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Genomic similarity of carbapenem-resistant Enterobacterales collected from mothers and their neonates

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ABSTRACT

Objective: New Delhi metallo-β-lactamase is endemic in India and the gut may act as a reservoir of carbapenemase-producing Enterobacterales (CPE). Maternal gut colonisation with bla_{NDM}-harbouring CPE increases the risk of neonatal gut colonisation. This study aimed to assess the vertical transmission of CPE from pregnant mothers (rectal) to neonates (rectal and blood).

Methods: Rectal samples were collected and processed for the presence of CPE, followed by bacterial identification and antibiotic susceptibility. Mother–neonate pairs colonised with the same species underwent pulsed-field gel electrophoresis and whole-genome sequencing to examine genetic relatedness. Detection of bla_{NDM} variants and their transmissibility was performed.

Results: Of the pregnant mothers (n = 86) and sick neonates (n = 93) analysed, eight mother–neonate pairs harboured similar carbapenem-resistant species, predominantly *Klebsiella pneumoniae*, followed by *Escherichia coli*. Pulsed-field gel electrophoresis and whole-genome sequencing revealed that most isolates from mother–neonate pairs were distinct and distributed within diverse sequence types, including epidemic clones (ST11/15/147/405/410). $bla_{NDM-1/5/7}$ were detected in CPE and predominantly associated with conjugative IncFII and IncFII(K) replicons. Genomic analysis supported one case of vertical transmission (ST147; bla_{NDM-1}-positive *K. pneumoniae*) from mother to a neonate. Further investigation of exogenous sources is required to understand the acquisition of bacteria. No evidence of transmission of bla_{NDM}-harbouring plasmids within mother–neonate pairs carrying distinct isolates was observed, indicating the independent acquisition of bacteria.

Conclusions: Although limited evidence of mother-to-neonate transmission was observed in this study, screening of the gut is necessary to understand CPE transmission in hospital settings and beyond. Targeted surveillance and infection-prevention policies are needed to curb CPE spread.

1. Introduction

Transmission of antimicrobial resistance genes (ARGs) between human, animal, and environmental bacteria has facilitated the global emergence of antimicrobial resistance (AMR) [1,2]. The human gut teeming with bacteria acts as a niche for the exchange of ARGs within a bacterial community [3]. Colonisation of the neonate begins immediately after birth, although recent evidence suggests it may also start before birth, in utero [4]. Neonates following birth are exposed to diverse microbial communities, which may include opportunistic pathogens acquired from the mother or the hospital environment, increasing the likelihood of neonatal colonisation with multidrug-resistant (MDR) bacteria [4]. In countries where the burden of MDR bacteria is high and the mother's gut harbours MDR strains, the possibility of transmission of such bacteria from mother to neonate increases. Colonisation of the

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neonatal gut is a normal phenomenon, but the presence of MDR strains in their gut may predispose the neonate to sepsis, particularly those who are premature and of low birth weight [5,6]. The vulnerability of the host is often an advantage for drug-resistant pathogens to cause diseases that are difficult to treat due to the diminishing options of active antimicrobials against MDR strains.

Carbapenems remain one of the most potent 'last-resort' drugs for the treatment of sepsis with MDR bacteria, but widespread non-judicious use of carbapenems has led to the emergence of carbapenem resistance worldwide. Carbapenem resistance is primarily mediated via the production of carbapenemases, which are enzymes that hydrolyse most β-lactam antibiotics, including carbapenems, by Gram-negative bacteria globally. Out of three classes of carbapenemases—class A (e.g., *Klebsiella pneumoniae* carbapenemase [KPC]), class B (metallo-β-lactamases such as New Delhi metallo-β-lactamase [NDM]), and class D (e.g., OXA-48-like carbapenemases)—NDM is the most widely spread carbapenemase and is epidemiologically linked to the Indian subcontinent [7]. NDM can hydrolyse most β-lactams, including carbapenems, but not monobactams [8]. To date, 75 bla_{NDM} variants have been reported worldwide (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#NDM). Of the different variants, $bla_{\text{NDM-1}}$ is the globally disseminated variant, followed by $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-7}}$ [7,9]. Transmission of bla_{NDM} is associated with different mobile genetic elements such as plasmids (IncX3, IncFII, IncFIIs, IncFIA, IncFIB, IncC, IncL/M, IncHI1b, and so on), transposons (Tn*125*, Tn*3000*), and insertion sequences (ISs; IS*Aba125*, IS*1*, IS*5*, IS*26*, IS*903*, IS*3000*, IS*Ec33*, IS*Kpn14*, and so on), with dominant sequence types (STs; ST167, ST410, or ST617, ST11, ST14) leading to the successful spread of bla_{NDM} across the globe [7].

Although there have been several reports of bla_{NDM}-possessing bacteria causing sepsis in neonates both from India [10–13] and other parts of the globe [14–17], understanding of the mother-to-neonate transmission of MDR bacteria is limited by several studies reporting the transmission of extended spectrum β-lactamase (ESBL)-producing bacteria [18–21]. However, studies inferring the transmission of bla_{NDM}-producing bacteria from mother to neonate have not been explored extensively. A multi-centric study (Burden of Antibiotic Resistance in Neonates from Developing Societies; BARNARDS) carried out in low- and middle-income countries reported the prevalence of bla_{NDM} in Southeast Asia and Africa [22]. The study described the increased carriage of bla_{NDM} (42%) and $bla_{\text{OXA-48}}$ (7%) in the neonatal gut compared with the maternal gut (bla_{NDM} = 8%, $bla_{\text{OXA-48}}$ = 2%) in the Indian population. Overall carriage of ESBLs and carbapenemase genes has been reported in the BARNARDS study [22]. However, the previous study did not explore the transmission of bla_{NDM}-possessing Enterobacterales from mothers to neonates in Indian samples. As an extension of the BARNARDS study, the present study specifically focused on the genetic similarity of carbapenem-resistant Enterobacterales between mothers and their neonates to determine whether transmission could have occurred.

2. Materials and methods

2.1. Study design, site and participants

Isolates analysed here were part of a collaborative study, BARNARDS, involving seven different countries, including India (July–November 2017). The present study involved samples collected from the India site (IPGME&R and SSKM hospital, Kolkata), both from pregnant mothers and sick neonates (period of collection: July–December 2017). In the BARNARDS study, women in labour (preferably) or immediately postpartum were enrolled, and rectal swabs were collected. Rectal swabs and blood samples were collected from neonates suspected of sepsis, excluding healthy neonates, as mentioned in the BARNARDS study [22]. In this study, only sick neonates were included. As this study focused on understanding the probability of transmission of carbapenemase-producing Enterobacterales (CPE) from mother to neonate by assessing isolate similarity, samples from mother–neonate dyads were included.

2.2. Culture, isolation, and identification of carbapenem-resistant Gram-negative bacteria

Rectal swabs and blood cultures were processed as described previously [22,23]. Briefly, rectal swabs were streaked on chrome agar plates (BD BBL, MD, USA) supplemented with vancomycin (10 mg/L; MP Biomedicals, CA, USA) and ertapenem (2 mg/L; VE; Sigma-Aldrich, USA). Chrome agar was used to differentiate bacteria in the rectal swabs and further similar bacteria (based on colour) were picked up from both rectal swabs of the mother and neonates for further analysis. Carbapenem-resistant Gram-negative bacteria growing on these plates were screened for the presence of carbapenemases (bla_{NDM}, bla_{KPC}, and $bla_{\text{OXA-48-like}}$) by polymerase chain reaction (PCR) [22]. Samples positive for carbapenemases ($bla_{\text{NDM/OXA-48}}$) were plated for single colonies to differentiate bacterial species. Following the repeat PCR for $bla_{\text{NDM/OXA-48}}$, carbapenem-resistant species were cryopreserved. The clonality of morphologically similar carbapenemase-producing isolates from each sample was checked by repetitive extragenic palindromic elements-PCR [24], and the resulting distinct representative isolates were further identified by Enterosystem 18R (Liofilchem S.r.l., Italy) and confirmed using a VITEK2 compact system (BioMérieux, Marcy l'Etoile, France). Mother–neonate dyads colonised with the same species of carbapenemase-producing bacteria designated as CPE, were considered for further analysis to understand the genomic similarity of the bacteria to determine whether mother-to-neonate transmission had occurred.

2.3. Antibiotic susceptibility testing of CPE collected from mother–neonate dyads

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion method (Liofilchem, Italy) [22] for nine different antibiotics (ceftazidime, ertapenem, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, tigecycline, trimethoprim-sulfamethoxazole). For meropenem and colistin, the minimum inhibitory concentration (MIC) was determined via the broth microdilution method. *E. coli* ATCC 25922 was used as a quality control, and the results were interpreted according to CLSI (2023) guidelines. For tigecycline, interpretation was done using EUCAST guidelines 2023.

2.4. Pulsed-field gel electrophoresis

Mothers and neonates colonised with the same species of CPE underwent pulsed-field gel electrophoresis (PFGE) following the PulseNet standardised procedure (http://www.cdc.gov/pulsenet/protocols.htm) in a CHEF Mapper apparatus (Bio-Rad Laboratories, CA, USA). Overnight DNA digestion was carried out using *Xba*I (New England Biolab, MA, USA) at 37 °C followed by electrophoresis in a 1% pulse field certified agarose gel (Bio-Rad Laboratories) for 19 h. *Salmonella* serotype Braenderup H9812 was used as the marker and bands were visually interpreted according to Tenovar criteria [25].

