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Letter to the Editor

Incidence of the novel *rmtF* and *rmtG* methyltransferases in carbapenem-resistant Enterobacteriaceae from a hospital in India

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Dear Editor,

Aminoglycosides play a critically important role in antimicrobial therapy, mostly against the rapidly evolving β-lactamase-producing Enterobacteriaceae, often for their co-drug effect with β-lactams. However, the evolution of acquired 16S rRNA methyltransferases, which confer high-level and broad-spectrum aminoglycoside resistance, and their frequent co-occurrence with carbapenemases, constitute a major threat to this class of antibiotic [1]. Reported cases of aminoglycoside methyltransferase within carbapenemase-producing bacteria were relatively rare until the extensive worldwide spread of New Delhi metallo-beta-lactamase 1 (NDM-1) [2]. Recently, novel variants of methyltransferases have emerged, although their true prevalence and distribution are yet to be ascertained. In the present study, the prevalence of methyltransferase activity was determined in clinical isolates of carbapenem-resistant Enterobacteriaceae (CRE) from our center. Furthermore, incidence of the novel *rmtF* and *rmtG* methyltransferase genes within these isolates was reported.

From 512 previously characterized (n = 761) clinical isolates of multidrug-resistant Enterobacteriaceae (MDRE) [3], carbapenem-non-susceptible isolates (CNSI) (defined as isolates intermediate or resistant to any of the carbapenems, namely ertapenem, imipenem, meropenem, and doripenem) were determined by the Kirby-Bauer disk susceptibility test. Further susceptibility tests to tigecycline, colistin, carbapenems, and third-generation cephalosporins; ceftazidime, ceftriaxone, and cefotaxime by minimum inhibitory concentration (MIC) breakpoints determined by the Clinical and Laboratory Standards Institute (CLSI)-referenced agar dilution method were performed on the CNSI and CRE, defined as isolates not susceptible to any of the carbapenems and resistant to all the third-generation cephalosporins [4]. Food and Drug Administration (FDA) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for susceptibility were used for tigecycline and colistin, respectively [5]. *E. coli* ATCC 25922 was used as a control.

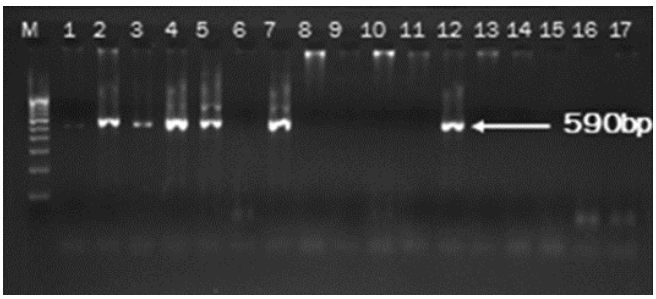
The disk diffusion test revealed 62.1% (318/512) MDRE to be CNSI. Resistance rate by MIC breakpoint determination was 47.2%, 34.3%, 34.0, 30.2%, 19.1%, and 4.0% to ertapenem, imipenem, meropenem, doripenem, tigecycline, and colistin, respectively (Table 1). The most active agent of the carbapenems was doripenem, and ertapenem was the least active agent. Although tigecycline and colistin retained the best activity against the isolates, declining activity of tigecycline was evidenced by its poor performance on *K. pneumoniae* (42.5% resistance) (Table 1). Comparatively, *in vitro* activity of carbapenems, tigecycline, and colistin on the isolates significantly differed from each other ($p = 0.00$). While the Wilcoxon post-hoc test revealed significant difference between tigecycline and colistin ($p = 0.04$), both significantly differed from the carbapenems (Table 1). Although doripenem was the most active among the carbapenems (Table 1), its activity did not differ significantly from meropenem ($p = 0.07$); however, it differ significantly from imipenem ($p = 0.03$). On the other hand, meropenem did not differ significantly from imipenem ($p = 0.03$), indicating that doripenem had only marginal *in vitro* susceptibility advantage over imipenem and meropenem on MDRE,

which suggests that therapeutic choices among these antibiotics may depend on other factors but not entirely on *in vitro* susceptibility advantage. The susceptibility profiles of *K. pneumoniae* and *E. coli* revealed consistently higher resistance rates among *K. pneumoniae* across carbapenems and tigecycline, except in colistin, where a low resistance rate of 8% (9/113) was observed (Table 1).

CRE was 55.9% (178/318) with *E. coli* (n = 64) and *K. pneumoniae* (n = 75), constituting the predominant number of isolates in this study. Polymerase chain reaction (PCR) amplification for the carbapenemase-encoding genes ($bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$) was performed using primers specific for each gene [6]. CRE isolates able to grow on brain-heart infusion (BHI) agar supplemented with 200 µg/mL each of gentamicin and amikacin were recognized to be positive for 16S rRNA methyltransferase activity, and PCR amplification for the novel N7 G1405 methyltransferase genes (*rmtF* and *rmtG*) was determined using specific primers [1,7,8].

