

EMERGENCE OF *armA* AND *rmtB* GENES AMONG VIM, NDM, AND IMP METALLO- β -LACTAMASE-PRODUCING MULTIDRUG-RESISTANT GRAM-NEGATIVE PATHOGENS

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In the recent years, it has been noted that microorganisms with acquired resistance to almost all available potent antibiotics are increasing worldwide. Hence, the use of antibiotics in every clinical setup has to be organized to avoid irrational use of antibiotics. This study was aimed to establish the pattern of antibiotic sensitivity and relevance of antimicrobial resistance in aerobic Gram-negative bacilli. A total of 103 aerobic Gram-negative bacteria namely *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Citrobacter koserii*, *Proteus* spp., and *Pseudomonas aeruginosa* were collected from tertiary care centers around Chennai. Kirby–Bauer Disk Diffusion test and study for genes of cephalosporin, carbapenem, and aminoglycoside resistance were done. A descriptive analysis of the data on altogether 103 clinical urine isolates was performed. All strains showed susceptibility to colistin. The frequency of genes encoding 16S rRNA methylases *armA* and *rmtB* were 7.8% and 6.8%, respectively. Among metallo- β -lactamases, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM-1} were detected in 6.8%, 3.8%, and 3.8%, respectively. One *E. coli* strain harbored *bla*_{SIM-1} gene. Cumulative analysis of data suggested that 30% of the strains carried more than one resistance gene. The current research evidenced the increasing frequency of resistance mechanisms in India. Combined approach of antibiotic restriction, effective surveillance, and good infection control practices are essential to overcome antibiotic resistance.

Keywords: multidrug resistance, ESBL, MBL, *armA*, *rmtB*

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Introduction

Enterobacteriaceae and non-fermentative Gram-negative *Pseudomonas* and *Acinetobacter* species are responsible for majority of hospital- and community-acquired infections globally due to their accelerated antibiotic resistance and difficulty in treatment by conventional antibiotics. Colistin and polymyxin B have become the only drugs of choice for serious infections caused by the beta-lactamases-producing aerobic Gram-negative bacilli. The incidence of infections caused by these organisms has increased in recent years [1]. To minimize the spread of these bacteria in the community, there is an increasing need for clinical laboratory to identify and characterize the strains up to the molecular level especially for drug resistance. Moreover, the inappropriate use of antibiotics and acquisition of mobile genetic elements by horizontal transfer may result in a “superbug,” with resistance to all licensed antibiotics. Deliberate and perpetual surveillance studies are warranted since the epidemiology of beta-lactamase-producing bacteria have become complex. In India, many studies have identified disseminated multidrug resistance among Gram-negative bacilli due to extended-spectrum beta-lactamases (ESBL) and carbapenemases [1].

Owing to the dramatic increase in bacterial resistance to β -lactam antibiotics, aminoglycosides have become the substitutes for the therapeutics due to their broad antimicrobial spectrum and also their synergistic effect in association with other antibiotics. Increased prescription of aminoglycosides for therapy accounts for the growing resistance to aminoglycoside antibiotics. Three groups of enzymes produced by bacteria confer aminoglycoside resistance namely, acetyltransferases, nucleotidyl transferases, and phosphotransferases, which inactivate gentamicin (GEN) and tobramycin (TOB). However, recently plasmid-mediated 16S rRNA methylases have emerged which confer remarkably high-level resistance to arbekacin, amikacin (AK), TOB, and GEN (3) through post-transcriptional methylation of the aminoglycoside-binding site leading to the loss of affinity [2].

Until now, 10 plasmid-mediated 16S rRNA methylases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, *rmtG*, *rmtH*, and *npmA*) have been identified in at least 30 countries worldwide [2–11]. Since the 16S rRNA methylase genes are frequently located on plasmids, there is an increased probability of other resistance mechanisms to interplay, thus leading to multidrug resistance. In particular, very recently, this coexistence is seen with genes encoding ESBL and metallo- β -lactamases (MBL). As a consequence, those strains remain resistant to aminoglycosides, beta-lactams, and carbapenems.

