ORIGINAL ARTICLE

Prevalence of 16S rRNA methylase genes among β-lactamase-producing Enterobacteriaceae clinical isolates in Saudi Arabia

Yazeed A. Al Sheikh[1,2], Mohammed Ali M. Marie[1$]*, James John[3,4], Lakshmana Gowda Krishnappa[1$] and Khaled Homoud M. Dabwab[1]

[1]Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia; [2]Chair of Medical and Molecular Genetics Research, Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia; [3]Department of Clinical Microbiology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India; [4]Department of Biotechnology, School of Life Sciences, Pondicherry University, Pondicherry, India

Background: Co production of 16S rRNA methylases gene and β-Lactamase gene among Enterobacteriaceae isolates conferring resistance to both therapeutic options has serious implications for clinicians worldwide.

Methods: To study co existence of 16S rRNA methylases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, and *npmA*) and β-Lactamase (bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-14}$) genes, we screened all phenotypic positive β-Lactamase producing enterobacteriaceae by polymerase chain reaction (PCR) targeting above genes. A total of 330 enterobacteriaceae strains were collected during study period out of that 218 isolates were identified phenotypically as β-Lactamase producers, which include 50 (22.9%) *Escherichia coli*; 92 (42.2%) *Klebsiella pneumoniae*, 44 (20.2%), *Citrobactor freundii* and 32 (14.7%) *Enterobacter* spp.

Results: Among this 218, only 188 isolates harbored the resistant gene for β-Lactamase production. Major β-Lactamase producing isolates were bla_{TEM-1} type. 122 (56 %) isolates were found to produce any one of the 16S rRNA methylase genes. A total of 116 isolates co produced β-Lactamase and at least one 16S rRNA methylases gene Co production of *armA* gene was found in 26 isolates with *rmtB* and in 4 isolates with *rmtC*. The *rmtA* and *rmtD* genes were not detected in any of the tested isolates. Six isolates were positive for a 16S rRNA methylase gene alone.

Conclusion: β-Lactamase producing isolates appears to coexist with 16S rRNA methylase predominantly *armA* and *rmtB* genes in the same isolate. We conclude the major β-Lactamase and 16S rRNA methylases co-producer was *K. pneumoniae* followed by *E. coli*. We suggest further work on evaluating other β-lactamases types and novel antibiotic resistance mechanisms among Enterobacteriaceae.

Keywords: *β-lactamase*; *Enterobacteriaceae*; *16S rRNA methylase gene*

*Correspondence to: Mohammed Ali M. Marie, Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Kingdom of Saudi Arabia, Mobile: +966531384490, Email: dr.mmarie2000@gmail.com

The widespread use of antibacterial drugs has resulted in the emergence of multidrug and pan-drug resistant Gram negative bacterial strains, which pose challenges in an era when new antibiotic choices are limited (1, 2). Multidrug resistant strains are intermediate or resistant to at least three drugs in the classes of β-lactams, carbapenems, aminoglycosides, and fluoroquinolones, whereas pan-drug resistant strains are treatable only with a colistin (3). Pan-drug resistant pathogens with acquired resistance to almost all available antimicrobial agents have emerged in recent years and challenged the treatment of infections (2, 3). Strains in the Enterobacteriaceae family have been well documented for their resistance to clinically used antibiotics (4, 5). Extended spectrum β-lactamase (ESBL) and metallo β-lactamase (MBL) production are the commonly recognized resistance mechanisms in Enterobacteriaceae strains worldwide (6).

Usually, aminoglycosides such as amikacin and tobramycin are used to treat infections by Gram negative pathogens, in combination with β-lactam agents.

$Equally contributed.

But increasing resistance to aminoglycosides by novel mechanisms is being reported (7, 8). 16S rRNA methylases have emerged recently as a mechanism of high level aminoglycoside resistance to most of the clinically important aminoglycosides, including arbekacin, amikacin, tobramycin, and gentamicin (4, 9). In 2003, the first 16S rRNA methylase gene, *armA*, was identified. Since then, eight plasmid-mediated 16S rRNA methylases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, and *npmA*) have been found in clinical isolates of Gram negative bacilli (10–12). Recently, *rmtD* (which was first identified in a pan-resistant *Pseudomonas aeruginosa* strain isolated in Brazil and hitherto only found in Latin America), was also detected in a *Klebsiella pneumoniae* isolate in Brazil (13–15). *armA* and *rmtB* have been found in many species of Gram negative bacilli in Asia (4, 16).

Dissemination of β-lactamase producing clinical isolates with acquired 16S rRNA methylase genes has become a global concern. Unreasonable use of β-lactams, including cephalosporins, carbapenams, and aminoglycosides for the treatment of Gram-negative nosocomial infections has facilitated the emergence of pan-resistant strains associated with therapeutic failures (3, 4). Coexistence of 16S rRNA methylase genes and β-lactamase genes among Enterobacteriaceae isolates confers resistance to both therapeutic options. This study was undertaken to determine the prevalent mechanisms of resistance among β-lactamase positive Enterobacteriaceae strains and also to investigate by polymerase chain reaction (PCR) the coexistence of β-lactamase and 16S rRNA methylase antibiotic resistance genes in these strains.

