EH.TC1	Conjugation unsuccessful								
	IN-MR165AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	NF	32	128	N/A	No	
	IN-MR165AB.TC1	Conjugation unsuccessful								
	IN-MR165EC	*Escherichia coli*	bla_{NDM-4}	Yes	bla_{TEM}, bla_{CIT}, *rmtB, oqxAB*	32	512	Col(BS512), FIA-FII, I(Gamma), I2 (Delta)	Yes	pEC-MR165-NDM-4; 138kb; IncFIA-InFII [F36:A4:B-]
	IN-MR165EC.TC1	*E. coli* J53	bla_{NDM-4}		bla_{TEM}	64	64	FIA-FII		
IN-MR210	IN-MR210AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	-	32	256	N/A	No	
	IN-MR210AB.TC1	Conjugation unsuccessful								
	IN-MR210KP	*Klebsiella pneumoniae*	bla_{NDM-1}	No	bla_{CTX-M}, bla_{SHV}, bla_{TEM}, *aac(6')-Ib, oqxAB, qnrB, qnrS*	64	64	FIB(K), FIB(pQil), FII(K)	Yes	pKP-MR210-NDM-1 ; 149kb; IncFIB(pQil)-IncFII(K) [K5:A-:B-]
	IN-MR210KP.TC1	Conjugation unsuccessful								
	IN-MR210EC	*Escherichia coli*	bla_{NDM-1}, bla_{NDM-5}	No	bla_{TEM}, bla_{DHA}, *armA, aac(6')-Ib*	128	512	C, HI1A-HI1B(R27)	Yes	pEC-MR210-NDM-1; 265kb; IncHI1A-IncHI1B [unknown]

									pEC-MR210-NDM-5; 137kb; IncC [ST1]
	IN-MR210EC.TC1	Conjugation unsuccessful							
IN-MR774EC	IN-MR774EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, *aac(6')-Ib-cr*	64	128	Col (BS512), ColpEC648, FIA, FIB, FIB(pNDM-Mar), FII, FII (pRSB107), HI1B(pNDM-MAR), X3	Yes	pEC-MR774-NDM-5; 46kb; IncX3
	IN-MR774EC.TC2	*E. coli* J53	bla_{NDM-5}		bla_{TEM}	16	64	X3		
	IN-MR774KP	*Klebsiella pneumoniae*	bla_{NDM-7}	Yes	bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{CIT}, *armA, rmtC, oqxAB, qnrB, qnrS*	64	128	C, FIB(K), FII(K)	No	Long-read sequencing was not carried out as the isolate was clonal with BR919KP
	IN-MR 774KP.TC1	*E. coli* J53	bla_{NDM-7}		bla_{TEM}, bla_{CIT}, *rmtC, oqxAB*	8	32	A/C		
IN-MR1225	IN-MR1225AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	*armA*	64	512	N/A	No	
	IN-MR 1225AB.TC1	Conjugation unsuccessful								
	IN-MR1225KP	*Klebsiella pneumoniae*	bla_{NDM-1}	Yes	bla_{CTX-M}, bla_{OXA-1}, bla_{SHV}, bla_{TEM}, bla_{DHA}, *armA, aac(6')-Ib, aac(6')-Ib-cr, oqxAB, qnrB*	8	8	Col440I, ColpVC, IncFIB(K), IncFII(K), IncHI1A, IncHI1B(R27)	Yes	Poor assembly
	IN-MR 1225KP.TC1	*E. coli* J53	bla_{NDM-1}		bla_{TEM}, *aac(6')-Ib, aac(6')-Ib-cr, oqxAB*	32	64	FII(K)		
IN-MR1226	IN-MR1226KP	*Klebsiella pneumoniae*	bla_{NDM-1}	No	bla_{CTX-M}, bla_{SHV}, *aac(6')-Ib, oqxAB, qnrS*	16	16	FIB(K), FIB(pQil), FII(K)	No	Long-read sequencing was not carried out as the isolate was clonal with BR548KP, BR707KP
	IN-MR 1226KP.TC1	Conjugation								