Limited studies that are available in India show the percentage of 16S rRNA methylases-mediated aminoglycoside resistance in Gram-negative isolates.

Nevertheless, surveillance in Southern and Eastern parts of Europe reported approximately 10%–50% aminoglycoside resistance in invasive strains with consistent increase every year. Moreover, *armA* and *rmtB* are often identified genes among the methylases in Gram-negative bacilli from East Asia and South America [3]. Hence, we intended to investigate the prevalence of *armA* and *rmtB* genes among the ESBL- and/or MBL-producing *Enterobacteriaceae* and non-fermenting Gram-negative bacilli prospectively recovered from patients at multiple centers in Chennai.

Materials and Methods

Clinical strains

About 103 aerobic Gram-negative urinary isolates resistant to at least one antibiotic were collected from multicenters, in and around Chennai, Tamil Nadu. Identification of bacterial isolates was done to the species level by cultural characteristics and biochemical reactions by conventional methods.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was carried out on all strains by Kirby–Bauer Disk Diffusion Agar method using commercial disks (HiMedia) according to the criteria recommended by Clinical and Laboratory Standards Institute (CLSI) [12]. The antibiotics tested were ampicillin (AMP), ciprofloxacin (CIP), ceftazidime (CAZ), CAZ/clavulanic acid (CAC), piperacillin (PIP), AK, GEN, TOB, imipenem (IPM), meropenem (MEM), ceftiofur (CX), aztreonam (AZT), cefepime (CPM), and PIP/tazobactam (PTZ). All isolates were tested for colistin susceptibility by E-test colistin strip (ranging from 0.016 to 256 mg/ml) according to the manufacturer's guidelines (Liofilchem, Italy). The minimum inhibitory concentration (MIC) was read at the point of complete inhibition of all growth, including hazes. The interpretive criteria used were those established in CLSI. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for antibiotic susceptibility testing and E-test.

Phenotypic screening for ESBLs and MBLs

ESBL production was detected by double-disk synergy test as recommended by the CLSI guidelines (CLSI, 2010). The strains showing any synergy between CAZ/CAC disk and CPM/CAZ disk were considered to be ESBL producer [13–17].

Combined disk test with IPM/MEM–EDTA-impregnated disk was performed for MBL screening as previously described by Arunagiri et al. [18].

Isolation of genomic DNA

A single colony was inoculated into 5-ml Luria–Bertani broth and incubated with shaking at 37 °C for 20 h. Overnight culture was harvested by centrifugation for 5 min and the pellet was resuspended in 500 µl distilled water. The cells were lysed by heating at 95 °C for 5 min and centrifuged for 1 min. After centrifugation, 5 µl of supernatant was used for PCR amplification.

Genotypic ESBL and MBL characterization

Amplification of DNA was performed in a thermal cycler for the detection of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} ESBLs and *bla*_{VIM}, *bla*_{IMP}, *bla*_{SIM-1}, *bla*_{GIM-1}, *bla*_{SPM-1}, and *bla*_{NDM-1} MBLs. Primers, PCR amplicon size, and annealing temperature were indicated in Table I. PCR-amplified products were subjected to electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light to observe the anticipated PCR product.

*Detection of *armA* and *rmtB* genes*

The *armA* and *rmtB* genes were detected by PCR as described by Doi and Arakawa [3]. Primers for *armA* and *rmtB* are described in Table I. Primers for *armA* detected a region of 315 bp and *rmtB* detected a region of 173 bp. PCR amplification was done; the products were electrophoresed in 1.5% agarose gels, and visualized under UV light. The cycling conditions were initial DNA denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing as given in Table I, extension at 72 °C for 1 min, and a final elongation at 72 °C for 5 min.

Results

During the study period, 103 clinical isolates resistant to at least one antibiotic were collected from clinical urine samples from multicenters, in and around Chennai, Tamil Nadu. Samples included 66 (64.1%) females and 37 (35.9%) males whose age group ranged from 1 to 68 years with mean age of 38.4 ± 19.1 years. Isolates were distributed as *E. coli* (73.7%), *Klebsiella pneumoniae* (10.6%), *P. aeruginosa* (9.7%), *Enterobacter* (2.9%), *Citrobacter koseri* (1.9%), and *Proteus* spp. (0.9%).