Materials and methods

Bacterial strains

Between January 2011 and December 2011, 330 non-duplicate Enterobacteriaceae isolates were consecutively collected from patients at El Iman Hospital, Riyadh: *E. coli*, *K. pneumoniae*, *C. freundii*, and *Enterobactor* spp (*E. aerogenes* and *E. cloacae*). The strains were obtained mostly from urine (72%) and blood (11%), but also from wounds, sputum, and other body fluids. Identification of the clinical isolates was confirmed by using GNI cards on the VITEK-60 system (bioMerieux, Marcy l'Etoile, France) and conventional methods, namely, Gram staining, morphology on differential media (MacConkey agar), and biochemical tests (manitol motility, Mannite, citrate utilization, indole production, triple sugar ion, and methyl red and Voges Proskauer tests).

Antimicrobial susceptibility testing

Antimicrobial susceptibilities were determined by using GNS cards on the VITEK-60 system (bioMerieux, France). Antimicrobial susceptibility to amikacin, tobramycin, and gentamicin was confirmed by the disk diffusion test using commercial disks for cefotaxime, ceftazidime, ciprofloxacin, levofloxacin, cefoxitin, imipenem, tetracycline, and trimethoprim/sulfamethoxazole (Himedia, Mumbai, India) according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) (17). Minimum inhibitory concentrations (MICs) of amikacin, tobramycin, and gentamicin were determined by agar dilution according to CLSI guidelines and interpretative criteria. *E. coli* ATCC 25922, *Enterococci* ATCC 29212, and *K. pneumoniae* ATCC 27853 were used as quality control strains for antimicrobial susceptibility testing.

Phenotypic identification of β-lactamase strains

β-Lactamase-producing strains were identified by resistance to third generation cephalosporins by using a VITEX-60 system (bioMerieux) and by production of β-lactamase utilizing the confirmatory double-disk combination test (17). *K. pneumoniae* ATCC 700603 was used as the β-lactamase positive control. β-lactamase producers were further characterized for the presence of different β-lactamase genes.

Extraction of total DNA from β-lactamase-producing strains

Whole genomic DNA was extracted from colonies grown overnight (14–16 h) at 37°C on blood agar (Remel, Lenexa, KS) using the QIAamp DNA Mini Kit and the QIAcube instrument (Qiagen, Valencia, CA) according to the manufacturers' instructions. Total DNA was extracted and stored at −20°C for further assays.

Genotypic characterization of β-lactamase and 16S rRNA methylase genes

bla genes were detected in β-lactamase-producing isolates by PCR using the previously reported oligonucleotide primers for bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-14}$ (18), and the *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, and *npmA* genes by using the following previously described primers (9, 13, 19–21):

Primer	Sequence
armA-f	5′ TATGGGGGTCTTACTATTCTGCCTAT
armA-r	5′ TCTTCCATTCCCTTCTCCTTT
rmtA-f	5′ CTAGCGTCCATCCTTTCCTC
rmtA-r	5′ TTTGCTTCCATGCCCTTGCC
rmtB-f	5′ TCAACGATGCCCTCACCTC
rmtB-r	5′ GCAGGGCAAAGGTAAAATCC
rmtC-f	5′ GCCAAAGTACTCACAAGTGG
rmtC-r	5′ CTCAGATCTGACCCAACAAG
rmtD-f	5′ CTGTTTGAAGCCAGCGGAACGC
rmtD-r	5′ GCGCCTCCATCCATTCGGAATAG
npmA-f	5′ CTCAAAGGAACAAAGACGG
npmA-r	5′ GAAACATGGCCAGAAACTC

Sequencing analysis of multiplex PCR products

Isolates positive for *bla* or 16S rRNA methylase genes were identified by PCR. The identities of the genes were confirmed by DNA sequencing on an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA) following the standard protocol, and the sequences were compared with those in the GenBank nucleotide database at http://www.ncbi.nlm.nih.gov/blast/. Thereafter, positive isolates were used as positive controls for the rest of the respective PCR reactions.

Results

Of the 330 isolated strains of Enterobacteriaceae, 218 isolates were identified as β-lactamase producers by the double-disk combination test: 50 (22.9%) *E. coli*, 92 (42.2%) *K. pneumoniae*, 44 (20.2%) *C. freundii*, and 32 (14.7%) *Enterobacter* spp. All 330 isolates showed the highest resistance to ciprofloxacin (70%) followed by tobramycin (68%), gentamicin (58%), aztreonam (57%), amikacin (54%), and co-trimoxazole (54%). A significantly higher resistance to nitrofurantoin was seen in *K. pneumoniae* strains (82%) than in *E. coli* (24%). Strong resistance was noted for carbapenems: meropenem (49%) and imipenem (38%). Most of the isolates were susceptible to colistin (95%) and tigecycline (93%). MIC values of all 330 isolates were ≥ 16 μg/ml for gentamicin and tobramycin, and more than 32 μg/ml for amikacin.