2.5. Whole-genome sequencing

Genomic DNA was extracted from 16 CPEs (rectal and blood), and 13 were processed for paired-end sequencing on an Illumina NovaSeq 6000 platform (San Diego, CA, USA), with three

isolates sequenced on an Ion Torrent platform (Thermo Fisher Scientific, MA, USA) based on platform availability. Bacterial whole-genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Spain) and the Qiagen DNeasy Ultraclean Microbial Kit, and quantified using Qubit 4.0 (Thermo Fisher Scientific). Paired-end libraries were constructed using a Nextera XT Kit (USA) and NEBNext Ultra II DNA Library Prep Kit (USA) according to the manufacturer's protocol. Next-generation sequencing was performed using an Illumina NovaSeq 6000 with a read length of (2 × 150 bp). Sequencing of a few samples was carried out on the Ion 540 chip with the Ion S5 system (Thermo Fisher Scientific) using the Ion Xpress Plus fragment library kit (Thermo Fisher Scientific) according to manufacturer instructions. Reads were subjected to quality control and trimming using fastp (v.0.20.1; https://github.com/OpenGene/fastp). Single reads produced from Ion Torrent sequencing were assembled using SPAdes (v.3.15.4; https://github.com/ablab/spades), and Shovill (v.0.9.0; https://github.com/tseemann/shovill) was used to assemble paired-end trimmed sequences from Illumina. The following online databases were used for analysis: MLST (2.0; https://cge.food.dtu.dk/services/MLST/) for multilocus sequence typing (MLST), for core genome ST (cgST) cgMLSTFinder (1.2) was used for *E. coli* (https://cge.food.dtu.dk/services/cgMLSTFinder/)- and PathogenWatch for *K. pneumoniae* (https://pathogen.watch/). In the case of novel alleles, genomes of *K. pneumoniae* were submitted to the BIGSdb-Pasteur website (https://bigsdb.pasteur.fr) for assignment of new cgST. Phylogroups of *E. coli* and *K. pneumoniae* were assessed through ClermonTyping (http://clermontyping.iame-research.center) and BIGSdb-Pasteur (https://bigsdb.pasteur.fr/cgibin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef&page=sequenceQuery), respectively. Along with this Resfinder (4.5.0; http://genepi.food.dtu.dk/resfinder) for ARGs, PlasmidFinder (2.1; https://cge.food.dtu.dk/services/PlasmidFinder/) for plasmid replicon types, and pMLST (2.0; https://cge.food.dtu.dk/services/pMLST/) for IncF typing (pMLST) were performed. The genetic background of bla_{NDM}- and $bla_{OXA-181}$-harbouring CPE isolates were assessed through SnapGene viewer (7.1; https://www.snapgene.com/snapgene-viewer), which was restricted to the size of the contigs harbouring carbapenemase genes.

Average nucleotide identity (ANI) was performed on genomes using EZ Biocloud (https://www.ezbiocloud.net/), and mash distance estimations were generated between pairs using mash dist (v.2.2) (https://github.com/marbl/mash). Snippy (v.4.6.0) (https://github.com/tseemann/snippy) was performed to detect single-nucleotide polymorphisms (SNPs) and variants between two bacterial isolates using one genome in the pair as the local reference to map reads against the second bacterial isolate of the pair.

2.6. Phylogenetic analysis

CSI Phylogeny version 1.4 (https://cge.food.dtu.dk/services/CSIPhylogeny/) was used to call SNPs and perform genome alignment. The complete genome sequence of *E. coli* K-12 strain MG1655 (GCF_904425475.1) and *K. pneumoniae* strain HS11286 (GCF_000240185.1) were used as references for the construction of phylogenetic trees for *E. coli* and *K. pneumoniae* study isolates, respectively, and visualised by iTOL. Details of resistance genes were annotated in the phylogenetic trees using iTOL editor v1_1.

2.7. Analysis of transmission of bla_{NDM} variants by conjugation

Conjugal transfer of different variants of bla_{NDM} to sodium azide-resistant *E. coli* J53 was carried out using a solid mating assay at 37 °C. Isolates co-harbouring $bla_{OXA-181}$ were also tested for conjugal transfer. The transconjugants were recovered from Luria Bertani agar plates supplemented with either: i) sodium azide (100 mg/L) and ertapenem (0.5 mg/L) or ii) sodium azide (100 mg/L) and cefoxitin (10 mg/L) (Sigma-Aldrich) and confirmed for the presence of bla_{NDM} along with other resistance genes by PCR using primers based on earlier reports [11,12]. Plasmid types in transconjugants were identified by PCR-based replicon typing (DIATHEVA, Italy). The transconjugants that harboured similar plasmid incompatibility groups with the same/different bla_{NDM} variants within mother–neonate pairs were processed for plasmid DNA isolation using a PureYield plasmid Midiprep System kit (Promega Corporation, USA) according to the manufacturers' protocol and run on 0.8% agarose gel.

3. Results

3.1. Carriage and characterisation of CPE in mother–neonate pairs: identification, antibiotic susceptibility profile, and STs

A total of 86 pregnant mothers giving birth to 93 neonates who were clinically diagnosed with sepsis (twin deliveries; $n = 7$) were included in the study (Fig. 1). Of them, 44 maternal rectal samples (MR) and 58 neonatal rectal samples (BR) exhibited the presence of carbapenem-resistant Gram-negative bacteria in VE-supplemented chrome agar plates. Twenty MR and 42 BR samples were positive for carbapenemases ($bla_{NDM/OXA-48}$) by PCR. Out of 14 Gram-negative bacteria isolated from neonatal blood samples (BB), 11 were carbapenem-resistant. The overall prevalence of $bla_{NDM/OXA-48}$-positive MR and BR samples was found to be 23% ($n = 20/86$) and 45% ($n = 42/93$) respectively. Carbapenemase-positive Gram-negative bacteria (n=29) isolated from 20 MR samples showing the presence of $bla_{NDM/OXA-48}$ by PCR were distributed as: *E. coli* ($n = 13$), *K. pneumoniae* ($n = 8$), and *Acinetobacter baumannii complex* ($n = 6$). Similarly, carbapenemase-positive Gram-negative bacteria (n=51) isolated from 42 BR samples found positive for $bla_{NDM/OXA-48}$ by PCR exhibited a prevalence of *E. coli* ($n = 21$), *K. pneumoniae* ($n = 15$), and *A. baumannii* ($n = 7$). The distribution of $bla_{NDM/OXA-48}$ producing Gram-negative bacteria primarily found in blood were as follows: *K. pneumoniae* ($n = 5$), *A. baumannii* ($n = 3$), *E. coli* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 1$). Apart from these organisms, other carbapenem-resistant ($bla_{NDM/OXA-48}$-positive) Gram-negative bacteria were also detected (Fig. 1). Eight pairs of mothers and their neonates were found to be colonised with the same carbapenem-resistant species (seven with the same species in their rectal swabs and one involving a swab and the blood of a neonate), suggesting probable mother-to-neonate transmission. Among the CPEs, organisms found among these eight pairs of mother–neonates with the same carbapenem-resistant species were *E. coli* and *K. pneumoniae*. Among these mother–neonate pairs, five pairs were colonised with *K. pneumoniae* ($n = 10$) and three pairs had *E. coli* ($n = 6$). These isolates ($n = 16$) were resistant to carbapenems and other antibiotics *viz*. cephalosporins, aminoglycosides, fluoroquinolones, and sulfonamides except tigecycline and colistin. The MIC of meropenem was distributed between 4 and 128 mg/L. Isolates belonging to pairs MR/BR434, MR/BR548, and MR/BR774 showed differences in susceptibility toward gentamicin, tigecycline, and trimethoprim/sulfamethoxazole, while the other pairs exhibited similar susceptibility profiles (Table 1).

MLST revealed the presence of diverse STs in *E. coli* (ST156, ST405, ST410, ST648, and ST2851) and *K. pneumoniae* (ST11, ST15, ST147, ST567, ST889, and ST1310). *K. pneumoniae* were distributed into the Kp1 phylogroup and *E. coli* belonged to phylogroups B1 ($n = 1$), C ($n = 2$), D ($n = 2$), and F ($n = 1$) (Table 1). Within these study isolates, presence of epidemic clones (*E. coli*- ST156, ST405,

Table 1
Genetic characterisation of carbapenemase-producing Enterobacterales (CPE) isolated from eight mother–neonate pairs.