Table 1. Oligonucleotide primers used for gene detection by PCR amplification (nucleotide sequence)

Target gene	Primer sequence (5'→3')	Product size (bp)	Annealing temperature	Ref.
<i>bla_{TEM}</i>	TEM-F GGTCTGACAGTTACCAATGC	1,074	55 °C for 1 min	[18]
<i>bla_{SHV}</i>	SHV-F CGCGGGTATTCTTATTGTGCG	1,016	68 °C for 1 min	[18]
<i>bla_{CTX-M}</i>	SHV-R TCTTCCGATGCCGCCAGTCA	544	51 °C for 1 min	[19]
	CTX-M-F TTTGGCATGTGCAGTACCAGTAA			
<i>bla_{VIM}</i>	CTX-M-R CGATATCGTTGGTGGTGCATA	500	46°C for 1min	[20]
	VIM-F TTTGGTCGATATCGCAACG			
<i>bla_{IMP}</i>	VIM-R CCAITCAGCCAGATCGGCAT	432	45 °C for 1 min	[20]
	IMP-F GTTTATGTTTCATACWTCG			
<i>bla_{GIM-1}</i>	IMP-R GGTTTAAAYAAAACAACCCAC	477	52 °C for 40 s	[21]
	GIM-F TCGACACACCTTGGTCTGAA			
<i>bla_{SIM-1}</i>	GIM-R AACTTCCAACTTGGCCATGC	570	52 °C for 40 s	[21]
	SIM-F TACAAGGGATTCTGGCATCG			
<i>bla_{SPM-1}</i>	SIM-R TAATGGCCTGTTCCCATGTG	271	52 °C for 40 s	[21]
	SPM-F AAAATCTGGGTACGCAACG			
<i>bla_{NDM-1}</i>	SPM-R ACATTATCCGCTGTGAACAGG	475	55 °C for 1 min	[22]
	NDM-F GGTTGGCGATCTGGTTTC			
<i>armA</i>	NDM-R CGGAATGGCTCATCACGATC	315	51 °C for 1 min	[3]
	armA-F ATTCTGCCCTATCCTAAATTGG			
<i>rmtB</i>	armA-R ACCTATACTTTATCGTCGTC	173	58 °C for 1 min	[3]
	rmtB-F GCTTTCTGGGGCGATGTAA			
	rmtB-R ATGCAATGCCGCGCTCGTAT			

Antimicrobial susceptibility testing

All the 103 isolates were sensitive to colistin by E-test method with MIC \leq 2 μ g/ml and were found resistant to AMP. The resistant trend decreased with respect to MEM, CIP, PIP, AZT, CPM, CAZ, and GEN substantially. More than 50% were resistant to five antibiotics, such as AMP, MEM, CIP, PIP, AZT, and CPM, and more than 50% were sensitive to GEN, CX, AK, CAC, TOB, PTZ, and IPM.

β -lactamase characterization

Of the 103 isolates, 57 (55.3%) were positive for ESBL screening as it showed synergy between CAZ/CAC and CPM/CAZ disk and 14 (13.5%) strains were positive for MBL screening since they exhibited significant zone size enhancement with the ethylenediaminetetraacetic acid-impregnated disk when compared with the IPM/MEM disk. Ten isolates showed positive for both ESBL and MBL screening. The most prevalent gene was *bla*_{CTX-M} as it appeared among the clinical isolates as single resistance gene (16.5%) or in combination with other resistance determinants (23.3%), such as *bla*_{TEM}, *bla*_{VIM}, *bla*_{SHV}, *bla*_{IMP}, and *bla*_{NDM-1}. The second most prevalent genes were TEM and SHV present alone in 6.7% and 3.8%, respectively. Genes *bla*_{VIM}, *bla*_{IMP}, *bla*_{SIM-1}, and *bla*_{NDM-1} were found in 5.8%, 2.9%, 0.9%, and 3.8% of strains, respectively, alone or in combination with other resistance genes. Altogether, 38/103 (36.8%) were found to be negative for any gene, which constitutes of 35 *E. coli* and 3 *K. pneumoniae*.

