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Website: www.jlponline.org DOI: 10.4103/JLP.JLP_59_19

Co occurrence of two 16S rRNA methyltrasferases along with *NDM* and *OXA* 48 *like* carbapenamases on a single plasmid in *Klebsiella pneumoniae*

K. V. L. Aishwarya, P. V. Geetha, M. Shanthi, S. Uma

Abstract:

BACKGROUND: The carbapenemase-encoding genes, bla_{NDM} and $bla_{OXA-48-like}$, confer resistance to all the known beta-lactams and are encountered along with other beta-lactamase-encoding genes and/or 16S ribosomal RNA (rRNA)-methylating genes. The co-occurrence of bla_{NDM} and $bla_{OXA-48-like}$ on a single plasmid is a rare occurrence.

AIM AND OBJECTIVE: The purpose of the study was to characterize the plasmids in *Klebsiella pneumoniae* isolates producing 16S rRNA methyltransferase along with *bla*_{NDM}, *bla*_{OXA-48-like}, and other resistance encoding genes.

MATERIALS AND METHODS: One-hundred and seventeen *K. pneumoniae* clinical isolates which were resistant to aminoglycosides were collected. Polymerase chain reaction-based screening for 16S rRNA methyltransferase genes *armA*, *rmtB*, and *rmtC*; carbapenamase genes *bla_{NDM}*, *bla_{OXA-48-like}*, *bla_{IMP}*, *bla_{VIM}*, and *bla_{KPC}*; and other resistance genes such as *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*, and *qnr* (*A*, *B*, and *S*) determinants *acc* (*6'*) *Ib-cr* was performed. Conjugation experiment was carried out for seven isolates that anchored *bla_{NDM}* and *bla_{OXA-48-like}* along with any one of the 16S rRNA methyltransferases. The plasmid-based replicon typing for different plasmid-incompatible (Inc) group was performed on the conjugatively transferable plasmids.

RESULTS: Among the 16S rRNA methyltransferases, *armA* was more predominant. bla_{NDM} and $bla_{OXA-49-like}$ were present in 56 (47.86%) and 22 (18.80%) isolates, respectively. Out of seven isolates which were conjugatively transferable, only four had bla_{NDM} and $bla_{OXA-49-like}$ on the same plasmid and they belonged to Inc N and A/C replicon. Three isolates co-harbored 16S rRNA methyltransferases *armA*, *rmtB*, and *rmtC*, and out of the them, one isolate harbored two 16S rRNA methyltransferases *armA* and *rmtB*, on the single-plasmid replicon A/C.

CONCLUSION: This is the first report revealing the coexistence of *bla_{NDM}* and *bla_{OXA-48-like}* co-harboring two 16S rRNA methylases on a single conjugative plasmid replicon belonging to incompatibility group A/C.

Key words:

16S ribosomal RNA methyltransferase, A/C replicon, *bla_{NDM}*, *bla_{OXA-48 like}*, co-harboring, *Klebsiella pneumoniae*

Introduction

A minoglycosides are highly potent broad-spectrum antibiotics

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with desirable properties for treating life-threatening infections. The resistance mechanisms exhibited by bacteria against aminoglycoside are as follows: (1) acquired lipid modification that makes the cell wall

How to cite this article: L. Aishwarya KV, Geetha PV, Shanthi M, Uma S. Co occurrence of two 16S rRNA methyltrasferases along with *NDM* and *OXA* 48 *like* carbapenamases on a single plasmid in *Klebsiella pneumoniae*. J Lab Physicians 2019;11:305-11.

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Submission: 08-04-2019 Accepted: 07-11-2019 impermeable; (2) aminoglycoside-modifying enzymes that modify the antibiotic at specific sites, making it inactive; and (3) active expulsion of the drug by efflux mechanism.

