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# Co occurrence of two 16S rRNA methyltransferases 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub>, 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub>, 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*; carbapenemase genes *bla*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>KPC</sub>; and other resistance genes such as *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub> and *qnr* (A, B, and S) determinants *acc* (6') *lb-cr* was performed. Conjugation experiment was carried out for seven isolates that anchored *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> were present in 56 (47.86%) and 22 (18.80%) isolates, respectively. Out of seven isolates which were conjugatively transferable, only four had *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub> co-harboring, *Klebsiella pneumoniae*

Department of  
Microbiology, Sri  
Ramachandra Institute  
for Higher Education  
and Research, Chennai,  
Tamil Nadu, India

## Address for correspondence:

Dr. K. V. L. Aishwarya,  
Department of  
Microbiology, Sri  
Ramachandra Institute  
for Higher Education  
and Research,  
Chennai - 600 116,  
Tamil Nadu, India.  
E-mail: mailaishmicro@  
gmail.com

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## Introduction

Aminoglycosides 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

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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*,<sup>[1,2]</sup> 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.<sup>[3]</sup> Till date, 11 RMTases have been identified, of which *armA*, *rmtB*, and *rmtC* are more frequent among *Enterobacteriaceae*.<sup>[4,5]</sup>

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.<sup>[3,5]</sup> In *Enterobacteriaceae*, 16S rRNA methyltransferases have been found to occur with *bla*<sub>NDM</sub><sup>[6]</sup> and rarely with *bla*<sub>OXA-48-like</sub>.<sup>[7,8]</sup>

*Enterobacteriaceae* harboring all the above but with single 16S rRNA methyltransferases have been reported from India, Singapore, Turkey, Oman, Switzerland, and France.<sup>[9-11]</sup> However, the co-occurrence of two 16S rRNA methyltransferases along with *bla*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub> 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.<sup>[5]</sup> 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.<sup>[12]</sup>

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*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub>), carbapenemase-encoding genes (*bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub> and *bla*<sub>OXA-like</sub>), 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.<sup>[13]</sup> The antibiotics tested were amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), piperacillin/tazobactam (100 µg/10 µg), cefotaxime (30 µg), cefazolin (30 µg), and cefuroxime (30 µg) (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<sup>[14-19]</sup> 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 MgCl<sub>2</sub> (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

**Table 1: Primers used for performing polymerase chain reaction for amplifying resistant genes**

Multiplex set	gene	Primer	Annealing temperature (°C)	Amplicon size (bp)
1	<i>armA</i>	F-CCGAAATGACAGTTCCTATC	52	864
		R-GAAAATGAGTGCCTTGGAGG		
	<i>rmtB</i>	F-GCT TTC TGC GGG CGA TGT AA		173
2	<i>rmtC</i>	R-ATG CAA TGC CGC GCT CGT AT	60	711
		F-CGA AGA AGT AAC AGC CAA AG		
	<i>bla<sub>TEM</sub></i>	R-ATC CCA ACA TCT CTC CCA CT		404
3	<i>bla<sub>SHV</sub></i>	F- TTTTCGTGTCGCCCTTATTCC	55	294
		R-ATCGTTGTCAGAAGTAAGTTGG		
	<i>bla<sub>CTX-M</sub></i>	F- CGCTGTGTATTATCTCCCT		754
4	<i>bla<sub>IMP</sub></i>	R- CGAGTAGTCCACCAGATCCT	52	232
		F- GGAATAGAGTGGCTTAAYTC		
	<i>bla<sub>VIM</sub></i>	R- GGCTGGGTGAAGTAAGTGAC		390
	<i>bla<sub>OXA-48-like</sub></i>	F-GATGGTGTGGTGCATA		438
		R- CGAATGCGCAGCACCAG		
<i>bla<sub>NDM</sub></i>	F- GCGTGGTTAAGGATGAACAC	621		
Simplex	<i>acc (6') lb-cr</i>	R- CATCAAGTTCAACCCAACCG	53	260
		F- GGTGGTGGCGATCTGGTTTTTC		
		R- CGGAATGGCTCATCACGATC		
4	<i>bla<sub>KPC</sub></i>	F- GGTGGTGGCGATCTGGTTTTTC	52	516
		R- CGGAATGGCTCATCACGATC		
	<i>qnrA</i>	F- CGTCTAGTTCTGCTGTCTTG		469
4	<i>qnrB</i>	R- CTTGTCATCCTTGTAGGCG	52	417
		F- TCAGCAAGAGGATTTCTCA		
	<i>qnrS</i>	R- GGCAGCACTATTACTCCA		417
Simplex	<i>acc (6') lb-cr</i>	F- GATCGTGAAAGCCAGAAAGG	53	260
		R- ACGATGCCTGGTAGTTGTCC		
		F- ACGACATTCGTCAACTGCAA		
Simplex	<i>acc (6') lb-cr</i>	R- TAAATTGGCACCCCTGTAGGC	53	260
		F- TTGGAAGCGGGGACGGA		
		R- ACACGGCTGGACCATA		