		unsuccessful							
	IN-MR1226EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{CTX-M}, bla_{TEM}, *rmtB*	128	64	FIA-FIB-FII (pHN7A8), I1-I(Alpha)	Yes	pEC-MR1226-NDM-5; 145kb; IncFIA-IncFIB-IncFII [F33:A4:B58]
	IN-MR 1226EC.TC3	*E. coli* J53	bla_{NDM-5}		bla_{CTX-M}, bla_{TEM}, *rmtB*	8	16	FIA-FIB-FII		
IN-BR16	IN-BR16KP	*Klebsiella pneumoniae*	bla_{NDM-7}	Yes	bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, *aac(6')-Ib-cr*, *oqxAB*, *qnrB*	128	64	Col440I, FIB(K) (pCAV1099-114), FIB (pNDM-Mar), HI1B(pNDM-MAR), X3	Yes	pKP-BR16-NDM-7; 52kb; IncX3
	IN-BR16KP.TC1	*E. coli* J53	bla_{NDM-7}		bla_{OXA-1}, bla_{TEM}	8	16	X3		
	IN-BR16PM	*Pseudomonas moselii*	bla_{VIM-2}	No	NF	32	128	Not found	No	
	IN-BR16PM.TC1	Conjugation unsuccessful								
IN-BR26	IN-BR26EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{TEM}, bla_{CIT}, *rmtB*	32	64	FIA, FII (pCoo), I1-I(Alpha)	Yes	Poor assembly
	IN-BR26EC.TC1	*E. coli* J53	bla_{NDM-5}		bla_{TEM}, *rmtB*	8	16	FIA, FII		
	IN-BR26KP	*Klebsiella pneumoniae*	bla_{NDM-7}	No	bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, bla_{SHV}, *aac(6')-Ib-cr*, *oqxAB*, *qnrB*	64	32	FIB(K)(pCAV1099-114), FIB (pNDM-Mar), HI1B(pNDM-MAR), X3	No	Long read sequencing was not carried out as the isolate was clonal with IN-BR16KP
	IN-BR26KP.TC1	Conjugation unsuccessful								
IN-BR244	IN-BR244AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	*armA*	32	512	N/A	No	
	IN-BR244AB.TC1	Conjugation unsuccessful								
	IN-BR244EC	*Escherichia coli*	bla_{NDM-5}, $bla_{OXA-181}$	No	bla_{TEM}, bla_{CIT}, *rmtB*, *qnrS*	128	512	ColKP3, ColpEC648, FIA-FIB-FII (pAMA1167-NDM-5), FIB(H89-PhagePlasmid), I(Gamma), X3	Yes	pEC-BR244-NDM-5; 98kb; IncFIA-IncFIB-IncFII [F1:A1:B49]