A novel mechanism of resistance to aminoglycoside was first discovered in 2003 in *Pseudomonas* and *Enterobacteriaceae*,^[1,2] in which the aminoglycoside-binding site is modified enzymatically by 16S ribosomal RNA (rRNA) methyltransferase, commonly known as RMTases. The 16S methyltransferases which are intrinsically found in both *Streptomyces* spp. and *Micromonospora* spp. have now been identified in other bacteria as a result of the uptake of a plasmid containing the RMTase encoding gene.^[3] Till date, 11 RMTases have been identified, of which *armA*, *rmtB*, and *rmtC* are more frequent among *Enterobacteriaceae*.^[4,5]

Aminoglycosides are often used as adjunct drugs for treatment, in combination with other classes of antibiotics. The 16S methylases are frequently associated with extended-spectrum beta-lactamases (ESBL), carbapenemases, and plasmid-mediated quinolone-resistant (PMQR) genes.^[3,5] In *Enterobacteriaceae*, 16S rRNA methyltransferases have been found to occur with bla_{NDM} ^[6] and rarely with $bla_{OXA-48-like}$.^[7,8]

Enterobacteriaceae harboring all the above but with single 16S rRNA methyltransferases have been reported from India, Singapore, Turkey, Oman, Switzerland, and France.^[9-11] However, the co-occurrence of two 16S rRNA methyltransferases along with $bla_{NDM'}$ $bla_{OXA-48-like'}$ and other resistant determinants on a single-plasmid replicon has not been reported till date. The presence of these genes on plasmid enables their spread to other bacterial species by horizontal gene transfer. The emergence of such multidrug-resistant bacteria is a cause for concern globally.

Classification of plasmids into incompatible (Inc) groups is necessary because specific Inc groups have been associated with virulence and are of epidemiological importance.^[5] The plasmid Inc grouping is based on the fact that two plasmids belonging to the same Inc group cannot proliferate in the same cell line. With this as basis, Carattoli *et al.* in 2005 designed a polymerase chain reaction (PCR)-based replicon typing, in which 18 sets of primers were used to recognize the coding sequence of *repA* genes, the cis-repeats of the origin of replication, and the counter transcript RNA interference.^[12]

In this context, the aim of this study was to detect the co-occurrence of the most common 16S methylases *armA*, *rmtB*, and *rmtC* with ESBL-encoding genes ($bla_{TEM'}$, bla_{SHV} , and bla_{CTX-M}), carbapenemase-encoding genes ($bla_{IMP'}$, $bla_{VIM'}$, $bla_{KPC'}$, $bla_{NDM'}$ and $bla_{OXA-like}$), and PMQR-encoding genes (*qnr* determinants and *aac* (*6'*)-*Ib*-*cr*).

Materials and Methods

Ethical approval

The study was approved by the institutional ethical committee (IEC-NI/15/APR/6/18).

Bacterial isolates

The study included 117, nonrepetitive, amikacin-resistant clinical isolates of *Klebsiella pneumoniae*, collected over a period of 6 months (June 2015–December 2015). The isolates were obtained from specimens such as urine (65), exudates (27), blood (14), and respiratory secretions (11). All the isolates were speciated based on conventional methods or VITEK-2 system (Vitek-2 GN-card; BioMerieux, Brussels, Belgium).

Antibiotic susceptibility testing and minimum inhibitory concentration

The disc diffusion technique and determination of minimum inhibitory concentration (MIC) were performed in accordance with the Clinical Laboratory Standard Institute, 2016.^[13] The antibiotics tested were amikacin ($30 \mu g$), gentamicin ($10 \mu g$), tobramycin ($10 \mu g$), imipenem ($10 \mu g$), ciprofloxacin ($5 \mu g$), piperacillin/ tazobactam ($100 \mu g/10 \mu g$), cefotaxime ($30 \mu g$), cefazolin ($30 \mu g$), and cefuroxime ($30 \mu g$) (Himedia laboratories, Mumbai, Maharashtra, India). The MIC for amikacin was determined by agar dilution method.

Template DNA preparation

A single bacterial colony was inoculated into Luria-Bertani broth (Himedia laboratories, Mumbai, Maharashtra, India) and incubated overnight at 37°C, and it was then centrifuged at 10,000 rpm for 10 min. The pellet was re-suspended in 250 µl of Millipore water, boiled at 100°C for 10 min, and cooled and centrifuged at 10,000 rpm for 10 min. The supernatant served as the template DNA.

Polymerase chain reaction

Four sets of multiplex PCRs were carried out using the previously described primers and conditions^[14-19] for all the study isolates. The multiplex primers used for different sets of genes, their annealing temperature, and the amplicon size are listed in Table 1.

Each reaction volume contained 2 µl of the DNA template added to the master mix which includes 10 pmol of the forward and reverse primers (Sigma-Aldrich, Missouri, United States), 10 Mm dNTPs (Takara, Shiga, Japan), 5U taq polymerase (Takara, Shiga, Japan), and 10X buffer with MgCl₂ (Takara, Shiga, Japan).