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<sub>NDM</sub>* and *bla<sub>OXA-48-like</sub>* 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<sup>[20]</sup> and 4 µg of amikacin<sup>[21]</sup> (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.*<sup>[12]</sup> 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

**Table 2: Primers used for replicon typing**

Multiplex set	Replicon type	Primer	Annealing temperature (°C)	Amplicon size (bp)
1	HI1	F- GGAGCGATGGATTACTTCAGTAC R-TGCCGTTTCACCTCGTGAGTA	60	471
	HI2	F- TTTCTCCTGAGTCACCTGTTAACAC R- GGCTCACTACCGTTGTATCCT		644
	I1	F- CGAAAGCCGGACGGCAGAA R-TCGTCTGTTCCGCCAAGTTCGT		139
2	X	F-AACCTTAGAGGCTATTTAAGTTGCTGAT R-TGAGAGTCAATTTTTATCTCATGTTTTAGC	60	376
	L/M	F- GGATGAAAACATCAGCATCTGAAG R- CTGCAGGGGCGATTCTTTAGG		785
	N	F- GTCTAACGAGCTTACCGAAG R-GTTTCAACTCTGCCAAGTTC		559
3	FIA	F-CCATGCTGGTTCTAGAGAAGGTG R -GTATATCCTTACTGGCTTCCGCAG	60	462
	FIB	F-GGAGTTCTGACACACGATTTTCTG R-CTCCCGTCGCTTCAGGGCATT		308
	W	F-CCTAAGAACAACAAAGCCCCCG R-GGTGCGCGGCATAGAACCGT		242
4	Y	F-AATTCAAACAACACTGTGCAGCCTG R-GCGAGAATGGACGATTACAAAACCTT	60	765
	P	F-CTATGGCCCTGCAAACGCGCCAGAAA R-TCACGCGCCAGGGCGCAGCC		534
	FIC	F-GTGAAGTGGCAGATGAGGAAGG R-TTCTCCTCGTCGCCAACTAGAT		262
5	A/C	F-GAGAACCAAAGACAAAGACCTGGA R-ACGACAAACCTGAATTGCCTCCTT	60	465
	T	F-TTGGCCTGTTTGTGCCTAAACCAT R-CGTTGATTACACTTAGCTTTGGAC		750
	FII <sub>s</sub>	F-CTGTCGTAAGCTGATGGC R-CTCTGCCACAAACTTCAGC		270
Simplex 1	F <sub>repB</sub>	F-TGATCGTTTAAGGAATTTTG R-GAAGATCAGTCACACCATCC	52	270
Simplex 2	K/B	F-GCGGTCCGGAAAGCCAGAAAA R-TCTTTCACGAGCCCCGCAAAA	60	160
Simplex 3	B/O	F-GCGGTCCGGAAAGCCAGAAAA R-TCTGCGTTCCGCCAAGTTCGA	60	159

(MIC range of >512 µg/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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> were detected in 60 (51.28%) and 25 isolates (21.36%), respectively. The co-occurrence of *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> carbapenemase was observed in seven isolates, along with one of the 16S rRNA methyltransferases. Other carbapenemases such as *bla*<sub>IPM</sub>, *bla*<sub>VIM</sub> and *bla*<sub>KPC</sub> were not encountered.