	IN-BR244EC.TC1	Conjugation unsuccessful								
IN-BR302	IN-BR302EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{CTX-M}, bla_{TEM}, bla_{CIT}, *rmtB*	64	128	FIA-FIB-FII (pHN7A8), I1-I(Alpha)	No	Long read sequencing was not carried out as the isolate was clonal with IN-BR499EC
	IN-BR302EC.TC1	*E. coli* J53	bla_{NDM-5}		bla_{CTX-M}, bla_{TEM}, bla_{CIT}, *rmtB*	8	16	FIA-FIB-FII, I1α		
	IN-BR302KP	*Klebsiella pneumoniae*	bla_{NDM-1}	Yes	bla_{CTX-M}, bla_{TEM}, *aac(6')-Ib, oqxAB, qnrS*	128	128	FIB(pQil)-FII(K), R	Yes	pKP-BR302-NDM-1; 111kb; IncFIB(pQil)-IncFII(K) [K2:A-:B-]
	IN-BR302KP.TC1	*E. coli* J53	bla_{NDM-1}		bla_{CTX-M}, bla_{TEM}, *aac(6')-Ib, qnrS*	32	32	FIBKQ-FII(K), R		
IN-BR499	IN-BR499EC	*Escherichia coli*	bla_{NDM-5}	Yes	$bla_{CTX-M,}$ bla_{TEM}, *rmtB*	64	128	FIA-FIB-FII (pHN7A8), I1-I(Alpha)	Yes	pEC-BR499-NDM-5;144kb; IncFIA-IncFIB-IncFII [F33:A4:B1]
	IN-BR499EC.TC1	*E. coli* J53	bla_{NDM-5}		$bla_{CTX-M,}$ bla_{TEM}	8	16	FIA-FIB-FII		
	IN-BR499KP	*Klebsiella pneumoniae*	bla_{NDM-5}	Yes	bla_{OXA-1}, bla_{TEM}, *rmtB, aac(6')-Ib-cr, oqxAB*	32	64	Col (BS512), Col440I, ColpVC, FIA(HI1), FII, FII(K), R	Yes	pKP-BR499-NDM-5; 97kb; IncFII [F2:A-:B-]
	IN-BR499KP.TC1	*E. coli* J53	bla_{NDM-5}		bla_{OXA-1}, bla_{TEM}, *rmtB*	8	16	FII		
IN-BR548	IN-BR548EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{CTX-M}, bla_{TEM}, *rmtB*	128	512	FIA-FIB-FII (pHN7A8), I1-I(Alpha)	Yes	pEC-BR548-NDM-5; 144kb; IncFIA-IncFIB-IncFII [F33:A4:B1]
	IN-BR548EC.TC1	*E. coli* J53	bla_{NDM-5}		$bla_{CTX-M,}$ bla_{TEM}, *rmtB*	32	32	FIA-FIB-FII, I1α		
	IN-BR548KP	*Klebsiella pneumoniae*	bla_{NDM-1}	Yes	bla_{CTX-M}, *aac(6')-Ib, oqxAB, qnrS*	64	32	FIB(K)-FII(K), FIB(pQil), FII(pKP91)	Yes	pKP-BR548-NDM-1; 111kb; IncFIB(pQil)-IncFII(K) [K2:A-:B-]
	IN-BR548KP.TC1	*E. coli* J53	bla_{NDM-1}		bla_{CTX-M}, *aac(6')-Ib*	4	16	FIBKQ-FII(K)		