$bla_{\text{NDM-1}}$ has emerged globally as the most common type of carbapenemase-encoding gene, and its occurrence has surpassed that of other types of carbapenemases. $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ are endemic in India, and a prevalence rate ranging between 31.3% and 91.6% for $bla_{\text{NDM-1}}$ in CRE isolates has been reported [9]. The high $bla_{\text{NDM-1}}$ (47.2%) and $bla_{\text{OXA-48}}$ (43.8%) carriage rate observed in this study highlights the importance of these genes in the development of resistance in our center. In addition, an increasing trend in the occurrence of $bla_{\text{NDM-1}}$ in Enterobacteriaceae isolates was observed. A study conducted in 2010 and a multicenter study conducted earlier reported a 6.7% and 2% $bla_{\text{NDM-1}}$ occurrence rate from this center [10,11]. In this study, an overall occurrence rate of 11.0% (n = 761 [3], $bla_{\text{NDM-1}}$ = 84) (Table 2) was observed, signifying an increasing trend of NDM-1 in our center.

Figure 1. PCR amplification of *rmtF* gene



M: 100 bp DNA ladder; *rmtF* gene: 590 bp

Methyltransferase activity was observed in 57.3% (102/178) of isolates (Table 2). Chi-square analysis revealed significant association between methyltransferase activity and $bla_{\text{NDM-1}}$ carriage (p = 0.00). Incidence of the novel *rmtF* gene (Figure 1) among isolates positive for methyltransferase activity was 25.5% (26/102), and a high rate of *rmtF* positive isolates was found to harbor the $bla_{\text{NDM-1}}$ gene (73.1% [19/26]) (Table 2). This finding corroborated a similar study on Indian isolates, which revealed that 24.2% of isolates expressing methyltransferase activity were positive for *rmtF* and 58.8% co-harbored *rmtF* and $bla_{\text{NDM-1}}$ genes [1]. The identification of this novel resistance determinant in Enterobacteriaceae from our center further confirms the assertion that the *rmtF* gene may have emerged some time ago and continued

Table 1. Resistance spectrum of carbapenem-non-susceptible Enterobacteriaceae

Bacterial isolates	CNSI (n)	Number and proportion (%) of isolates resistant to antibiotic								
		CTR	CTX	CAZ	ERT ≥ 2	MER ≥ 4	IMI ≥ 4	DOR ≥ 4	TG ≥ 8	COL > 2
E. coli	142	140 (98.6)	139 (97.9)	125 (88.0)	44 (31.0)	32 (22.5)	33 (23.3)	28 (19.7)	2 (1.4)	1 (0.7)
K. pneumoniae	113	107 (94.7)	108 (95.6)	103 (91.1)	76 (67.3)	61 (54.0)	57 (50.4)	55 (48.7)	48 (42.5)	9 (8.0)
K. oxytoca	12	10 (83.3)	10 (83.3)	9 (75.0)	8 (66.7)	4 (33.3)	6 (50.0)	3 (25.0)	3 (25.0)	1 (8.3)
C. freundii	7	7 (100)	7 (100)	7 (100)	5 (71.4)	4 (57.1)	4 (57.1)	4 (57.1)	2 (28.6)	1 (14.2)
C. koseri	17	16 (94.1)	16 (94.1)	13 (76.5)	9 (52.9)	3 (17.6)	2 (11.2)	2 (11.8)	0 (0.0)	0 (0.0)
E. aerogenes	5	5 (100)	4 (80)	5 (100)	3 (60.0)	2 (40.0)	2 (40.0)	2 (40.0)	2 (40.0)	0 (0.0)
E. cloacae	2	2 (100)	2 (100)	1 (50)	1 (50.0)	1 (50.0)	2 (100)	1 (50.0)	0 (0.0)	0 (0.0)
M. morganii	1	1 (100)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA	NA
P. mirabilis	13	11 (84.6)	12 (92.3)	12 (92.3)	4 (30.0)	1 (7.7)	2 (15.4)	1 (7.7)	NA	NA
P. vulgaris	6	4 (66.6)	6 (100)	5 (83.3)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	NA	NA
Total	**318**	307 (96.5)	307 (96.5)	287 (90.3)	**150 (47.2)[a]**	**108 (34.0)[bc]**	**109 (34.3)[b]**	**96 (30.2)[c]**	**57 (19.1)[d]**	**12 (4.0)[e]**

Total/percentage with same superscript are not significantly different (p > 0.05); CTR: ceftriaxone; CTX: cefotaxime; CAZ: ceftazidime; ERT: ertapenem; MER: meropenem; IMI: imipenem; DOR: doripenem; TIG: tigecycline; COL: colistin; NA: not applicable