Prevalence of methylase genes

Out of 103 isolates, only 12 (11.6%) were harboring methylase gene *armA* and *rmtB* and only four of them were presented as individual gene. The remaining eight isolates were positive for these genes together with other beta-lactamase resistance genes.

Discussion

Beta-lactamase production has been identified as one of the most important mechanism of resistance among Gram-negative strains leading to global threat. Recently, the high prevalence of aminoglycoside resistance mechanisms among these strains is alarming. In this study, resistance rates of the Gram-negative isolates to GEN were more than 50.0%, but resistance to AK and TOB were

relatively low. Yu et al. [23] reported similar resistance to GEN among the *E. coli* isolates but showed higher resistance to TOB.

The *armA* gene was initially sequenced from a *Citrobacter freundii* strain in Poland but first characterized from *K. pneumoniae* BM4536 in France in 2000, and the *rmtB* gene was first identified in *Serratia marcescens* S-95 isolated from a Japanese patient in 2002. Since then these two genes have been found in *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter baumannii* in Asia-Pacific region [23–26]. In this study, the overall prevalence rate of 16S rRNA methylase genes *armA* and *rmtB* alone/in combination with beta-lactamase genes was 11.6%. This is higher than the percentage of *armA* and *rmtB* previously reported in a Chinese study (5.4%), a Taiwanese study (0.4%), and a study from Shanghai, China (3.4%) [24, 26]. Very few studies have reported *armA* and *rmtB* genes from India [26]. Unlike other reported studies, the data from this study showed *armA* to be more prevalent than *rmtB* gene [24, 28, 29].

In this study, 16S rRNA methylase genes have been identified to be linked with other beta-lactamase resistance determinants *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM-1}, and cotransferred with other resistance determinants on self-transferable plasmids to recipients by conjugation as reported previously [23–25, 27], thus, conferring resistance to multiple antibiotics. The *armA* gene was detected as a single gene in one (0.9%) *K. pneumoniae* and two (1.9%) *P. aeruginosa* strains. However, in various combinations with other beta-lactamase genes, it was seen in four (3.9%) *E. coli* strains and one (0.9%) *K. pneumoniae* strain. Similarly, *rmtB* gene was detected as a single gene in one (0.9%) *P. aeruginosa* strain but four (3.9%) *E. coli* and one (0.9%) *K. pneumoniae* strains harbored this gene in combination with other beta-lactamase gene. Overall, out of the 14 isolates carrying 16S rRNA methylases, one (0.9%) strain harbored *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{NDM-1}, *armA*, and *rmtB* genes and was highly resistant to all the tested antibiotics except colistin. *Enterobacter*, *C. koseri*, and *Proteus* spp. did not carry any 16S rRNA methylase gene either alone or in combination.

Numerous previous studies have reported ESBL production ranging from 68% to 72% [30, 31] and MBL production 28% to 42% [32, 33] among the Gram-negative strains. In this study, genotypic screening for ESBLs and MBLs identified 55.3% strains carried at least one ESBL gene and 13.5% strains carried at least one of the MBL gene by PCR. The presence and diversity of ESBLs and MBLs in different isolates as a single gene and in association with other resistance genes are shown in Table II. Among the ESBLs, CTX-M was the most common gene found (16.5%) both individually and in combination (22.3%), followed by TEM-type ESBLs (9.7%) individually and (15.5%) in association and SHV-type ESBLs (4.8%) individually and (1.9%) in association. PCR for MBLs showed *bla*_{NDM-1} and *bla*_{VIM} (6.7%) to be the most dominant type found followed by *bla*_{IMP} (3.8%)