Patient ID	Carbapenemase-producing organism isolated	ST	Phylogroup	Antibiotic susceptibility	MIC (mg/L)		Carbapenemase present	Resistance genes	Replicon types	cgMLST and pairwise SNP distance	Other carbapenem-resistant Gram-negative bacteria isolated from rectal swab [R] and blood [B] of neonates
					MEM	COL					
IN-MR16	*Klebsiella pneumoniae* (IN-MR16KP)	11	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT-NS	64	0.25	$bla_{\text{NDM-1}}$, $bla_{\text{OXA-181}}$	*aac(6′)-Ib3, aph(3′)-VI,* $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-6}}$, *dfrA14, fosA6, oqxA, oqxB, qnrB1, rmtC,* $bla_{\text{SHV-11}}$, *sul1, tet(A)*	ColKP3, ColRNAI, IncC, IncFIB(K), IncFII(K)	747	*E. coli* [R]
IN-BR16	*Klebsiella pneumoniae* (IN-BR16KP)	1310	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT-NS	128	0.25	$bla_{\text{NDM-7}}$	*aph(6)-Id, aac(6′)-Ib-cr, aac(3)-IId, aph(3′′)-Ib, catB3,* $bla_{\text{CTX-M-15}}$, *dfrA30, fosA5, fosA7,* $bla_{\text{OXA-1}}$, *oqxA, oqxB, qnrB1,* $bla_{\text{SHV-12}}$, *sul2,* $bla_{\text{TEM-1B}}$, *tet(B)*	Col440I, ColpVC, IncFIB(K)(pCAV1099–114), IncFIB(pNDM-Mar), IncHI1B(pNDM-MAR), IncX3	39640*	*P. mosselii* [R] NF [B]
IN-MR42	*E. coli* (IN-MR42EC)	2851	C	CAZ, ETP, IMP, MEM, CN, AK, CIP, SXT-NS; TGC-S	64	0.25	$bla_{\text{NDM-5}}$, $bla_{\text{OXA-181}}$	*aadA2,* $bla_{\text{CMY-42}}$, $bla_{\text{CTX-M-15}}$, *dfrA12, mph(A), qnrS1, rmtB, sul1,* $bla_{\text{TEM-1B}}$, *tet(A)*	ColKP3, IncFIA, IncFII, IncI(Gamma), IncX3	57686	NF [R]
IN-BB42	*E. coli* (IN-BB42EC)	410	C	CAZ, ETP, IMP, MEM, CN, AK, CIP, SXT-NS; TGC-S	4	2	$bla_{\text{NDM-5}}$	*aadA2, dfrA12, rmtB, sul1,* $bla_{\text{TEM-1B}}$, *tet(A)*	Col(IRGK), Col156, IncFIA, IncFIB, IncFII, IncFII(29), IncX4	149299	*A. baumannii* [R]
IN-MR434	*E. coli* (IN-MR434EC)	648	F	CAZ, ETP, IMP, MEM, AK, CIP, SXT- NS; **CN**, TGC- S	64	0.5	$bla_{\text{NDM-5}}$	*aadA2, dfrA12, mph(A), sul1,* $bla_{\text{TEM-1B}}$, *tet(A)*	IncFIA, IncFIB, IncFII	192000	NF [R]
IN-BR434	*E. coli* (IN-BR434EC)	405	D	CAZ, ETP, IMP, MEM, **CN**, AK, CIP, SXT-NS; TGC-S	64	0.5	$bla_{\text{NDM-5}}$	*aadA2,* $bla_{\text{CMY-42}}$, *dfrA12, rmtB, sul1,* $bla_{\text{TEM-1B}}$	Col(MG828), IncFIB(H89-PhagePlasmid), IncFII, IncI(Gamma)	141146	*K. pneumoniae* [B]
IN-MR548	*Klebsiella pneumoniae* (IN-MR548KP)	15	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, **SXT-**NS; TGC (S)	64	0.5	$bla_{\text{NDM-5}}$	*aadA2, aac(6′)-Ib-cr,* $bla_{\text{CTXM-15}}$, *catB3, dfrA12, dfrA14, ermB, fosA6, mph(A),* $bla_{\text{OXA-1}}$, *oqxA, oqxB, rmtB,* $bla_{\text{SHV-28}}$, *sul1,* $bla_{\text{TEM-1B}}$, *tet(D)*	Col(BS512), ColpVC, Col440I, IncFIA(HI1), IncFII, IncFII(K)	39641*	NF [R]
IN-BR548	*Klebsiella pneumoniae* (IN-BR548KP)	889	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP- NS; TGC, **SXT**-S	64	1	$bla_{\text{NDM-1}}$	*aac(6′)-Ib, aac(6′)-Ib-cr, aadA1, aph(3′)-VI,* $bla_{\text{CTX-M-15}}$, *fosA6,* $bla_{\text{OXA-9}}$, *oqxA, oqxB, qnrS1,* $bla_{\text{SHV-108}}$	IncR, IncFIB(K), IncFIB(pQil), IncFII(K)	39642*	*E. coli* [R] NF [B]
IN-MR598	*E. coli* (IN-MR598EC)	405	D	CAZ, ETP, IMP, MEM, CN, AK, CIP, SXT-NS; TGC-S	32	2	$bla_{\text{NDM-5}}$, $bla_{\text{OXA-181}}$	*aph(6)-Id, aph(3′′)-Ib, aadA5,* $bla_{\text{CMY-42}}$, *dfrA17, mph(A), qnrS1, rmtB, sul1, sul2,* $bla_{\text{TEM-1B}}$, *tet(A)*	Col156, ColKP3, IncFIA, IncFIB, IncFII(pRSB107), IncI(Gamma), p0111	194425	NF [R]
IN-BR598	*E. coli* (IN-BR598EC)	156	B1	CAZ, ETP, IMP, MEM, CN, AK, CIP, SXT- NS; TGC-S	128	2	$bla_{\text{NDM-5}}$	*aadA2,* $bla_{\text{CTX-M-15}}$, *catA1, dfrA12, dfrB4, qepA8, rmtB, sul1,* $bla_{\text{TEM-1B}}$, *tet(A)*	IncFIA, IncFII, IncX4	91838	*K. pneumoniae* [B]

(*continued on the next page*)

Table 1
(*continued*)

Patient ID	Carbapenemase-producing organism isolated	ST	Phylogroup	Antibiotic susceptibility	MIC (mg/L)		Carbapenemase present	Resistance genes	Replicon types	cgMLST and pairwise SNP distance		Other carbapenem-resistant Gram-negative bacteria isolated from rectal swab [R] and blood [B] of neonates
					MEM	COL						
IN-MR774	*Klebsiella pneumoniae* (IN-MR774KP)	147	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, SXT- NS; **TGC**-S	64	1	bla_{NDM-7}	*aph(6)-Id, aph(3″)-Ib, aac(6′)-Ib3, aph(3′)-Ia, armA, aadA2,* $bla_{CTX-M-15}$, bla_{CMY-6}, *catA2, dfrA12, fosA, mph(A), mph(E), msr(E), oqxA, oqxB, qnrB1, rmtC,* bla_{SHV-11}, *sul1, sul2,* bla_{TEM-1A}, *tet(A)*	IncC, IncFIB(K), IncFII(K)	39643*		*E. coli* [R]
IN-BR774	*Klebsiella pneumoniae* (IN-BR774KP)	567	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, **TGC**, SXT- NS	32	2	bla_{NDM-1}	*aph(3′)VI, aph(6)-Id, aph(3″)-Ib, aadA1, aac(6′)-Ib,* $bla_{CTX-M-15}$, *fosA6,* bla_{OXA-9}, *oqxA, oqxB, qnrS1,* bla_{SHV-11}, *sul2,* bla_{TEM-1B}	IncFIB(K), IncFIB(pQil), IncFII(K), IncFII(pKP91), IncR	39644*		NF [B]
IN-MR1137	*Klebsiella pneumoniae* (IN-MR1137KP)	147	NF	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC-NS; SXT- S	32	1.5	bla_{NDM-1}	*ARR-3, aadA1, aac(6′)-Ib3, aph(3′)-VI,* $bla_{CTX-M-15}$, *catB3, fosA,* bla_{OXA-1}, bla_{OXA-9}, *oqxA, oqxB, qnrS1,* bla_{SHV-11}, *sul1,* bla_{TEM-1A}	IncFIB(pKPHS1), IncFIB(pQil), IncR	ND	1 SNP (27 insertions or deletions) among each other	NF [R]
IN-BR1137	*Klebsiella pneumoniae* (IN-BR1137KP)	147	NF	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC-NS; SXT- S	16	1.5	bla_{NDM-1}	*ARR-3, aadA1, aac(6′)-Ib-cr, aac(6′)-Ib3, aph(3′)-VI,* $bla_{CTX-M-15}$, *catB3, fosA,* bla_{OXA-1}, bla_{OXA-9}, *oqxA, oqxB, qnrS1,* bla_{SHV-11}, *sul1,* bla_{TEM-1A}	IncFIB(pKPHS1), IncFIB(pQil), IncR	ND		NF [B]
IN-MR1225	*Klebsiella pneumoniae* (IN-MR1225KP)	15	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT- NS	4	2	bla_{NDM-1}	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIa, aac(6′)-Ib3, armA, aadA1,* $bla_{CTX-M-15}$, *dfrA14,* bla_{DHA-1}, *mph(E), msr(E),* bla_{OXA-9}, *oqxA, oqx*B, *qnrB1,* bla_{SHV-28}, *sul1, sul2,* bla_{TEM-1A}, *tet(A)*	Col440I, ColpVC, IncFIB(K), IncFIB(pKPHS1), IncFII(K), IncHI1A, IncHI1B(R27)	39645*	3904 SNP variants among each other	*A. baumannii* [R]
IN-BR1225	*Klebsiella pneumoniae* (IN-BR1225KP)	15	Kp1	CAZ, ETP, IMP, MEM, CN, AK, CIP, TGC, SXT- NS	32	2	bla_{NDM-5}	*aadA2, aac(6′)-Ib-cr,* $bla_{CTX-M-15}$, *catB3, dfrA12, dfrA14, fosA6,* bla_{OXA-1}, *oqxA, oqxB, rmtB,* bla_{SHV-28}, *sul1,* bla_{TEM-1B}, *tet(D)*	Col(BS512), ColpVC, Col440I, IncFIA(HI1), IncFII, IncFII(K), IncR	39646*		NF [B]

Differences in the susceptibility pattern of antibiotics within individual mother–neonate pairs are marked in bold (IN-MR/BR434EC, IN-MR/BR548KP and IN-MR/BR774KP.).
Sequence typing (ST) schemes: the Warwick scheme for *E. coli*, Pasteur scheme for *K. pneumoniae* were used.
*A novel cgMLST (core genome multilocus sequence typing) values *for K. pneumoniae.* Pairwise single-nucleotide polymorphism distance was performed for mother–neonate pairs with the same ST only. For IN-MR/BR1137KP pair, cgMLST value could not be obtained, as few loci were missing from the complete genome assemblies.
AK: Amikacin; BB: Neonatal blood samples; BR: Neonatal rectal samples; CAZ: Ceftazidime; CIP: Ciprofloxacin; CN: Gentamicin; COL: Colistin; ETP: Ertapenem; IMP: Imipenem; IN: India; MEM: Meropenem; MIC: Minimum inhibitory concentration; MR: Maternal rectal samples; ND: Not determined; NF: Not found; NS: Non-susceptible; S: Susceptible; SXT: Trimethoprim/sulfamethoxazole.