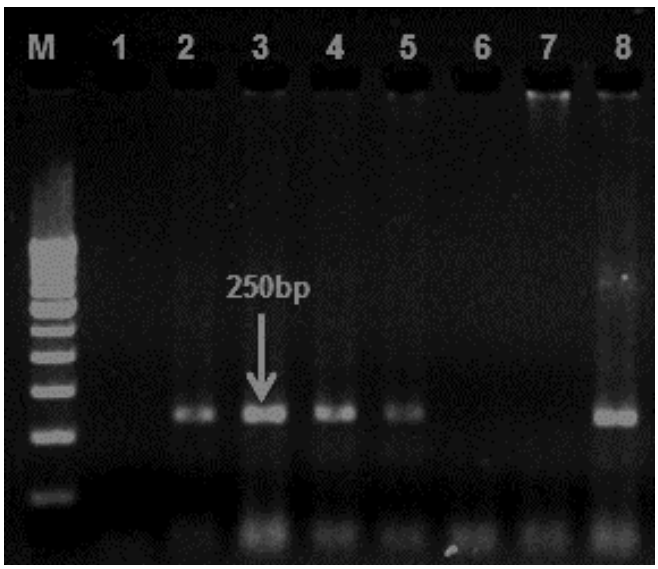
Table 2. Distribution of $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, methyltransferase activity, and *rmtF* and *rmtG* genes in carbapenem-resistant Enterobacteriaceae

Bacteria	Number and proportion (%) of isolates							
	CNSI	CRE†	$bla_{\text{NDM-1}}$††	$bla_{\text{OXA-48}}$††	$bla_{\text{NDM-1}}$ †† $bla_{\text{OXA-48}}$	MTA†††	*rmtF*‡‡	*rmtG*‡‡
E. coli	142 (65.1)	64 (45.1)	27 (42.1)	38 (59.4)	10 (15.6)	30 (46.9)	3 (10.0)	3 (10.0)
K. pneumoniae	113 (65.3)	75 (66.3)	40 (53.3)	29 (38.7)	7 (9.3)	53 (70.7)	20 (37.7)	7 (13.2)
K. oxytoca	12 (48.0)	8 (66.7)	4 (50.0)	4 (50.0)	1 (12.5)	4 (50.0)	0 (0.0)	0 (0.0)
C. freundii	7 (24.1)	6 (85.7)	3 (50.0)	1 (16.7)	0 (0.0)	4 (66.7)	1 (25.0)	0 (0.0)
C. koseri	17 (47.2)	10 (58.8)	3 (30.0)	7 (30.0)	1 (10)	4 (40.0)	1 (25.0)	0 (0.0)
E. aerogenes	5 (100)	3 (60.0)	2 (66.6)	1 (33.3)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
E. cloacae	2 (100)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
M. morganii	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (00.0)	0 (0.0)	0 (0.0)
P. mirabilis	13 (81.3)	7 (53.8)	3 (42.9)	2 (42.6)	3 (42.7)	3 (42.9)	1 (33.0)	0 (0.0)
P. vulgaris	6 (100)	3 (50.0)	1 (33.3)	1 (33.3)	0 (0.0)	0 (00.0)	0 (0.0)	0 (0.0)
Total	**318 (62.1)**	178 (56.0)	84 (47.2)	78 (43.8)	22 (12.4)	**102 (57.3)**	26 (25.5)	10 (9.8)

CNSI: carbapenem-non-susceptible isolates; CRE: carbapenem-resistant isolates; MTA: methyltransferase activity; † Proportion calculated with CNSI as n; †† Proportion calculated with CRE as n; ‡‡ Proportion calculated with MTA as n

to spread in association with the $bla_{\text{NDM-1}}$ gene among clinical isolates unbeknownst. This is more likely, as screening for these determinants is yet to be incorporated into routine screening panels, and Enterobacteriaceae with such characteristic determinants were not targeted or resistance was viewed as being conferred by aminoglycosides-modifying enzymes. The emergence of *K. pneumoniae* co-producing NDM-1 and RmtF methyltransferase was reported recently in the United States [12]. Similarly, *K. pneumoniae* accounted for the highest RmtF production and RmtF and NDM-1 co-production in this study. The reported emergence of *rmtF* from the United Kingdom (UK), India, Nepal, the United States (US), and Saudi Arabia highlights the likely global dissemination of the *rmtF* gene among Enterobacteriaceae [1,12-14].