Amplification reactions were performed under the following conditions: initial denaturation at 95°C for 4 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing based on the primer employed for 30 s

Multiplex set	gene	Primer	Annealing temperature (°C)	Amplicon size (bp)
1	armA	F-CCGAAATGACAGTTCCTATC	52	864
		R-GAAAATGAGTGCCTTGGAGG		
	rmtB	F-GCT TTC TGC GGG CGA TGT AA		173
		R-ATG CAA TGC CGC GCT CGT AT		
	rmtC	F-CGA AGA AGT AAC AGC CAA AG		711
		R-ATC CCA ACA TCT CTC CCA CT		
2	bla _{tem}	F- TTTCGTGTCGCCCTTATTCC	60	404
		R-ATCGTTGTCAGAAGTAAGTTGG		
	bla _{shv}	F- CGCCTGTGTATTATCTCCCT		294
		R- CGAGTAGTCCACCAGATCCT		
	bla _{стх-м}	F- CGCTGTTGTTAGGAAGTGTG		754
		R- GGCTGGGTGAAGTAAGTGAC		
3	bla _{ımP}	F- GGAATAGAGTGGCTTAAYTC	55	232
		R-TCGGTTTAAYAAAACAACCACC		
	bla _{vim}	F-GATGGTGTTTGGTCGCATA		390
		R- CGAATGCGCAGCACCAG		
	bla _{oxA-48-like}	F- GCGTGGTTAAGGATGAACAC		438
		R- CATCAAGTTCAACCCAACCG		
	bla _{nDM}	F -GGTTTGGCGATCTGGTTTTC		621
		R -CGGAATGGCTCATCACGATC		
	bla _{кPC}	F- CGTCTAGTTCTGCTGTCTTG		798
		R- CTTGTCATCCTTGTTAGGCG		
4	qnrA	F- TCAGCAAGAGGATTTCTCA	52	516
		R- GGCAGCACTATTACTCCCA		
	qnrB	F- GATCGTGAAAGCCAGAAAGG		469
		R- ACGATGCCTGGTAGTTGTCC		
	qnrS	F- ACGACATTCGTCAACTGCAA		417
		R- TAAATTGGCACCCTGTAGGC		
Simplex	acc (6') lb-cr	F- TTGGAAGCGGGGACGGA	53	260
		R- ACACGGCTGGACCATA		

	Table	1:	Primers	used	for	performing	pol	vmerase	chain	reaction	for	amplifving	resistant	aenes
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with an extension at 72°C for 50 s, and a final extension for one cycle at 72°C for 5 min. The PCR product was then run on a 1.5% agarose gel for detection of the amplified fragment. Strains previously confirmed by PCR were sequenced and used as positive controls.

Bacterial conjugation

Mating-out assays were performed at 37°C using *Escherichia coli J53* as recipient, and the donors were the isolates which were positive for 16S methylases along with bla_{NDM} , and $bla_{OXA-48-like}$ genes. The transconjugants were screened on MacConkey agar plate containing 100 µg of sodium azide (Himedia Laboratories, Mumbai, Maharashtra, India) along with 10 µg of cefoxitin^[20] and 4 µg of amikacin^[21] (SRL Pvt. Ltd, Mumbai, Maharashtra, India) each. The transconjugants were subjected to PCR detection of the antibiotic-resistant genes to confirm the transfer from the donor to the recipients.

Plasmid-based replicon typing

Plasmid-Inc group for the transconjugants was determined by plasmid-based replicon typing (PBRT) performed as described by Carattoli *et al.*^[12] The 18 Inc groups tested were HI1, HI2, I1- $\alpha\gamma$, X, L/M, N, FIA, FIB,

W, Y, P, FIC, A/C, T, FIIAs, F, K, and B/O.

PBRT was carried out in Applied Biosystems (Vetri 96-well thermal cycler, Pittsburgh, Pennsylvania) with five different multiplex-PCRs and three simplex-PCRs under the following conditions: all the amplifications were performed with the following amplification scheme: cycle of denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for all the Inc groups except that of F Inc group simplex which anneals at 52°C for 30 s with an extension at 72°C for 50 s, and a final extension for one cycle at 72°C for 5 min. The amplicon was then run on a 1.5% agarose gel for detection of the amplified fragment, and its corresponding Inc group was identified. The primers used for both multiplex- and simplex-PCRs performed are summarized in Table 2.