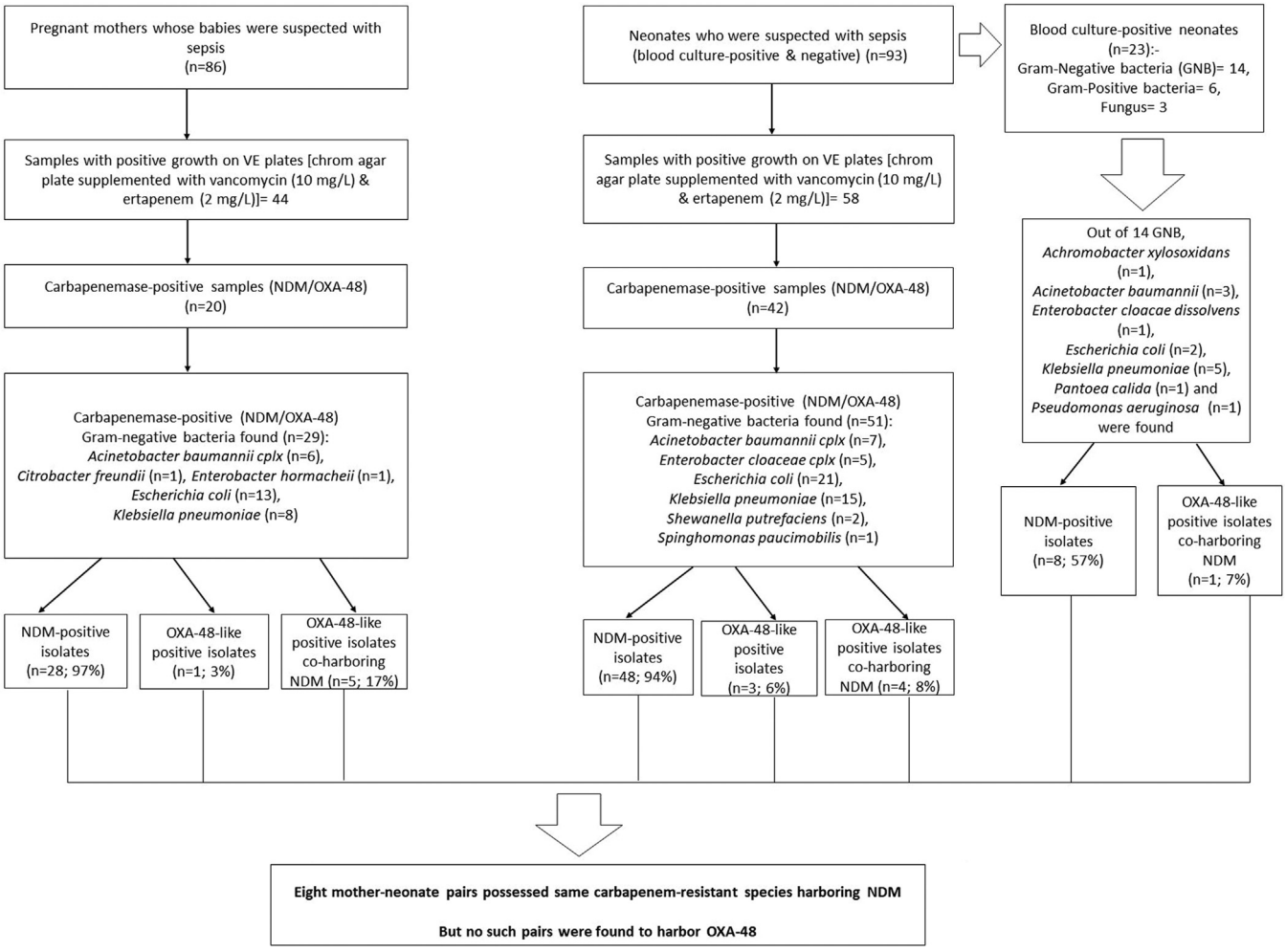

Fig. 1. Flowchart representing the enrolment of pregnant women and respective neonates, isolation of carbapenem-resistant Gram-negative bacteria and selection of mother–neonate pairs harbouring carbapenemase-producing Enterobacterales (CPE) for further analysis.

ST410 and *K. pneumoniae*- ST15, ST147) were mostly found in BR isolates (Table 1).

3.2. Resistance determinants and diversity of plasmid replicons among mother–neonate pairs

CPE isolates exhibited two types of carbapenemases: bla_{NDM} ($n = 16$; bla_{NDM-1} [$n = 6$], bla_{NDM-5} [$n = 8$], bla_{NDM-7} [$n = 2$]) and $bla_{OXA-181}$ ($n = 3$). None of them harboured bla_{KPC}. *K. pneumoniae* ($n = 10$) showed diverse bla_{NDM} variants (bla_{NDM-1} [$n = 6$], bla_{NDM-5} [$n = 2$], and bla_{NDM-7} [$n = 2$]), while *E. coli* ($n = 6$) harboured only bla_{NDM-5}. $bla_{CTX-M-15}$ ($n = 12$) was the dominant ESBL detected in the isolates. Additionally, $bla_{OXA-181}$ was detected in bla_{NDM}-harbouring *K. pneumoniae* ($n = 1$) and *E. coli* ($n = 2$). Other resistance determinants such as genes conferring resistance to fluoroquinolones, aminoglycosides, phenicols, sulphonamides, and so on were present in different combinations in *E. coli* and *K. pneumoniae* (Table 1).

Various replicons were detected in CPEs (Table 1). The predominant plasmid types detected were IncFII and IncFII(K) ($n = 7$) > IncFIA, IncFIB(K), and IncR ($n = 5$) > IncFIB (pQIL) ($n = 4$). IncFII(K) ($n = 7$) and IncFIB(K) ($n = 5$) were prevalent in *K. pneumoniae*, whereas IncFIA-IncFII ($n = 5$) and IncFIB ($n = 3$) were prevalent in *E. coli*. Isolates possessing $bla_{OXA-181}$ co-existing with bla_{NDM} (IN-MR16KP, IN-MR42EC, and IN-MR598EC) additionally harboured the ColKP3 plasmid (Table 1).

3.3. Genetic context of bla_{NDM} and $bla_{OXA-181}$ in CPE

All $bla_{NDM-1/5}$-positive CPEs throughout this study showed a similar genetic context around the bla_{NDM} gene, whereas the genetic environment of bla_{NDM-7} was different. Isolates revealed a conserved genetic environment of $bla_{NDM-1,5}$ comprising IS*Aba125* (full or truncated) (upstream) and ble_{MBL}-*trpF-dsbD* (downstream). Additionally, IS*630-tnpA* was identified at the 5′-end of bla_{NDM-1} in the IN-MR1137/BR1137 pair and in IN-BR774KP. In bla_{NDM-5}-possessing *E. coli*, *dsbD* was followed by IS*91*-like IS*CR1 tnpA*, whereas various IS elements, such as IS*CR27* or IS*6*-like IS*26 tnpA* or IS*91*-like IS*CR1 tnpA*, were found downstream of bla_{NDM-1} in *K. pneumoniae*. *sul1-qacE-aadA-DUF1010-dfrA12-intI1* present on bla_{NDM-5}-harbouring plasmids implied the presence of class 1 integrons. bla_{NDM-7} was preceded by intact/truncated IS*5-tnpA* (Fig. 2a).

For isolates possessing $bla_{OXA-181}$, the ARGs were bracketed between IS*Ecp1* (full) upstream, while Δ*ereA* and Col replicase (*repA*) was downstream, except for one isolate (IN-MR598EC) where Tn*3*-like IS*3000* was located upstream of $bla_{OXA-181}$ (Fig. 2b).

3.4. Genetic relatedness of bla_{NDM}-positive CPEs and assessment of similarity within mother–neonate isolate pairs

PFGE showed mostly distinct pulsotypes within eight mother–neonate isolate pairs, of which IN-MR1137KP and IN-BR1137KP were clonal (Fig. 3a). The PFGE data of these clonal isolates was

Fig. 2. Schematic diagram of the genetic environment of carbapenemase genes found in the study isolates. (a) $bla_{NDM-1/5/7}$ and (b) $bla_{OXA-181}$. Coloured arrows represent open reading frames. Genes are abbreviated according to their corresponding proteins—*trpF*: N-(5′-phosphoribosyl) anthranilate isomerase; *dsbD*: thiol:disulfide interchange protein; *cutA*: divalent-cation tolerance protein; *groES, groEL*: heat-chaperonin protein; *tnpA*: transposase; IS: insertion sequence; *sul1*: dihydropteroate synthase; *qacE*: quaternary ammonium compound-resistance protein; *aadA*: aminoglycoside (3'') (9) adenylyl transferase; *dfr*: dihydrofolate reductase; *intI1*: class 1 integrase; *mph(A)*: macrolide 2′-phosphotransferase; *aph(3′)-VI*: aminoglycoside 3′-phosphotransferase; *armA*: 16S rRNA (guanine(1405)-N(7))-methyltransferase; *mobA, mobC*: mobilization relaxosome proteins; *vbhA*: antitoxin; Δ*ereA*: truncated erythromycin esterase; *qnrS1*: quinolone resistance determinant; ble_{MBL}: bleomycin resistance gene; *hyp*: hypothetical protein; Δ: truncation. Due to the constraint of contig size because of short-read sequencing, we could not explore the other regions of the plasmid harbouring the carbapenemase genes.