Figure 2. PCR amplification of *rmtG* gene



M: 100 bp DNA ladder; *rmtG* gene: 250 bp

Another novel variant, *rmtG*, recently reported in Brazil, shares the highest similarity with *rmtD*, which is confined to South America [8]. However, 9.8% (10/102) isolates comprising *K. pneumoniae* (n = 7) and *E. coli* (n = 3) were positive for *rmtG* genotype (Figure 2 and Table 2), indicating that, unlike regionally confined *rmtD*, *rmtG* may have a worldwide dissemination. To the best of our knowledge, this is the first reported case of *rmtG* genotype among isolates of Enterobacteriaceae outside South America. Thus, more studies are needed to ascertain worldwide dissemination of this resistance determinant. The emergence and simultaneous identification of these novel methyltransferases in Asia, the UK, and the US is a reminder that it is critical not only to detect new acquired resistance determinants, but also to analyze our environment for known traits in order to gain the most knowledge [2].

Although a major limitation of this work is our inability to determine other carbapenemase-encoding genes besides $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ and other 16S rRNA methyltransferases besides the novel variants, the incidence of *rmtF* and *rmtG* genes in CRE from our center stresses the need to constantly monitor clinical isolates for emerging resistance patterns. As aminoglycoside and carbapenem activity becomes increasingly compromised, and because tigecycline and colistin are not ideal drugs for the treatment of infection due to MDRE, prompt identification and tracking of possible sources of infection and implementation of standard infection control practices are crucial.

References

1. Hidalgo L, Hopkins KL, Gutierrez B, Ovejero CM, Shukla S, Douthwaite S, Prasad KN, Woodford N, Gonzalez-Zorn B (2013) Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in enterobacteriaceae isolated in India and the UK. J Antimicrob Chemother 68: 1543-1550.
2. Hidalgo L (2013) Identification and characterization of an emergent aminoglycoside resistance mechanism the 16S rRNA methyltransferases. Available: http://www.print.ucm.es/24917/1/T35237.pdf. Accessed 20 November 2014.
3. Filgona J, Baneerje T, Anupurba S (2014) Antimicrobial resistance pattern of multidrug resistant *enterobacteriaceae* (MDRE) isolated from clinical samples with special reference to carbapenemase production and susceptibility to tigecycline. Br Microbiol Res J 4: 1035-1045.
4. Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement. M100-S23. Wayne: CLSI.
5. Galani L, Ioannidis K, Plakias G, Karaiskos I, Baziaka F, Paskalis C, Vakalis N, Giamarellou H (2012) In the era of polymyxins use: emergence of colistin resistance in Klebsiella pneumoniae. Twenty-second European Congress of Clinical Microbiology and Infectious Diseases.
6. Poirel L, Timothy RW, Vincent C, Nordmann P (2011) Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119-123.
7. Galimand M, Courvalin P, Lambert T (2012) RmtF, a new member of the aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family. Antimicrob Agents Chemother 56: 3960-3962.
8. Bueno MF, Francisco GR, O'Hara JA, de Oliveira Garcia D, Doi Y (2013) Coproduction of 16S rRNA methyltransferase RmtD or RmtG with KPC-2 and CTX-M group extended-spectrum β-lactamases in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 57: 2397-2400.
9. Kamalanathan A, Madhavan HN, Malathi J, Sekar U, Sekar B, Shanthi M, Sowmiya M (2013) Clonal diversity of New Delhi metallobetalactamase-1producing Enterobacteriaceae in a tertiary care centre. Indian J Med Microbiol 31: 237-241.
10. Kumari S, Sen MR, Upadhyay S, Bhattacharjee A (2011) Dissemination of the New Delhi metallo-b-lactamase-1 (NDM-1) among enterobacteriaceae in a tertiary referral hospital in north India. J Antimicrob Chemother 66: 1646-1647. doi:10.1093/jac/dkr180.
11. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10: 597-602.
12. Lee CS, Vasoo S, Hu F, Patel R, Doi Y (2014) *Klebsiella pneumoniae* ST147 coproducing NDM-7 carbapenemase and RmtF 16S rRNA methyltransferase in Minnesota. J Clin Microbiol 52: 4109-4110.
13. Tada T, Miyoshi-Akiyama T, Dahal RK, Mishra SK, Ohara H, Shimada K, Kirikae T, Pokhrel BM (2013) Dissemination of multidrug-resistant *Klebsiella pneumoniae* clinical isolates with various combinations of carbapenemases (NDM-1 and OXA-72) and 16S rRNA methylases (ArmA, RmtC and RmtF) in Nepal. Int J Antimicrob Agents 42: 372-374.
14. Al Sheikh YA, Marie MAM, John J, Krishnappa LG, Dabwab KHM (2014) Prevalence of 16S rRNA methylase genes among β-lactamase-producing Enterobacteriaceae clinical isolates in Saudi Arabia. Libyan J Med 9: 10.3402/ljm.v9.24432. doi: 10.3402/ljm .v9. 24432.

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