IN-BR707	IN-BR707EC	*Escherichia*	bla_{NDM-5}	Yes	bla_{CTX-M}, bla_{TEM}, *rmtB*	128	128	FIA-FIB-FII(pHN7A8),	Yes	pEC-BR707-NDM-5; 144kb;

		coli						I1-I(Alpha)	IncFIA-IncFIB-IncFII [F33:A4:B58]
	IN-BR707EC.TC1	*E. coli* J53	bla_{NDM-5}		bla_{CTX-M}, bla_{TEM}, *rmtB*	16	32	FIA-FIB-FII, I1α	
	IN-BR707KP	*Klebsiella pneumoniae*	bla_{NDM-1}	Yes	bla_{CTX-M}, bla_{SHV}, *aac(6')-Ib-cr, aac(6')-Ib, oqxAB, qnrS*	16	16	FIB(K), FIB(pQil)-FII(K), FII (pKP91)	Yes	pKP-BR707-NDM-1; 110kb; IncFIB (pQil)-IncFIIK [K2:A-:B-]
	IN-BR707KP.TC1	*E. coli* J53	bla_{NDM-1}		bla_{CTX-M}, *aac(6')-Ib, qnrS*	4	32	FIBKQ-FII(K)		
IN-BR919	IN-BR919AB	*Acinetobacter baumannii*	bla_{NDM-1}	No	*armA*	128	512	N/A	No	
	IN-BR919AB.TC1	Conjugation unsuccessful								
	IN-BR919EC	*Escherichia coli*	bla_{NDM-5}	Yes	bla_{TEM}, bla_{CIT}, *rmtB*	64	128	FIA-FIB-FII, I(Gamma)	Yes	pEC-BR919-NDM-5; 146kb IncFIA-IncFIB-IncFII [F36:A1:B20]
	IN-BR919EC.TC1	*E. coli* J53	bla_{NDM-5}		bla_{TEM}	64	256	FIA-FIB-FII, I1-I(Alpha)		
	IN-BR919KP	*Klebsiella pneumoniae*	bla_{NDM-7}, $bla_{OXA-181}$	Yes	bla_{CTX-M}, bla_{TEM}, bla_{CIT}, *armA, rmtC, aac(6')-Ib-cr, oqxAB, qnrB*	128	32	C, FIB(K), FII(K)	Yes	pKP-BR919-NDM-7; 271kb IncC [ST1]
	IN-BR919KP.TC1	*E. coli* J53	bla_{NDM-7}, $bla_{OXA-181}$		bla_{TEM}, bla_{CIT}, *rmtC, aac(6')-Ib-cr*	16	64	A/C		
IN-BR1045	IN-BR1045KP	*Klebsiella pneumoniae*	bla_{NDM-1}, $bla_{OXA-181}$	Yes	bla_{CTX-M}, bla_{OXA-1}, bla_{SHV}, bla_{TEM}, bla_{CIT}, *aac(6')-Ib, oqxAB, qnrS*	32	32	ColKP3, FIA(HI1)-FIIK, FIB(K), FII(K), X1	Yes	pKP-BR1045-NDM-1; 83kb; IncFII(K) [K2:A-:B-]
	IN-BR1045KP.TC1	*E. coli* J53	bla_{NDM-1}, $bla_{OXA-181}$		bla_{CTX-M}, bla_{OXA-1}, bla_{TEM}, *aac(6')-Ib, oqxAB, qnrS*	8	8	FII(K), ColKP3		
	IN-BR1045EC	*Escherichia*	bla_{NDM-5}	Yes	bla_{TEM}, bla_{CIT}, *rmtB,*	32	64	Col(BS512), FIA- FIB-	Yes	pEC-BR1045-NDM-5; 114kb