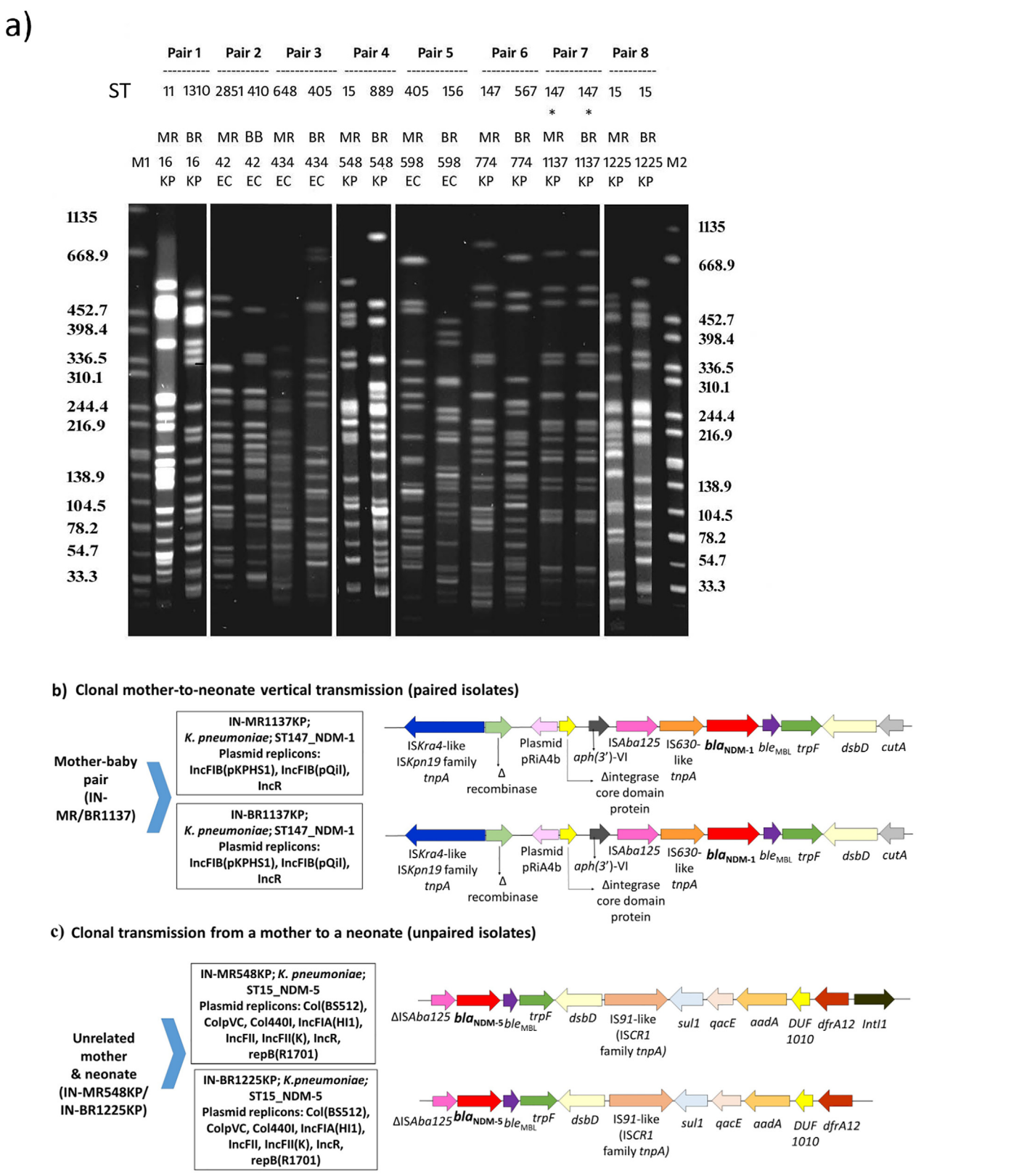

Fig. 3. Molecular typing of similar carbapenemase-producing Enterobacterales (CPE) isolated from mother–neonate pairs and genomic comparison of pairs exhibiting probable transmission events using (a) pulsed-field gel electrophoresis fingerprint pattern of *XbaI* digested bacterial DNA of eight mother–neonate pair CPE isolates. Lanes 1 and 18: *Salmonella* serotype Braenderup H9812 as the reference standard (band sizes are denoted in kilobases); Lane 2–17: isolates of individual mother–neonate pairs (Pair 1: MR16KP and R16KP; Pair 2: MR42EC and BB42EC; Pair 3: MR434EC and BR434EC; Pair 4: MR548KP and BR548KP; Pair 5: MR598EC and BR598EC; Pair 6: MR774KP and BR774KP; Pair 7: MR1137KP and BR1137KP; Pair 8: MR1225KP and BR1225KP); *Mother–neonate pairs that are clonal or have indistinguishable band pattern. (b) Schematic representation of genetic environment of bla_{NDM-1}-possessing *K. pneumoniae* in a clonal mother–neonate pair (IN-MR1137 and IN-BR1137). (c) Schematic representation of the genetic environment of bla_{NDM-5}-possessing *K. pneumoniae* in an unrelated mother and neonate (IN-MR548KP and IN-BR1225KP). Genes and their transcription orientations are indicated by horizontal arrows. *trpF*: N-(5′-phosphoribosyl) anthranilate isomerase; *dsbD*: thiol:disulfide interchange protein; *cutA*: divalent-cation tolerance protein; IS: insertion sequence; *sul1*: dihydropteroate synthase; *qacE*: quaternary ammonium compound-resistance protein; *aadA*: aminoglycoside (3′′) (9) adenylyl transferase; *dfr*: dihydrofolate reductase; *intI1*: class 1 integrase; *aph(3′)-VI*: aminoglycoside 3′-phosphotransferase; ble_{MBL}: bleomycin resistance gene; *tnpA*: transposase; Δ: truncation.

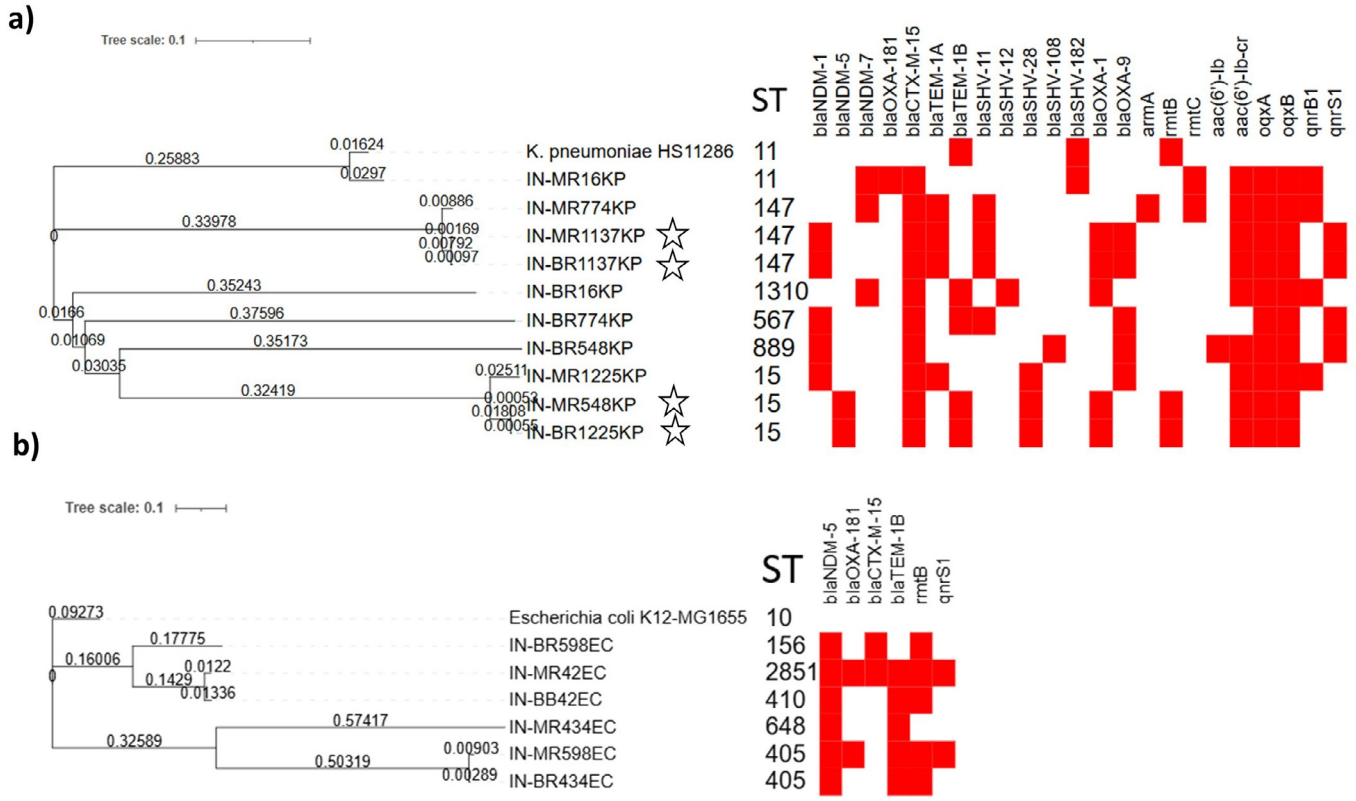

Fig. 4. Core genome phylogeny of bla_{NDM}-positive *E. coli* and *K. pneumoniae* isolated from rectal swabs (IN-MR/BR) and blood (IN-BB) of mother–neonate pairs. The sequence types of *E. coli* (Warwick scheme) and *K. pneumoniae* (Pasteur scheme) are mentioned for all isolates. The presence (*red colour*) or absence (*white colour*) of acquired resistance genes is depicted in the figure. Branch lengths have been depicted in the figure indicating evolutionary relationships between the isolates. Star shapes indicate a close relationship between mother–neonate pair isolates (IN-MR/BR1137KP) and unpaired isolates (IN-MR548KP and IN-BR1225KP). *E. coli* K12-MG1655 and *K. pneumoniae* HS11286 are used as references.