Table II. Prevalence of resistance determinants in clinical isolates

Type	Resistance genes		Organism producing	Total
Existence of a single genotype	Extended-spectrum beta-lactamase genes	<i>bla</i> _{CTX-M}	<i>E. coli</i> (11), <i>P. aeruginosa</i> (1), <i>K. pneumoniae</i> (1), and <i>Enterobacter</i> (1)	14
		<i>bla</i> _{TEM}	<i>E. coli</i> (7), <i>P. aeruginosa</i> (2), and <i>Proteus</i> spp. (1)	10
Metallo-β-lactamase genes	16S rRNA methylase genes	<i>bla</i> _{SHV}	<i>E. coli</i> (4) and <i>P. aeruginosa</i> (1)	5
		<i>bla</i> _{VIM}	<i>K. pneumoniae</i> (1)	1
		<i>armA</i>	<i>P. aeruginosa</i> (2) and <i>K. pneumoniae</i> (1)	3
		<i>rmtB</i>	<i>P. aeruginosa</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM}	<i>E. coli</i> (8) and <i>P. aeruginosa</i> (1)	9
Existence of two or more than two genotypes	Association of ESBL and MBL genes	<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} + <i>bla</i> _{SHV}	<i>K. pneumoniae</i> (1) and <i>C. koseri</i> (1)	2
		<i>bla</i> _{CTX-M} + <i>bla</i> _{VIM} + <i>bla</i> _{IMP} + <i>bla</i> _{NDM-1}	<i>K. pneumoniae</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>bla</i> _{VIM-1}	<i>E. coli</i> (2) and <i>K. pneumoniae</i> (1)	3
		<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} + <i>bla</i> _{NDM-1}	<i>E. coli</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>bla</i> _{IMP}	<i>E. coli</i> (1)	1
Combination of 16S rRNA methylase genes with other genes		<i>bla</i> _{IMP} + <i>bla</i> _{NDM-1}	<i>P. aeruginosa</i> (1)	1
		<i>bla</i> _{SIN-1}	<i>E. coli</i> (1)	1
		<i>bla</i> _{TEM} + <i>bla</i> _{VIM}	<i>E. coli</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>armA</i>	<i>E. coli</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>rmtB</i>	<i>E. coli</i> (3)	3
Negative		<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} + <i>bla</i> _{NDM-1} + <i>armA</i> + <i>rmtB</i>	<i>E. coli</i> (1)	1
		<i>bla</i> _{CTX-M} + <i>bla</i> _{TEM} + <i>armA</i>	<i>E. coli</i> (2)	2
		<i>bla</i> _{CTX-M} + <i>bla</i> _{SHV} + <i>rmtB</i>	<i>E. coli</i> (1)	1
		<i>bla</i> _{IMP} + <i>armA</i>	<i>K. pneumoniae</i> (1)	1
		<i>bla</i> _{SHV} + <i>bla</i> _{VIM} + <i>rmtB</i>	<i>K. pneumoniae</i> (1)	2
			38	

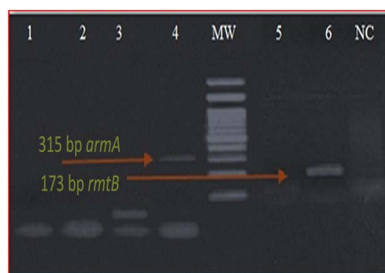


Figure 1. PCR amplification of 16S rRNA methylase genes

and *bla*_{SIM-1} (0.9%) gene similar to a study by Somily et al. [34]. One *E. coli* strain was found to harbor SIM-1 gene and another *E. coli* strain, which was identified with high resistance to all the antibiotics except colistin was positive for *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{NDM-1}, *armA*, and *rmtB* genes. Similarly, the emergence of the multidrug-resistant *E. coli* and *K. pneumoniae* strains producing both ESBLs and 16S rRNA methylases was identified in a previous study in Taiwan. Thus, the antibiotic resistance in Gram-negative strains lead to increased morbidity, mortality at hospital settings as revealed by surveillance studies from Europe, Asia-Pacific region, Latin America, and North America over the last 3–5 years [35].

The antibiotic resistogram showed an increasing resistance to various antibiotics. However, in contrast to the other reported studies, colistin was the most active drug with 100% sensitivity followed by IMP and PIP/PTZ [36]. Hence, colistin is the only choice available for the treatment of these multidrug-resistant strains, but it should be used in accordance with the antimicrobial consumption policy to prevent the dissemination of drug-resistant clones.