		coli			*oqxAB*			FII		IncFIA-IncFIB-IncFII [F2:A1:B49]
	IN-BR1045EC.TC1	*E. coli* J53	*bla*$_{NDM-5}$		NF	16	16	FIA-FIB-FII		
IN-BR1285	IN-BR1285KP	*Klebsiella pneumoniae*	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$	Yes	*bla*$_{CTX-M}$, *bla*$_{OXA-1}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *rmtB, aac(6')-Ib-cr, oqxAB, qnrS*	128	256	Col440II, ColKP3, FIA (pBK30683), FIB(K), FII(K), X3	Yes	Poor assembly
	IN-BR1285KP.TC1	*E. coli* J53	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$		*bla*$_{CTX-M}$, *bla*$_{OXA-1}$, *bla*$_{TEM}$, *rmtB, aac(6')-Ib-cr, oqxAB, qnrS*	32	128	ColKP3, FIA, FIB(K)N, FII(K)		
	IN-BR1285AB	*Acinetobacter baumannii*	*bla*$_{NDM-1}$	No		64	256	N/A	No	
	IN-BR1285AB.TC1	Conjugation unsuccessful								

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612 **Table 3. Genomic characteristics of *bla*$_{NDM}$-bearing plasmids in terms of *bla*$_{NDM}$ variants, replicon types and conjugation modules resolved**

613 **by illumina and nanopore sequencing in carbapenem-resistant Enterobacterales (CRE).**

Isolate Id	***bla*$_{NDM}$-variants**	**Plasmid Id**	**Resistance genes present on *bla*$_{NDM}$-carrying plasmid**	**GC content (%)**	**Size**	**Inc type [pMLST]**	**Plasmid addiction system**	**Conjugation**	**Conjugation module**				**IS element associated for *bla*$_{NDM}$ mobilization located upstream of *bla*$_{NDM}$**
									OriT	**Relaxase type**	**T4CP**	**T4SS**	
IN-MR16KP	*bla*$_{NDM-1}$	**pKP-MR16-NDM-1**	*aph(3')-VI, aac(6')-Ib3, bla*$_{CMY-6}$, *bla*$_{NDM-1}$, *rmtC, sul1*	51.7	142040 bp (~142kb)	IncC [ST1]	*parB, higBA*	Yes	Absent	MOBH	*t4cp2*	*G_tfc7, virb4, traBCEKLNUVW*	Truncated IS*Aba125*