Results

The study isolates exhibited resistance to all the antimicrobials tested by disc diffusion method.

A high degree of resistance to amikacin

Aishwarva.	et al.: ⁻	Two	16S	ribosomal	RNA	methy	vltransferases	s on a	a sinale-	plasmid	replicon
, ,											

Multiplex set	Replicon type	Primer	Annealing temperature (°C)	Amplicon size (bp)
1	HI1	F- GGAGCGATGGATTACTTCAGTAC	60	471
		R-TGCCGTTTCACCTCGTGAGTA		
	HI2	F- TTTCTCCTGAGTCACCTGTTAACAC		644
		R- GGCTCACTACCGTTGTCATCCT		
	11	F- CGAAAGCCGGACGGCAGAA		139
		R-TCGTCGTTCCGCCAAGTTCGT		
2	х	F-AACCTTAGAGGCTATTTAAGTTGCTGAT	60	376
		R-TGAGAGTCAATTTTTATCTCATGTTTTAGC		
	L/M	F- GGATGAAAACTATCAGCATCTGAAG		785
		R- CTGCAGGGGCGATTCTTTAGG		
	N	F- GTCTAACGAGCTTACCGAAG		559
		R-GTTTCAACTCTGCCAAGTTC		
3	FIA	F-CCATGCTGGTTCTAGAGAAGGTG	60	462
		R -GTATATCCTTACTGGCTTCCGCAG		
	FIB	F-GGAGTTCTGACACACGATTTTCTG		308
		R-CTCCCGTCGCTTCAGGGCATT		
	W	F-CCTAAGAACAACAAAGCCCCCG		242
		R-GGTGCGCGGCATAGAACCGT		
4	Y	F-AATTCAAACAACACTGTGCAGCCTG	60	765
		R-GCGAGAATGGACGATTACAAAACTTT		
	Р	F-CTATGGCCCTGCAAACGCGCCAGAAA		534
		R-TCACGCGCCAGGGCGCAGCC		
	FIC	F-GTGAACTGGCAGATGAGGAAGG		262
		R-TTCTCCTCGTCGCCAAACTAGAT		
5	A/C	F-GAGAACCAAAGACAAAGACCTGGA	60	465
		R-ACGACAAACCTGAATTGCCTCCTT		
	т	F-TTGGCCTGTTTGTGCCTAAACCAT		750
		R-CGTTGATTACACTTAGCTTTGGAC		
	FII s	F-CTGTCGTAAGCTGATGGC		270
	Ū.	R-CTCTGCCACAAACTTCAGC		
Simplex 1	F _{renB}	F-TGATCGTTTAAGGAATTTTG	52	270
	10pb	R-GAAGATCAGTCACACCATCC		
Simplex 2	K/B	F-GCGGTCCGGAAAGCCAGAAAA	60	160
		R-TCTTTCACGAGCCCGCCAAA		
Simplex 3	B/O	F-GCGGTCCGGAAAGCCAGAAAA	60	159
		R-TCTGCGTTCCGCCAAGTTCGA		

	Table	2:	Primers	used	for	replicon	typing
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(MIC range of >512 $\mu g/mL)$ was observed in all the study isolates.

PCR screening revealed that, of the 117 isolates, 79 (67.52%) harbored *armA*, 19 (16.23%) *rmtB*, and 16 carried (13.67%) *rmtC*. Of these, four isolates harbored more than one 16S rRNA methyltransferase. bla_{NDM} and $bla_{OXA-48-like}$ were detected in 60 (51.28%) and 25 isolates (21.36%), respectively. The co-occurrence of bla_{NDM} and $bla_{OXA-48-like}$ carbapenamase was observed in seven isolates, along with one of the 16S rRNA methyltransferases. Other carbapenemases such as $bla_{IPM'}$ $bla_{VIM'}$ and bla_{KPC} were not encountered.

The other resistance-encoding genes detected were *Qnr* determinant (48), *acc* (6') *lb-cr* (72), *bla*_{TEM} (4), and *bla*_{CTX-M} (9). Sixty isolates co-harbored *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}. The occurrence of these genes with *bla*_{NDM}, *bla*_{OXA-48-like}, and 16S rRNA methyltransferase is depicted in Table 3.