AMP showed highest percentage of resistance followed by CIP and MEM (Figure 1). Several published reports have documented reduced susceptibility to MEM among Gram-negative strains. Kaul et al. [37] reported increased carbapenem resistance in Gram-negative bacilli. A study by Srinivasa Rao et al. [38] has reported high-level resistance (>75%) to both carbapenem and other antibiotics routinely used for the treatment of Gram-negative bacilli.

Conclusions

Comparatively, high prevalence of plasmid-mediated *armA* and *rmtB* genes was described in this study among clinical aerobic Gram-negative isolates from urine samples from multicenter in Chennai. The majority of 16S rRNA methylase gene-positive isolates coproduced CTX-M, TEM, SHV-type ESBLs and IMP, VIM, NDM-1-type MBLs. Thus, both horizontal gene transfer and clonal spread

may occur leading to widespread dissemination of the *armA* and *rmtB* genes. A very rare gene is *bla_{SIM}* and it is usually found in *Acinetobacter* spp., but in this study, we have identified the presence of *bla_{SIM-1}* in *E. coli*, which may indicate the dissemination of the *bla_{SIM-1}* gene.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Manoharan, A., Barla, G. S., Peter, R., Sugumar, M., Mathai, D.: Multidrug resistance mediated by co-carriage of extended-spectrum beta-lactamases, AmpC and New Delhi metallo-beta-lactamase-1 genes among carbapenem-resistant *Enterobacteriaceae* at five Indian medical centres. *Indian J Med Microbiol* **34**, 359–361 (2016).
2. Samadi, N., Pakzad, I., Monadi Sefidan, A., Hosainzadegan, H., Tanomand, A.: Study of aminoglycoside resistance genes in *Enterococcus* and *Salmonella* strains isolated from Ilam and Milad hospitals, Iran. *Jundishapur J Microbiol* **8**, e18102 (2015).
3. Doi, Y., Arakawa, Y.: 16S ribosomal RNA methylation: Emerging resistance mechanism against aminoglycosides. *Clin Infect Dis* **45**, 88–94 (2007).
4. Deng, Y. T., Zeng, Z. L., Tian, W., Yang, T., Liu, J. H.: Prevalence and characteristics of *rmtB* and *qepA* in *Escherichia coli* isolated from diseased animals in China. *Front Microbiol* **4**, 198 (2013).
5. Bueno, M. F., Francisco, G. R., O'Hara, J. A., de Oliveira Garcia, D., Doi, Y.: Co-production of 16S ribosomal RNA methyltransferase RmtD and RmtG with KPC-2 and CTX-M-group ESBLs in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* **53**, 2397–2400 (2013).
6. Davis, M. A., Baker, K. N., Orfe, L. H., Shah, D. H., Besser, T. E., Call, D. R.: Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli*. *Antimicrob Agents Chemother* **54**, 2666–2669 (2010).
7. Galimand, M., Courvalin, P., Lambert, T.: Plasmid-mediated high-level resistance to aminoglycosides in *Enterobacteriaceae* due to 16S rRNA methylation. *Antimicrob Agents Chemother* **47**, 2565–2571 (2003).
8. Galimand, M., Courvalin, P., Lambert, T.: *RmtF*, a new member of the aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family. *Antimicrob Agents Chemother* **56**, 3960–3962 (2012).
9. O'Hara, J. A., McGann, P., Snesrud, E. C., Clifford, R. J., Waterman, P. E., Lesho, E. P., Doi, Y.: Novel 16S ribosomal RNA methyltransferase *RmtH* produced by *Klebsiella pneumoniae* associated with war-related trauma. *Antimicrob Agents Chemother* **57**, 2413–2416 (2013).
10. Wachino, J., Shibayama, K., Kurokawa, H., Kimura, K., Yamane, K., Suzuki, S., Shibata, N., Ika, Y., Arakawa, Y.: Novel plasmid-mediated 16S rRNA m1A1408 methyltransferase, NpmA, found in a clinically isolated *Escherichia coli* strain resistant to structurally diverse aminoglycosides. *Antimicrob Agents Chemother* **51**, 4401–4409 (2007).