***IN-MR159EC**	*bla*$_{NDM-5}$	**pEC-MR159-NDM-5**	*aadA2*, *dfrA12*, *erm(B)*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	52.1	102894 bp (~103kb)	IncFII [F2:A-:B-]	*pemI/K*, Hok/Gef-like protein	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFHKLNUW*, *trbC*	Truncated IS*Aba125*; IS*26* composite transposon present
***IN-MR165EC**	*bla*$_{NDM-4}$	**pEC-MR165-NDM-4**	*aac(3)-IIa*, *aadA2*, *aadA5*, *dfrA12*, *dfrA17*, *mph(A)*, *bla*$_{NDM-4}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$, *tet(A)*	53.3	137682 bp (~138kb)	IncFIA-IncFII [F36:A4:B-]	*parAB*, *pemI/K*, *ccdAB*, *vapBC*, Hok/Gef-like protein	Yes	Present	MOBF	Absent	*virB1*, *virb4*, *traBCEFHKLNUW*, *trbC*	Truncated IS*Aba125*
***IN-MR210KP**	*bla*$_{NDM-1}$	**pKP-MR210-NDM-1**	*aac(6')-Ib-Hangzhou*, *ARR-2*, *bla*$_{CTX-M-15}$, *catA1*, *bla*$_{NDM-1}$, *qnrS1*, *qnrB1*, *rmtF*, *bla*$_{TEM-1B}$	53.3	155978 bp (~156kb)	IncFIB(pQil)-IncFII(K) [K5:A-:B-]	*higBA*, *parAB*	No	Absent	Absent	Absent	Absent	Truncated IS*Aba125*
***IN-MR210EC**	*bla*$_{NDM-1}$	**pEC-MR210-NDM-1**	*armA*, *aac(6')-Ib*, *aac(6')-Ib-cr*, *aadA*, *bla*$_{DHA-1}$, *msr(E)*, *mph(E)*, *bla*$_{NDM-1}$, *bla*$_{OXA-9}$, *qacE*, *sul1*, *bla*$_{TEM-1A}$	46.5	264538 bp (~265kb)	IncHI1A-IncHI1B [unknown]	*parAB*, *parM*, *parR*, *relE/stbE*, *relB/stbD*	No	Absent	MOBH	*traD*	*virb4*	Truncated IS*Aba125*
	bla$_{NDM-5}$	**pEC-MR210-NDM-5**	*aadA2*, *bla*$_{CMY-6}$, *dfrA12*, *bla*$_{NDM-5}$, *qacE*, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	51.6	137468 bp (~137kb)	IncC [ST1]	*parAB*	No	MOBH	MOBH	Absent	Absent	Truncated IS*Aba125*
IN-MR774EC	*bla*$_{NDM-5}$	**pEC-MR774-NDM-5**	*bla*$_{NDM-5}$	46.5	46118 bp (~46kb)	IncX3	Absent	Yes	Absent	MOBP	Absent	*virB1*, *virb4*	IS*5*
IN-MR1226EC	*bla*$_{NDM-5}$	**pEC-MR1226-NDM-5**	*aadA2*, *bla*$_{CTX-M-55}$, *dfrA12*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	52.1	144570 bp (~145kb)	IncFIA-IncFIB(AP001918) - IncFII(pHN7A8) [F33:A4:B58]	*parAB*, *pemK/I*, Hok/Gef-like protein, *ccdAB*, *vapBC*	Yes	Absent	MOBF	*traD*, *t4cp2*	*traGH*	Truncated IS*Aba125*; IS*26* composite transposon present
IN-BR16KP	*bla*$_{NDM-7}$	**pKP-BR16-NDM-1**	*qnrB1*, *bla*$_{NDM-7}$	47	51641 bp (~52kb)	IncX3	Absent	Yes	Absent	MOBP	Absent	*virB1*, *virb4*	IS*5*
***IN-BR244EC**	*bla*$_{NDM-5}$	**pEC-BR244-NDM-5**	*aac(3)-IId*, *aadA2*, *aadA5*, *dfrA17*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$, *tet(B)*	51	97912 bp (~98kb)	IncFIA-IncFIB(AP001918) - IncFII(pAMA1167-NDM-5) [F1:A1:B49]	*parAB*, Hok/Gef-like protein, *ccdAB*, *vapBC*, *relB/stbD*	No	Absent	MOBF	Absent	Absent	Truncated IS*Aba125*
IN-BR302KP	*bla*$_{NDM-1}$	**pKP-BR302-NDM-1**	*aadA1*, *aph(3')-VI*, *aac(6')-Ib*, *bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-9}$, *qnrS1*	52.2	110719 bp (~111kb)	IncFIB(pQil)-IncFII(K) [K2:A-:B-]	*parAB*, *vapBC*, Hok/Gef-like protein, *higB*	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFGHKLNUVW*	Complete IS*Aba125*
[a]IN-BR499EC	*bla*$_{NDM-5}$	**pEC-BR499-NDM-5**	*aadA2*, *bla*$_{CTX-M-55}$, *dfrA12*, *bla*$_{NDM-5}$, *mph(A)*, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	52.1	144204 bp (~144kb)	IncFIA-IncFIB(AP001918) - IncFII(pHN7A8) [F33:A4:B1]	*parAB*, *pemK/I*, Hok/Gef-like protein, *ccdAB*, *vapBC*	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFGHKLNUW*, *trbC*	Truncated IS*Aba125*; IS*26* composite transposon present
IN-BR499KP	*bla*$_{NDM-5}$	**pKP-BR499-NDM-5**	*aadA2*, *dfrA12*, *erm(B)*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	52.8	97232 bp (97kb)	IncFII [F2:A-:B-]	*pemK/I*, Hok/Gef-like protein	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *traBEGHKLNUVW*, *trbC*	Truncated IS*Aba125*
[a]IN-BR548EC	*bla*$_{NDM-5}$	**pEC-BR548-NDM-5**	*aadA2*, *bla*$_{CTX-M-55}$, *dfrA12*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*,	51.8	143769 bp (~144kb)	IncFIA-IncFIB(AP001918)	*parAB*, *pemK/I*, Hok/Gef-like protein, *ccdAB*,	Yes	Present	MOBF	Absent	*virB1*, *virb4*, *traBCEFHKLNUW*, *trbC*	Truncated IS*Aba125*; IS*26* composite