Conjugation experiment was carried out for all the 7 out of 117 *K. pneumoniae* strains (KP1, KP48, KP98, KP124, KP231, KP297, and KP302) that co-harbored both bla_{NDM} and $bla_{OXA-48-like}$ carbapenamases, along with one of the 16S rRNA methyltransferases. The conjugation yield products, namely the single transconjugant, their plasmid replicon type, the resistance genes, and their associated resistant determinants, are tabulated [Table 4].

Out of the seven isolates that were conjugatively transferable, only four isolates had bla_{NDM} and $bla_{OXA-48-like}$ on the same plasmid, and they belonged to replicon-Inc N and A/C. Of them, two isolates harbored only a single plasmid. One *K. pneumoniae* harbored two 16S rRNA methyltransferases *armA* and *rmtB*.

Discussion

Genes	<i>armA</i> positives (<i>n</i> =79)	<i>rmtB</i> positives (<i>n</i> =19)	<i>rmtC</i> positives (<i>n</i> =16)	<i>armA+rmtB</i> positives (<i>n</i> =2)	<i>rmtB+rmtC</i> positives (<i>n</i> =2)	armA+rmtC/ armA+rmtB+rmtC (n=0)
<i>bla</i> _{NDM} (<i>n</i> =60)	36	12	12	-	-	-
bla _{OXA-48- like} (n=25)	20	3	2	-	-	-
bla _{NDM} + bla _{OXA-48} (n=7)	3	1	1	2	-	-
bla _{IMP/VIM/KPC} (n=0)	-	-	-	-	-	-
bla _{TEM} (n=4)	2	2	-	-	-	-
bla _{sHv} (n=0)	-	-	-	-	-	-
bla _{ctx-M} (n=9)	3	2	4	-	-	-
<i>bla_{TEM}+bla_{SHV} (n</i> =18)	12	3	2	1	-	-
bla _{sHV} +bla _{CTX-M} (n=7)	2	5	-	-	-	-
bla _{TEM} +bla _{CTX-M} (n=7)	3	1	1	-	2	-
bla _{TEM} +bla _{SHV} +bla _{CTX-M} (n=70)	45	5	9	1	-	-
qnr determinants (n=48)	32	6	4	1	-	-
aac (6')-lb-cr (n=71)	47	11	10	1	2	-

Table 3:	Number	of isolates	associated	with t	he most	common	16S rRNA	methyltransferases	along v	with	othe
resistan	t determi	nants									

Table 4: Isolates that co-harbored bla_{NDM} and $bla_{OXA-48-like}$ along with their resistant determinants and plasmid-incompatible groups

lsolate number	Source	Donor-resistant determinants	Transconjugant-resistant determinants	Plasmid-incompatible group
KP1	Exudates	bla_{NDM} , $bla_{OXA-48-like}$, $armA$, bla_{TEM} , bla_{SHV} , bla_{CTX-M}	bla _{oxa-48-like} , armA, bla _{tEM} , bla _{ctx-M}	L/M
KP48	Respiratory	bla _{NDM} , bla _{OXA-48-like} , rmtC	bla _{NDM} , bla _{OXA48-like} , rmtC	Ν
KP98	Urine	$bla_{NDM,} bla_{OXA-48-like}$, armA, rmtB, bla_{TEM} , bla_{SHV} , bla_{CTX-M} , aac (6')-lb-cr, qnrB	bla _{NDM} , armA, rmtB, bla _{CTX-M} , aac (6')-Ib-cr, qnrB	A/C
KP124	Urine	bla _{NDM,} bla _{OXA-48-like} , rmtB, bla _{SHV} . bla _{CTX-M} , qnrB, aac (6')-lb-cr	bla _{NDM,} bla _{OXA-48-like} , bla _{CTX-M} ,	Ν
KP231	Urine	bla _{NDM,} bla _{OXA-48-like} , armA, rmtB, bla _{TEM} , bla _{SHV} . aac (6')-Ib-cr	bla _{NDM,} bla _{OXA-48-like} , rmtB, armA, bla _{TEM} , aac (6')-lb-cr	A/C
KP297	Blood	bla _{NDM} , bla _{OXA-48-like} , armA, bla _{TEM} , bla _{SHV} , bla _{CTX-M}	bla _{oxa-48-like} , armA, bla _{TEM} , bla _{ctx-M}	Ν
KP302	Urine	bla _{NDM} bla _{OXA-48-like} , armA, bla _{CTX-M} , qnrB, aac (6')-lb-cr	bla _{NDM.} bla _{OXA-48-like} , armA, bla _{CTXM} , qnrB, aac (6')-lb-cr	A/C