11. Yokoyama, K., Doi, Y., Yamane, K., Kurokawa, H., Shibata, N., Shibayama, K., Yagi, T., Kato, H., Arakawa, Y.: Acquisition of 16S rRNA methylase gene in *Pseudomonas aeruginosa*. *Lancet* **362**, 1888–1893 (2003).
12. Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second International Supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA, 2012.
13. Aitta, A. A., Said, M. E., Dabaa, E. E., El Salam, M. A., Mahdy, H. M.: Detection of plasmid-mediated 16S rRNA methylase conferring high-level resistance to aminoglycosides in gram negative bacilli from Egypt. *Life Sci J* **9**, 2472–2479 (2012).
14. Kolar, M., Bardon, J., Chroma, M., Hricova, K., Stosova, T., Sauer, P., Koukalova, D.: ESBL and AmpC beta-lactamase-producing *Enterobacteriaceae* in poultry in the Czech Republic. *Vet Med* **55**, 119–124 (2010).
15. Abreu, A. G., Marques, S. G., Neto, V. M., Gonçalves, A. G.: Extended-spectrum beta-lactamase producing *Enterobacteriaceae* in community-acquired urinary tract infections in São Luís, Brazil. *Braz J Microbiol* **44**, 469–471 (2013).
16. Pitout, J. D., Reisbig, M. D., Venter, E. C., Church, D. L., Hanson, N. D.: Modification of the double-disk test for detection of *Enterobacteriaceae* producing extended spectrum and AmpC β -lactamases. *J Clin Microbiol* **41**, 3933–3935 (2003).
17. Huseyin, T., Hakki, B.: Molecular characterization of TEM- and SHV-derived extended-spectrum beta-lactamases in hospital-based *Enterobacteriaceae* in Turkey. *Jpn J Infect Dis* **58**, 162–167 (2005).
18. Arunagiri, K., Sekar, B., Sangeetha, G., John, J.: Detection and characterization of metallo-beta-lactamases in *Pseudomonas aeruginosa* by phenotypic and molecular methods from clinical samples in a tertiary care hospital. *West Indian Med J* **61**, 778–783 (2012).
19. Edelstein, M., Pimkin, M., Palagin, I., Edelstein, I., Stratchounski, L.: Prevalence and molecular epidemiology of CTX-M extended-spectrum- β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother* **47**, 3724–3732 (2003).
20. Amudhan, M. S., Sekar, U., Kamalanathan, A., Balaraman, S.: *bla*_{IMP} and *bla*_{VIM} mediated carbapenem resistance in *Pseudomonas* and *Acinetobacter* species in India. *J Infect Dev Ctries* **6**, 757–762 (2012).
21. Ellington, M. J., Kistler, J., Livermore, D. M., Woodford, N.: Multiplex PCR for rapid detection of genes encoding acquired metallo- β -lactamases. *J Antimicrob Chemother* **59**, 321–322 (2007).
22. Shanthi, M., Sekar, U., Kamalanathan, A., Sekar, B.: Detection of New Delhi metallo-beta-lactamase-1 (NDM-1) carbapenemase in *Pseudomonas aeruginosa* in a single centre in southern India. *Indian J Med Res* **140**, 546–550 (2014).
23. Yu, F. Y., Yao, D., Pan, J. Y., Chen, C., Qin, Z. Q., Parsons, C., Yang, L. H., Li, Q. Q., Zhang, X. Q., Qu, D., Wang, L. X.: High prevalence of plasmid-mediated 16S rRNA methylase gene *rmtB* among *Escherichia coli* clinical isolates from a Chinese teaching hospital. *BMC Infect Dis* **10**, 184 (2010).
24. Galimand, M., Courvalin, P., Lambert, T.: Plasmid-mediated high-level resistance to aminoglycosides in *Enterobacteriaceae* due to 16S rRNA methylation. *Antimicrob Agents Chemother* **47**, 2565–2571 (2003).
25. Yan, J. J., Wu, J. J., Ko, W. C., Tsai, S. H., Chuang, C. L., Wu, H. M., Lu, Y. J., Li, J. D.: Plasmid mediated 16S rRNA methylases conferring high-level aminoglycoside resistance