			bla$_{TEM-1B}$			- IncFII(pHN7A8) [F33:A4:B1]	*vapBC*						transposon present
[a]**IN-BR548KP**	*bla*$_{NDM-1}$	**pKP-BR548-NDM-1**	*aadA1*, *aph(3')-VI*, *aac(6')-Ib*, *bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-9}$, *qnrS1*	52.2	110719 bp (~111kb)	IncFIB(pQil)-IncFII(K) [K2:A-:B-]	*parAB*, Hok/Gef-like protein, *higB*, *vapBC*	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFGHKLNUV*, *trbC*	Complete IS*Aba125*
[a]**IN-BR707EC**	*bla*$_{NDM-5}$	**pEC-BR707-NDM-5**	*aadA2*, *bla*$_{CTX-M-55}$, *dfrA12*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	52.1	144455 bp (~144kb)	IncFIA-IncFIB(AP001918) - IncFII(pHN7A8) [F33:A4:B58]	*parAB*, *pemK/I*, Hok/Gef-like protein, *ccdAB*, *vapBC*	Yes	Present	MOBF	*traD*, *t4cp2*	*traGH*	Truncated IS*Aba125*; IS*26* composite transposon present
[a]**IN-BR707KP**	*bla*$_{NDM-1}$	**pKP-BR707-NDM-1**	*aadA1*, *aph(3')-VI*, *aac(6')-Ib*, *bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-9}$, *qnrS1*	52.2	110700 bp (~110kb)	IncFIB(pQil)-IncFIIK [K2:A-:B-]	*parAB*, Hok/Gef-like protein, *higB*, *vapBC*	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFGHKLNUW*, *trbC*	Complete IS*Aba125*
***IN-BR919EC**	*bla*$_{NDM-5}$	**pEC-BR919-NDM-5**	*aadA2*, *dfrA12*, *erm(B)*, *mph(A)*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$	51.9	145865 bp (~146kb)	IncFIA-IncFIB(AP001918) - IncFII [F36:A1:B20]	*parAB*, *pemK/I*, Hok/Gef-like protein, *ccdAB*, *vapBC*	Yes	Present	MOBF	Absent	*virB1*, *virb4*, *traBCEFKLNUVW*, *trbC*	Truncated IS*Aba125*; IS*26* composite transposon present
[a]**IN-BR919KP**	*bla*$_{NDM-7}$	**pKP-BR919-NDM-7**	*aadA2*, *armA*, *aac(6')-Ib3*, *bla*$_{CMY-6}$, *dfrA12*, *mph(E)*, *msr(E)*, *bla*$_{NDM-7}$, *rmtC*, *sul1*, *bla*$_{TEM-1A}$	50.4	271125 bp (~271kb)	IncC [ST1]	*parB*, *higBA*	Yes	Absent	MOBH	*traD*	*virB4*	IS*5*
IN-BR1045EC	*bla*$_{NDM-5}$	**pEC-BR1045-NDM-5**	*aadA2*, *catA1*, *dfrA12*, *bla*$_{NDM-5}$, *rmtB*, *sul1*, *bla*$_{TEM-1B}$, *tet(B)*	51.5	114412 bp (~114kb)	IncFIA-IncFIB(AP001918) - IncFII [F2:A1:B49]	*parAB*, *pemK/I*, *ccdAB*, *vapBC*, *relB/stbD*	Yes	Absent	MOBF	*traD*,	*virB1*, *virb4*	Truncated IS*Aba125*
IN-BR1045KP	*bla*$_{NDM-1}$	**p1KP-BR1045-NDM-1**	*aph(3')-VI*, *bla*$_{NDM-1}$, *bla*$_{OXA-181}$	52.2	83291 bp (~83kb)	IncFII(K) [K2:A-:B-]	*parAB*, *ccdAB*, *higBA*	Yes	Present	MOBF	*traD*, *t4cp2*	*virB1*, *virb4*, *traBCEFGHKLNUW*, *trbC*	Complete IS*Aba125*
	bla$_{NDM-1}$	**p2KP-BR1045-NDM-1**	*aadA1*, *aph(3')-VI*, *aac(6')-Ib*, *bla*$_{CTX-M-15}$, *catA1*, *bla*$_{NDM-1}$, *bla*$_{OXA-9}$, *qnrS1*, *bla*$_{TEM-1A}$, *bla*$_{TEM-1B}$, *tet(A)*	52	239621 bp (~240kb)	IncFIA(HI1)-IncFIIK [K12:A10:B-]	Hok/Gef-like protein	Yes	Present	MOBF	*traD*, *t4cp2*	*trbAB*, *traIKLMNOPQRUWY*	Complete IS*Aba125*

Footnotes

oriT: origin of transfer; T4CP: type IV coupling proteins; T4SS: type IV secretion system; IN-MR stands for Indian maternal rectal sample; IN-BR stands for Indian neonatal rectal sample; Inc stands for incompatibility. Long-read sequencing data of few co-resident CRE isolates were excluded in this table- IN-MR16EC, IN-BR26EC, IN-MR1225KP & IN-BR1285KP as they had poor assembly issues. In IN-MR165EH (*E.*

hormaechei), *bla*$_{NDM}$ was located on the chromosome. [a]IN-MR774KP, IN-MR1226KP and IN-BR302EC have not been mentioned in the table as they were clonal with IN-BR919KP, IN-BR548KP/BR707KP and IN-BR499EC/BR548EC/BR707EC respectively. *The samples which were co-colonized with CRNF isolates, nanopore sequencing was not conducted for these isolates and hence the data was not included in this table. For example: *P. mosellii* isolate (IN-BR16PM) and *A. baumannii* isolates (IN-MR159AB, IN-MR165AB, IN-MR210AB, IN-BR244AB, IN-BR-919AB).