The other resistance-encoding genes detected were *Qnr* determinant (48), *acc* (6') *Ib-cr* (72), *bla*<sub>TEM</sub> (4), and *bla*<sub>CTX-M</sub> (9). Sixty isolates co-harbored *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>. The occurrence of these genes with *bla*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub> 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> carbapenemases, 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*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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

**Table 3: Number of isolates associated with the most common 16S rRNA methyltransferases along with other resistant determinants**

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

**Table 4: Isolates that co-harbored *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> along with their resistant determinants and plasmid-incompatible groups**

Isolate number	Source	Donor-resistant determinants	Transconjugant-resistant determinants	Plasmid-incompatible group
KP1	Exudates	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>CTX-M</sub>	<i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>CTX-M</sub>	L/M
KP48	Respiratory	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>rmtC</i>	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>rmtC</i>	N
KP98	Urine	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>rmtB</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>CTX-M</sub> , <i>aac</i> (6')-Ib-cr, <i>qnrB</i>	<i>bla</i> <sub>NDM</sub> , <i>armA</i> , <i>rmtB</i> , <i>bla</i> <sub>CTX-M</sub> , <i>aac</i> (6')-Ib-cr, <i>qnrB</i>	A/C
KP124	Urine	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>rmtB</i> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>CTX-M</sub> , <i>qnrB</i> , <i>aac</i> (6')-Ib-cr	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>bla</i> <sub>CTX-M</sub>	N
KP231	Urine	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>rmtB</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>aac</i> (6')-Ib-cr	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>rmtB</i> , <i>armA</i> , <i>bla</i> <sub>TEM</sub> , <i>aac</i> (6')-Ib-cr	A/C
KP297	Blood	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>CTX-M</sub>	<i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>CTX-M</sub>	N
KP302	Urine	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>CTX-M</sub> , <i>qnrB</i> , <i>aac</i> (6')-Ib-cr	<i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA-48-like</sub> , <i>armA</i> , <i>bla</i> <sub>CTX-M</sub> , <i>qnrB</i> , <i>aac</i> (6')-Ib-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.<sup>[21]</sup>

All the 117 *K. pneumoniae* isolates exhibited high-level resistance to amikacin with an MIC >512 µg/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.<sup>[22]</sup>

The plasmid-mediated 16S rRNA methyltransferase usually exhibits a strong linkage with multidrug-resistant determinants.<sup>[23]</sup> Among the ESBLs, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> were the most frequently encountered [Table 3]. Similar to our observation, the coexistence of these in *Enterobacteriaceae* has been reported from India previously.<sup>[24-26]</sup>

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*<sub>CTX-M</sub> on the same plasmid and its location on an effective transposon Tn1548.<sup>[27]</sup>

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<sup>[5]</sup> where it was observed in 27.64% of *E. coli*.

In a report from Morocco,<sup>[28]</sup> only 2 out of 116 aminoglycoside-resistant *K. pneumoniae* harbored both *bla*<sub>NDM</sub> 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*<sub>NDM</sub>-harboring plasmids are usually untypeable,<sup>[29]</sup> whereas *bla*<sub>OXA-48-like</sub> plasmids are associated with IncL/M. The *bla*<sub>NDM</sub> producers can co-harbor other beta-lactamases and/or 16SrRNA methyltransferase genes (*armA*, *rmtB*, and *rmtC*). However, the co-occurrence of *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> in a single plasmid is rarely reported.<sup>[30]</sup>

In the present study, seven isolates harboring *bla*<sub>NDM</sub> and *bla*<sub>OXA-48-like</sub> 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<sup>[5]</sup> 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*<sub>NDM</sub> and *bla*<sub>OXA48-like</sub> 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*<sub>NDM</sub> and *bla*<sub>OXA48-like</sub> in IncHI3 replicon.<sup>[29]</sup>

## 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*<sub>NDM</sub> and *bla*<sub>OXA48-like</sub> 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.

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## Conflicts of interest

There are no conflicts of interest.

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