validated by comparison of the genomes; that is, both isolates belonged to ST147 and were 1 SNP distant from each other and had 27 insertions/deletions. The ANI and mash distance between the two genomes was found to be 99.98% and 996/1000, respectively, suggesting a high degree of genomic similarity. They also showed similar resistance (bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{SHV-11}, bla_{OXA-1}, bla_{TEM-1A}, *oqxA, oqxB, qnrS1*) and replicon profiles: IncFIB (pQil), IncFIB (pKPHS1), and IncR. Furthermore, the genetic context of bla_{NDM-1} (IS*630*-like transposase preceded by IS*Aba125* [5′-end] and ble_{MBL}-*trpF-dsbD-cutA* [3′-end]) was similar in both the isolates (Fig. 3b and Table 1). These findings suggest acquisition of the same strain by vertical transmission to the neonate from the mother.

Aside from this, two unpaired isolates (IN-MR548KP and IN-BR1225KP) were noted that belonged to ST15, exhibited indistinguishable PFGE band patterns, showed a similar resistance and replicon profile, and also displayed an identical genetic background for bla_{NDM-5} (Fig. 3a, c and Table 1). These isolates were 25 SNPs distant across the genome with a genomic mash distance of 1000/1000, perhaps suggesting circulation of the same strain within the hospital environment. Additionally, the MR sample (IN-MR42) whose species matched with the species of the neonatal blood sample (IN-BB42) was analysed and found to be distinct as per PFGE (Fig. 3a). Hence, no evidence of transmission was noted in this pair.

The core genome phylogenetic tree (Fig. 4) represents diversity among NDM-containing *E. coli* and *K. pneumoniae* isolates. Within the *K. pneumoniae* phylogenetic clade, two different clusters were observed- i) the mother–neonate paired cluster- IN-MR/BR1137KP (ST147) forming a subclade with IN-MR774KP (ST147); ii) unpaired cluster—IN-MR548KP and IN-BR1225KP (ST15) forming subclade with IN-MR1225KP (ST15) (Fig. 4a). These clusters matched with the band patterns of PFGE (Fig. 3). Among the *E. coli* clades, IN-MR42EC (ST2851) with BB42EC (ST410) and IN-MR598EC (ST405) with IN-BR434EC (ST405) exhibited phylogenetic relatedness, corroborating with PFGE results (Fig. 3 and Fig. 4b).

3.5. Comparison of bla_{NDM}-possessing plasmids among mothers and neonates

A comparison of the bla_{NDM}-harbouring plasmids was undertaken as most of the isolates between the individual mother–neonate pairs were distinct (except for one pair). Initially, conjugation and plasmid replicon typing were carried out to evaluate the transmissibility and similarity of plasmid replicon types between individual pairs. All plasmids possessing bla_{NDM} variants in *E. coli* and *K. pneumoniae* were found to be conjugative except for one, which was non-conjugative (IN-MR42EC) (Table 2). The bla_{NDM} variants ($bla_{NDM-1/5/7}$) were compared and of the eight pairs, three pairs harboured bla_{NDM-5} in *E. coli* and one pair carried bla_{NDM-1} in *K. pneumoniae*. The remaining pairs had different bla_{NDM} variants; that is, bla_{NDM-1} and bla_{NDM-5} in two pairs and bla_{NDM-1} and bla_{NDM-7} in two pairs in *K. pneumoniae* (Table 1). A variety of plasmid incompatibility types, such as IncFII, IncFIIK, IncFIBKQ, IncFIA, IncFIB, IncA/C, IncX3, and IncR, were detected in bla_{NDM} transconjugants. In bla_{NDM-5}-possessing transconjugants, IncFII ($n = 7$) was predominant while IncFIIK ($n = 4$) was predominant in bla_{NDM-1}-carrying transconjugants. IncX3 ($n = 1$) and IncA/C ($n = 1$) were found in bla_{NDM-7}-harbouring transconjugants (Table 2).

To understand the similarity of bla_{NDM}-possessing plasmids within individual pairs, plasmids were characterised via replicon types, pMLST-IncF type and plasmid size. Plasmid replicon types and pMLST of IncF plasmids varied in the majority of pairs.

Table 2
Characteristics of carbapenemase-producing Enterobacterales (CPE) and its transconjugants among mother–neonate pairs harbouring different NDM variants.

Mother–neonate pair	Isolate ID	Carbapenemase present	Other resistance genes present/transferred (PCR)	Plasmid replicon types present (WGS) /transferred (PBRT)	Plasmid MLST (pMLST) (FAB formula)	Plasmid DNA isolated from transconjugants with the same incompatibility groups within the pairs
Pair 1	IN-MR42EC	bla_{NDM-5}, $bla_{OXA-181}$	bla_{TEM}, bla_{CIT}, *qnrS, rmtB*	IncFIA, IncFII, IncI(Gamma), IncX3	[F2:A-:B-]	
	Conjugation unsuccessful					
	IN-BB42EC	bla_{NDM-5}	bla_{TEM}, *rmtB, armA, oqxB, qnrB, qnrS*	Col (IRGK), Col156, IncFIA, IncFIB, IncFII, IncFII(29), IncX4	[F31:A4:B1]	
	IN-BB42EC.TC1	bla_{NDM-5}	bla_{TEM}, *rmtB*	IncFII	Not done	No
Pair 2	IN-MR434EC	bla_{NDM-5}	bla_{TEM}, bla_{CIT}	IncFIA, IncFIB, IncFII	[F36:A1:B1]	
	IN-MR434EC.TC1	bla_{NDM-5}	bla_{TEM}	IncFIA, IncFIB, IncFII	Not done	Yes
	IN-BR434EC	bla_{NDM-5}	bla_{TEM}, *rmtB*	Col (MG828), IncFIB(H89-PhagePlasmid), IncFII, IncI(Gamma)	[F2:A-:B-]	
	IN-BR434EC.TC1	bla_{NDM-5}	bla_{TEM}, *rmtB*	IncFII	Not done	Yes
Pair 3	IN-MR598EC	bla_{NDM-5}, $bla_{OXA-181}$	bla_{TEM}, bla_{CIT}, *rmtB, qnrS*	Col156, ColKP3, IncFIA, IncFIB, IncFII(pRSB107), IncI(Gamma), p0111	[F1:A1:B16]	
	IN-MR598EC.TC1	bla_{NDM-5}, $bla_{OXA-181}$	bla_{TEM}, *rmtB, qnrS*	IncFIA, IncFIB, IncFII, ColKP3	Not done	Yes
	IN-BR598EC	bla_{NDM-5}	bla_{CTX-M}, bla_{TEM}, *rmtB*	IncFIA, IncFII, IncX4	[F2:A4:B-]	
	IN-BR598EC.TC1	bla_{NDM-5}	bla_{TEM}, *rmtB*	IncFII	Not done	Yes
Pair 4	IN-MR16KP	bla_{NDM-1}, $bla_{OXA-181}$	bla_{CTX-M}, bla_{CIT}, *rmtC, oqxAB, qnrB, aac(6′)-Ib-cr*	ColKP3, ColRNAI, IncC, IncFIB(K), IncFII(K)	[K1:A-:B-]	
	IN-MR16KP.TC1	bla_{NDM-1}, $bla_{OXA-181}$	bla_{CTX-M}, bla_{CIT}, *rmtC, oqxAB, aac(6′)-Ib-cr*	IncA/C, IncFIBKN, IncFII(K), ColKP3	Not done	No
	IN-BR16KP	bla_{NDM-7}	bla_{CTX-M}, bla_{TEM}, bla_{OXA-1}, *oqxAB, qnrB, aac(6′)-Ib-cr*	Col440I, ColpVC, IncFIB(K)(pCAV1099–114), IncFIB(pNDM-Mar), IncHI1B(pNDM-MAR), IncX3	[F-:A-:B-]	
	IN-BR16KP.TC1	bla_{NDM-7}	bla_{TEM}, bla_{OXA-1}	IncX3	Not done	No
Pair 5	IN-MR548KP	bla_{NDM-5}	bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *aac(6′)-Ib-cr*	Col (BS512), ColpVC, Col440I, IncFIA(HI1), IncFII, IncFII(K), IncR	[F2:A13:B-]	
	IN-MR548KP.TC1	bla_{NDM-5}	bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *aac(6′)-Ib-cr*	IncFII, IncFII(K), IncR	Not done	Yes
	IN-BR548KP	bla_{NDM-1}	bla_{CTX-M}, *oqxAB, qnrS, aac(6′)-Ib*	IncFIB(K), IncFIB(pQil), IncFII(K)	[K2:A-:B-]	
	IN-BR548KP.TC1	bla_{NDM-1}	bla_{CTX-M}, aac*(6′)-Ib*	IncFII(K)	Not done	Yes
Pair 6	IN-MR774KP	bla_{NDM-7}	bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{CIT}, *armA, rmtC, oqxAB, qnrB, qnrS*	IncC, IncFIB(K), IncFII(K)	[K2:A-:B-]	
	IN-MR774KP.TC1	bla_{NDM-7}	bla_{TEM}, bla_{CIT}, *rmtC, oqxAB*	IncA/C	Not done	No
	IN-BR774KP	bla_{NDM-1}	bla_{CTX-M}, bla_{TEM}, *oqxAB, qnrS*	IncFIB(K), IncFIB(pQil), IncFII(K), IncFII (pKP91), IncR	[K2:A-:B-]	
	IN-BR774KP.TC1	bla_{NDM-1}	bla_{CTX-M}, bla_{TEM}, *qnrS*	IncFIBKQ, IncFII(K), IncFII	Not done	No
Pair 7	IN-MR1137KP	bla_{NDM-1}	bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *oqxAB, qnrS, aac(6′)-Ib, aac(6′)-Ib-cr*	IncFIB(pKPHS1), IncFIB(pQil), IncR	[F-:A-:B-]	
	IN-MR1137KP.TC1	bla_{NDM-1}	bla_{TEM}, bla_{OXA-1}, *qnrS, aac(6′)-Ib, aac(6′)-Ib-cr*	IncFIBKQ, IncR	Not done	No
	IN-BR1137KP	bla_{NDM-1}	bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *oqxAB, qnrS, aac(6′)-Ib, aac(6′)-Ib-cr*	IncFIB (pKPHS1), IncFIB(pQil), IncR	[F-:A-:B-]	
	IN-BR1137KP.TC1	bla_{NDM-1}	bla_{TEM}, bla_{OXA-1}, *qnrS, aac(6′)-Ib, aac(6′)-Ib-cr*	IncFIBKQ, IncR	Not done	No