K. pneumoniae is a common enterobacterial pathogen causing healthcare-associated infections such as bacteremia, urinary tract infection, pneumonia, and meningitis. The rampant usage of antibiotics is modeling the bacteria to exhibit a phenomenal change in the drug susceptibility pattern with remarkable changes at their genetic level.^[21]

All the 117 *K. pneumoniae* isolates exhibited high-level resistance to amikacin with an MIC >512 μ g/mL. Such high levels of resistance are attributable to the use of S-adenyosyl-L-methionine as a co-substrate and methylation of the aminoglycoside-binding A-site of the 16S rRNA of the bacterial ribosome.^[22]

The plasmid-mediated 16S rRNA methyltransferase usually exhibits a strong linkage with multidrug-resistant determinants.^[23] Among the ESBLs, $bla_{TEM'}$ $bla_{SHV_}$ and bla_{CTX-M} were the most frequently encountered [Table 3]. Similar to our observation, the coexistence of these in *Enterobacteriaceae* has been reported from India previously.^[24-26]

In this study, the prevalence of *armA* (67.52%) was high when compared to that of *rmtB* (16.23%) and *rmtC* (13.67%). The greater occurrence of *armA* is presumably due to its co-existence with bla_{CTX-M} on the same plasmid and its location on an effective transposon Tn1548.^[27]

The presence of more than one 16S rRNA methyltransferase is seldom reported. Interestingly, we found four isolates (3.41%) harboring multiple 16S rRNA methyltransferases in combinations of armA + rmtB and rmtB + rmtC. The occurrence of more than one 16S rRNA methylases in our study was low as compared to a report from Northeast India^[5] where it was observed in 27.64% of *E. coli*.

In a report from Morocco,^[28] only 2 out of 116 aminoglycoside-resistant *K. pneumoniae* harbored both bla_{NDM} and *aac* (6')-*Ib-cr*. In this study, a higher proportion (46/117) co-harbored the above determinants, which is a cause for worry because it restricts the application of combination therapy.

Most of the bla_{NDM} -harboring plasmids are usually untypeable,^[29] whereas $bla_{OXA-48-like}$ plasmids are associated with IncL/M. The bla_{NDM} producers can co-harbor other beta-lactamases and/or 16SrRNA methyltransferase genes (*armA*, *rmtB*, and *rmtC*). However, the co-occurrence of bla_{NDM} and $bla_{OXA-48-like}$ in a single plasmid is rarely reported.^[30]

In the present study, seven isolates harboring $bla_{\rm NDM}$ and *bla_{OXA-48-like}* along with 16S rRNA methyltransferase were conjugatively transferable. The incompatibility typing of plasmids revealed that *armA* was encoded within L/M-, A/C-, and N-Inc groups, whereas, *rmtB* and *rmtC* were encoded within A/C- and N-Inc groups, respectively. Two isolates which anchored both *armA* and *rmtB* were encoded within a single Inc group A/C. This pattern is in accordance with the report from North India^[5] where multiple 16S methyltransferase genes were found on diverse Inc group types such as FIB, FIIS, I, T, X, N, Y, L/M, and FIA. By PBRT, the *bla*_{NDM} and *bla*_{OXA48-like} were encoded within plasmids belonging to L/M-, A/C-, and N-Inc groups. Previously, Soundari et al. have reported the presence of both *bla_{NDM}* and *bla_{OXA48-like}* in IncHI3 replicon.[29]

Conclusion

Our findings underline the emerging threat of multidrug-resistant pathogens that produce 16S rRNA methylase disseminating in this region.

This study is of epidemiological importance. There is co-occurrence of two 16SrRNA methyltransferases along with bla_{NDM} and $bla_{OXA48-like}$ on single-plasmid replicon, and they belong to Inc A/C. To the best of our knowledge, this is the first report of such an occurrence till date. However, multi- centric studies needs to be carried out with increased sample size to have a better understanding about the different plasmid Inc types involved, their diverse source of origin and acquisition which would aid in the control strategies.

Financial support and sponsorship

This study was supported by the Department of Science and Technology under INSPIRE-fellowship scheme (reference number IF150368).

Conflicts of interest

There are no conflicts of interest.

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