- in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. *J Antimicrob Chemother* **54**, 1007–1012 (2004).
26. Doi, Y., Yokoyama, K., Yamane, K., Wachino, J., Shibata, N., Yagi, T., Shibayama, K., Kato, H., Arakawa, Y.: Plasmid-mediated 16S rRNA methylase in *Serratia marcescens* conferring high-level resistance to aminoglycosides. *Antimicrob Agents Chemother* **48**, 491–496 (2004).
 27. Ali, S. Z., Tabish, M., Al-Shahrani, A.: Reassuring no *bla*_{NDM-1} harboring *K. pneumoniae* in neonatal intensive care unit of Aligarh Hospital, Uttar Pradesh, India. *Int J Res Pharm Sci* **6**, 25–29 (2016).
 28. Wu, Q., Zhang, Y., Han, L., Sun, J., Ni, Y.: Plasmid-mediated 16S rRNA methylases in aminoglycoside-resistant *Enterobacteriaceae* isolates in Shanghai, China. *Antimicrob Agents Chemother* **53**, 271–272 (2009).
 29. Yu, F., Wang, L., Pan, J., Yao, D., Chen, C., Zhu, T., Lou, Q., Hu, J., Wu, Y., Zhang, X., Chen, Z., Qu, D.: Prevalence of 16S rRNA methylase genes in *Klebsiella pneumoniae* isolates from a Chinese teaching hospital: Coexistence of *rmtB* and *armA* genes in the same isolate. *Diagn Microbiol Infect Dis* **64**, 57–63 (2009).
 30. Mittal, N., Nair, D., Gupta, N., Rawat, D., Kabra, S., Kumar, S., Prakash, S. K., Sharma, V. K.: Outbreak of *Acinetobacter* spp septicemia in a neonatal ICU. *Southeast Asian J Trop Med Public Health* **34**, 365–366 (2003).
 31. Kansal, R., Pandey, A., Asthana, A. K.: Beta-lactamase producing *Acinetobacter* species in hospitalized patients. *Indian J Pathol Microbiol* **52**, 456–457 (2009).
 32. Rawat, D., Nair, D.: Extended-spectrum β -lactamases in gram negative bacteria. *J Glob Infect Dis* **2**, 263–274 (2010).
 33. Uma Karthika, R., Srinivasa Rao, R., Sahoo, S., Shashikala, P., Kanungo, R., Jayachandran, S., Prasanth, K.: Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. *J Med Microbiol* **58**, 430–435 (2009).
 34. Somily, A. M., Garaween, G. A., Abukhalid, N., Absar, M. M., Senok, A. C.: Comparison of molecular and phenotypic methods for the detection and characterization of carbapenem resistant *Enterobacteriaceae*. *Acta Microbiol Immunol Hung* **63**, 69–81 (2016).
 35. Ko, K. S., Suh, J. Y., Kwon, K. T., Jung, S. I., Park, K. H., Kang, C. I., Chung, D. R., Peck, K. R., Song, J. H.: High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J Antimicrob Chemother* **60**, 1163–1167 (2007).
 36. Rynga, D., Shariff, M., Deb, M.: Phenotypic and molecular characterization of clinical isolates of *Acinetobacter baumannii* isolated from Delhi, India. *Ann Clin Microbiol Antimicrob* **14**, 40 (2015).
 37. Kaul, S., Brahmadathan, K. N., Jagannati, M., Sudarsanam, T. D., Pitchamuthu, K., Abraham, O. C., John, G.: One year trends in the gram-negative bacterial antibiotic susceptibility patterns in a medical intensive care unit in South India. *Indian J Med Microbiol* **25**, 230–235 (2007).
 38. Srinivasa Rao, R., Uma Karthika, R., Singh, S. P., Shashikala, P., Kanungo, R., Jayachandran, S., Prashanth, K.: Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*. *Indian J Med Microbiol* **26**, 333–337 (2008).