(*continued on next page*)

Table 2 (*continued*)

Mother–neonate pair	Isolate ID	Carbapenemase present	Other resistance genes present/transferred (PCR)	Plasmid replicon types present (WGS) /transferred (PBRT)	Plasmid MLST (pMLST) (FAB formula)	Plasmid DNA isolated from transconjugants with the same incompatibility groups within the pairs
Pair 8	IN-MR1225KP	bla_{NDM-1}	bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{DHA}, *armA, oqxAB, qnrB, aac(6′)-Ib-cr*	Col440I, ColpVC, IncFIB(K), IncFIB(pKPHS1), IncFII(K), IncHI1A, IncHI1B (R27)	[K7:A-:B-]	
	IN-MR1225KP.TC1	bla_{NDM-1}	bla_{TEM}, *oqxAB, aac(6′)-Ib-cr*	IncFII(K)	Not done	Yes
	IN-BR1225KP	bla_{NDM-5}	bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *rmtB, aac(6′)-Ib-cr*	Col (BS512), ColpVC, Col440I, IncFIA(HI1), IncFII, IncFII(K), IncR	[F2:A13:B-]	
	IN-BR1225KP.TC1	bla_{NDM-5}	bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *rmtB, aac(6′)-Ib-cr*	IncFII, IncFII(K), IncR	Not done	Yes

bla_{TEM} is present in *E. coli* J53, which was used as a recipient in conjugation experiments. Thus, the presence of bla_{TEM} in transconjugants (in this table) does not necessarily represent the true transmission of bla_{TEM} from donor to recipient (*E. coli* J53) and it may also represent the intrinsic bla_{TEM} of *E. coli* J53.
EC: *Escherichia coli*; FAB formula: F refers to IncFII, A and B refers to IncFIA, and IncFIB respectively; KP: *Klebsiella pneumoniae*; MLST: Multilocus sequence typing; PBRT: PCR-based replicon typing ; TC: Transconjugants; WGS: Whole-genome sequencing.

For most pairs, the distribution of resistance genes in mother–neonate pairs was different (Table 2). Plasmids were isolated from the transconjugants of those pairs that harboured similar plasmid incompatibility groups and carried the same bla_{NDM} variants (IN-MR434EC/BR434EC, IN-MR598EC/BR598EC) or different bla_{NDM} variants (IN-MR548KP/BR548KP, IN-MR1225KP/BR1225KP). Differences in plasmid size as well as in pMLSTs were noted within the pairs irrespective of bla_{NDM} variant (Table 2 and Fig. 5a, b). No plasmid transmission was observed, indicating the independent acquisition of plasmids.

Aside from a comparison of plasmids between individual mother–neonate pairs, analysis of pMLST using whole-genome sequencing (WGS) data suggested that F2:A-:B-/F2:A13:B- and K2:A-:B- (FAB formula) were the common plasmid types associated with bla_{NDM-5} and $bla_{NDM-1/7}$, respectively (Table 2).

4. Discussion

The bla_{NDM} gene is endemic in India [7], and with the gut being a potential reservoir of the prevailing antibiotic resistance genes [3], gut samples were evaluated for the presence of carbapenem-resistant organisms. This study assessed the similarity of CPE from samples collected from mothers and their neonates, with eight mother–neonate pairs having similar bacterial species. However, further molecular (PFGE) and genomic analysis revealed that the majority of carbapenemase-producing maternal and neonatal gut isolates were distinct. PFGE and whole-genomic analysis herein support one case of vertical transmission from mother to neonate at birth in a pre-term very low-birth-weight neonate. However, it is also plausible that the acquisition occurred from exogenous sources that have not been investigated.

Similar studies performed either in developing countries [22,26–28] or in developed countries [21,29–31] have assessed the vertical transmission of drug-resistant (ESBL/carbapenemase-producing/MDR) bacteria from mother to newborn. Some studies assessed the similarity of the mother or neonatal isolate by comparison of AMR profiles [32,33] and others evaluated isolates using genomic [19,20,22,26,28,34] or molecular analysis [31,35–38]. A meta-analysis by Bulabula et al. [18] reported 27% transmission of MDR Gram-negative bacteria from colonised mothers to neonates. However, most studies published later have found a low prevalence of vertical transmission of maternal gut colonisation in mother–neonate pairs [20,22,26,28]. The present study also noted a single case where the organism isolated from mother and neonate was similar. The present study was a part of the BARNARDS study [22], where carriage of carbapenem-resistant bacteria in mothers and neonates from this study site was high (bla_{NDM} [42%] and bla_{OXA-48} [7%] in neonates; bla_{NDM} [8%] and bla_{OXA-48} [2%] in mothers. Despite the high carriage of carbapenemases in individual mothers and neonates, transmission was limited. Out of 86 mothers in this study, 67% had undergone caesarean deliveries and the rest had vaginal deliveries (33%). We hypothesise that fewer cases of vaginal delivery could have reduced the transmission of flora from mother to neonate as the vaginal mode of delivery predisposes the neonate to acquisition of maternal flora as the baby passes through the birth canal [39]. In addition, the study focused on the transmission of CPEs, excluding all isolates (or their transmission thereof) that were not resistant to carbapenems.

As most of the isolates within mother–neonate pairs were diverse, we also tried to understand the transmission of bla_{NDM}-possessing plasmids between the isolates within the pairs. No similarity in the plasmids was observed as per pMLST (IncF) through WGS analysis in the majority of the pairs. Additionally, the plasmid sizes isolated from the transconjugants with the same plasmid incompatibility groups, irrespective of bla_{NDM} variants within the pairs, varied. Thus, plasmid transmission within individual mother–neonate pairs was not observed. Although in individual pairs the plasmid backbone was different, overall the three commonly identified bla_{NDM} variants were $bla_{NDM-1/5/7}$. bla_{NDM-5} was predominant followed by bla_{NDM-1} and bla_{NDM-7}. These bla_{NDM} variants were detected in different plasmid scaffolds predominantly residing in the IncFII and IncFII(K) conjugative plasmids. Additionally, IncFIA, IncFIB(K), IncFIB (pQIL), and IncR were common in the isolates. Also, few replicons such as IncX3, IncFIA, IncFIB, IncFII, IncI (gamma), IncFIB(K), IncFIB (pQil), IncFII(K), IncR, repB, and different Col replicons were common between mothers and neonates. Analysis of bla_{NDM} transconjugants revealed a probable association of bla_{NDM} variants with predominantly IncFII and IncFIIK plasmids. IncFII and IncFIIK are large, self-transmissible conjugative plasmids, prevalent in Southeast Asia and have the potential to disseminate resistance genes across diverse species, as reported in earlier studies [12,40]. The genetic context of bla_{NDM} variants and the replicon types detected in the study isolates were similar to bla_{NDM}-harbouring isolates reported in previous studies [7,41]. Some isolates possessed $bla_{OXA-181}$ in addition to bla_{NDM}, were found to be associated with the ColKP3 plasmid, and had a

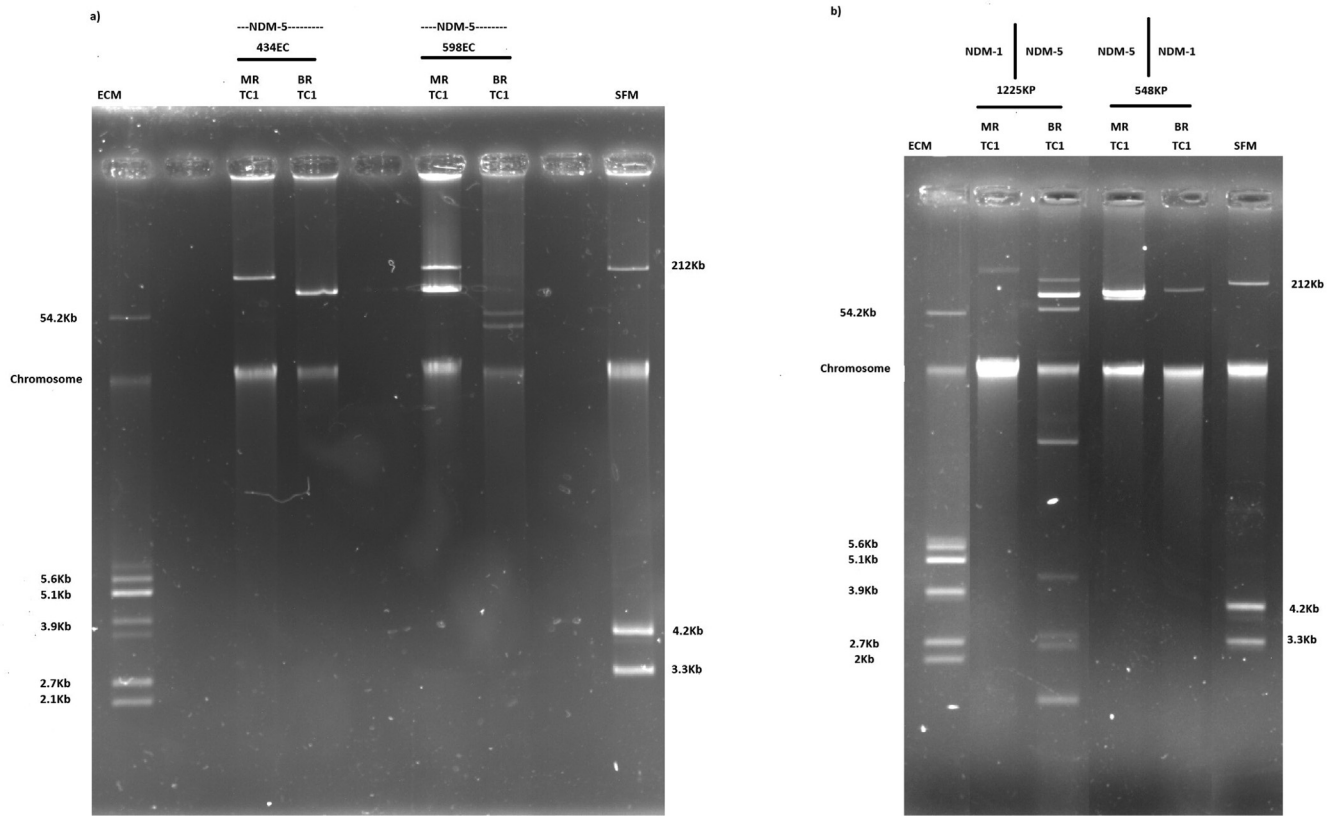

Fig. 5. Comparison of plasmid profiles of transconjugants from mothers and neonates harbouring NDM variants within the same plasmid incompatibility groups in (a) *E. coli* and (b) *K. pneumoniae*. (a) Lane 1: ECM-*Escherichia coli* K12 V517 marker; lane 3 and 4: plasmid DNA isolated from transconjugants of *E. coli* harbouring $bla_{\text{NDM-5}}$ in mother (MR434EC: IncFIA-FIB-FII) and neonate (BR434EC: IncFII); lane 6 and 7: plasmid DNA isolated from transconjugants of *E. coli* harbouring $bla_{\text{NDM-5}}$ in a mother (MR598EC: IncFIA-FIB-FII) and neonate (BR598EC: IncFII); lane 9: SFM: *The Shigella flexneri* YSH6000 marker was used as a guide to molecular size. (b) Lane 1: ECM-*Escherichia coli* K12 V517 marker; lane 2 and 3: plasmid DNA isolated from transconjugants of *K. pneumoniae* harbouring $bla_{\text{NDM-1}}$ in a mother (MR1225KP: IncFIIK) and $bla_{\text{NDM-5}}$ in a neonate (BR1225KP: IncFII-FIIK-R); lane 4 & 5: plasmid DNA isolated from transconjugants of *K. pneumoniae* harbouring $bla_{\text{NDM-5}}$ in a mother (MR548KP: IncFII-FIIK-R) and $bla_{\text{NDM-1}}$ in a neonate (BR548KP: IncFIIK); lane 6: *Shigella flexneri* YSH6000 marker was used as a guide to molecular size.

similar genetic background surrounding $bla_{\text{OXA-181}}$, as reported previously [42].

Aside from an understanding of carbapenem-resistant genomes and their similarities, our study also elucidated the diversity of *E. coli* and *K. pneumoniae*. In this study, the majority of *K. pneumoniae* isolates detected within the gut of mothers and neonates were of diverse STs (ST11 [$n = 1$], ST15 [$n = 3$], ST147 [$n = 3$], ST567 [$n = 1$], ST889 [$n = 1$], and ST1310 [$n = 1$]) and belonged to the Kp1 phylogroup, including high-risk AMR clones [43] such as ST11, ST15, and ST147. This finding aligns with previous reports indicating that Kp1 is the dominant phylogroup among *K. pneumoniae* strains colonising the human gut [44]. Previous studies have reported the colonisation of high-risk *K. pneumoniae* clones (ST11, ST15, and ST147) in the human gut, with ST15 recognised as an efficient coloniser [20,45–47]. The association between the Kp1 phylogroup and ST15 had been observed in pregnant women from low-income countries in a previous study [45]. These results suggest that the carriage of Kp1 *K. pneumoniae* is more common in the human gut and its association with high-risk AMR clones increases the likelihood of infections. Colonisation of neonates with such epidemic clones may predispose neonates to subsequent infections.

In the case of *E. coli*, five different STs (ST156 [$n = 1$], ST405 [$n = 2$], ST410 [$n = 1$], ST648 [$n = 1$], and ST2851 [$n = 1$]) were detected in the gut of mothers and neonates. Of them, ST156, ST405, ST410, and ST648 are epidemic clones reported worldwide [48] and found to be associated with $bla_{\text{NDM-5}}$ in this study. These high-risk clones disseminate globally, are often associated with resistance and virulence genes, and are able to colonise or persist within hosts, causing recurrent infections [48]. In this study ST2851 was found in one maternal gut sample, a rarely reported ST, which was previously detected in a urinary tract sample from India [49]. Previous studies have also reported carriage of carbapenem-resistant genes ($bla_{\text{NDM-5}}$, bla_{KPC}) in epidemic clones of *E. coli* in the gut of healthy adults [50,51]. *E. coli* belonged to diverse phylogroups (A, B1, B2, C, D, E, F), of which A and B1 are predominantly found in the gut as commensals [52]. Previous studies had also reported the presence of phylogroups B1, C, D, E, and F in the human gut [53–56]. Similarly, the carbapenem-resistant *E. coli* isolates in this study belonged to four phylogroups (B1 [$n = 1$], C [$n = 2$], D [$n = 2$], and F [$n = 1$]).

As discussed above, there are differences in the rates of mother-to-neonate transmission in different studies. These differences may arise because of sampling strategy, the carriage of resistant bacteria, the predominant mode of delivery, and so on. Our dataset was limited as we evaluated only the transmission of carbapenem-resistant bacteria. Isolates were pre-selected from bacteria cultured in the presence of ertapenem, thus they may not represent a true population survey. Although we isolated the plasmids for analysis, we did not have access to long-read sequencing to allow the construction of plasmid sequences and plasmid similarities were based on pMLST. The findings suggest the presence of genetically similar bacteria in mothers and unpaired neonates which indicates that the hospital environment might be the source of transmission

of CPE. However, local hospital surface swabs were not collected to compare the bacteria colonising surfaces to determine an alternative source of acquisition.

Although some studies have implicated a higher rate of mother–neonate transmission through vaginal delivery [57,58] the single case of similar maternal and neonatal bacterial strain (ANI 99.98%, one SNP) identified in this study was from a neonate delivered via caesarean section. Similar types of observations were made in some studies where no mother–neonate transmission was noted [27,34]. A cumulative PFGE and WGS approach has enhanced the clarity of our data by increasing the discriminatory interpretation of results [59]. Future research should collect data to allow deeper analyses that incorporate appropriate transmission modelling approaches with microbial genomics.

5. Conclusion

Despite having a high carriage of bla_{NDM}-producing carbapenem-resistant bacteria in the gut of mothers and neonates, only limited evidence supporting mother-to-neonate transmission was identified. Although neonates carried the same carbapenem-resistant species as that of their mother, they were genetically different, which indicated that these bacteria are mostly acquired from the environment. Diverse STs of *E. coli* and *K. pneumoniae* were identified with the presence of different NDM variants, and extensive AMR genes suggested that hospital and other external sources might be responsible for acquisition of CPE in the gut. The presence of CPE, some being epidemic clones, in maternal and neonatal rectal samples increases the probability of spread of such organisms within a hospital setting and later on discharge in environments beyond the hospitals. Gut specimens thus need screening for such organisms to mitigate the risk of infections in vulnerable neonates.

Author contributions

P.B. performed most experiments and S.N. performed some specific experiments. K.S., S.N., S.D., and P.B. contributed to analysing WGS data and bioinformatics analysis. SM and BS coordinated the collection of rectal specimens, blood cultures and maintenance of clinical data. P.B., S.N., K.S., and S.B. contributed to the writing of the manuscript. T.R.W. critically analysed the manuscript. S.B. conceived and designed the study protocols.

Ethical approval and consent to participate

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 taken prior to enrolment in the study. Patient information was anonymised and de-identified prior to analysis. All methods were performed in accordance with the relevant guidelines and regulations.

Data availability

Whole-genome sequences of isolates from this study have been submitted to NCBI under Bioproject number PRJNA939406. IN-BB42EC has been submitted to the European Nucleotide Archive under Bioproject number PRJEB33565.

Declaration of competing interest

The authors declare no competing interests.

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