β-Lactamase characterization

The major β-lactamase-producing isolates were of the bla_{TEM-1} type: at least one bla_{TEM-1} gene was present in 83.5% (182/218) of the isolates. All 218 isolates of *E. coli* and *K. pneumoniae* possessed one or more β-lactamase gene, whereas 14 of the *C. freundi* and 16 *Enterobacter* spp were negative for all tested β-lactamase genes.

In total 70 isolates (32%) harbored only bla_{TEM-1}: 26 *E. coli* and 44 *K. pneumoniae*. We detected the presence bla_{TEM-1} in combination with $bla_{CTX-M-14}$ in 51% of the isolates (111/218) and in combination with bla_{SHV-12} in 39.4% (86/218). Other important combinations were $bla_{CTX-M-14} + bla_{TEM-1} + bla_{SHV-12}$ (39%; $n = 85$) and $bla_{CTX-M-14} + bla_{TEM-1}$ (12%; $n = 26$) (Table 1).

16S rRNA methylase characterization of β-lactamase

Among the 218 isolates, 122 isolates (56%) were found to produce 16S rRNA methylase genes, including 24 *E. coli*, 52 *K. pneumoniae*, 30 *Citrobacter*, and 16 *Enterobacter* spp. *armA*, *rmtB*, *rmtC*, and *npmA* were found exclusively in 72, 10, 6, and 4 isolates, respectively. *armA* coexisted with *rmtB* in 26 isolates and with *rmtC* in 4 isolates. *rmtA* and *rmtD* were not detected in any of the isolates (Table 2).

Coexistence of β-lactamase and 16S rRNA methylase genes

A total of 116 isolates harbored both β-lactamase and 16S rRNA methylase genes, including 24 *E. coli*, 52 *K. pneumoniae*, 26 *C. freundi*, and 14 *Enterobactor* spp. Six isolates were positive for 16S rRNA methylase genes alone. Sixty-six β-lactamase-producing isolates did not harbor any methylase genes (Tables 3 and 4).

Discussion

We noted that almost all (83.5%) Enterobacteriaceae isolates were β-lactamase positive. This rate is even higher than reported in some other developing countries ($60 - 70\%$) (2, 5, 13, 22). By contrast, the prevalence of ESBL-producing isolates among Enterobacteriaceae in developed nations is much lower: $< 1\%$ in Sweden (23) and 1.7% in France (24). One review reported that the prevalence of ESBLs in Europe was higher than in the US but much lower than in Asia and South America (25). This issue is evidently a challenge in developing nations, and our study clearly shows that it is a matter of urgency in our region.

The isolates were tested only for the *bla* genes reported to be prevalent in this region (26) ($bla_{CTX-M-14} + bla_{TEM-1}$ and bla_{SHV}), and 83.5% of them were positive for bla_{TEM-1}. An Indian study (2) reported that 61.1% of MBL producers carried *bla(VIM)* and 2 (3%) carried *bla(IMP)*. However, in our study we did not examine these two genes but only the major ESBL genes. This is probably the reason why some phenotypically β-lactamase positive isolates were genotypically negative for *bla* genes.

Table 1. Coexistence of different β-lactamase genes in isolates phenotypically positive for β-lactamase

	blaTEM-1	blaTEM-1+CTX-M 14	bla TEM-1+SHV-12 like	blaTEM-1+SHV 12 like+CTX-M 14	bla negative*	Total
E. coli	26	3	1	20	00	50
K. pneumoniae	44	9	0	39	00	92
C. freundi	0	10	0	16	14	44
Enterbacter	0	4	0	10	18	32
Total	70	26	1	85	36**	218

*bla-negative isolates are attributed to presence of *bla* genes other than those analyzed.
**Six isolates were postive for 16S rRNA methylase genes (*armA* and *rmtB*).

Table 2. Coexistence of different 16sRNA methylase genes in isolates phenotypically positive for β-lactamase

	armA	*rmt B*	*rmt C*	*npmA*	*armA+rmtB*	*armA+rmtC*	*arm* negative*	Total
E. coli	16	2	2	0	2	2	26	50
K. pneumoniae	34	6	0	2	8	2	40	92
C. freundi	16	2	0	0	12	0	14	44
Enterobacter	6	0	4	2	4	0	16	32
Total	72	10	6	4	26	4	96	218

*Isolates negative for 16S rRNA methylase genes may be attributed to absence of methylase genes or to presence of methylase genes other than those that were analyzed in this study, such as *rmtE* and *rmtF*.

Previous study showed that the most prevalent microorganism was *E. coli*, followed by *K. pneumonia*, and other *Enterobacteriaceae* (5, 6, 21, 27). A study from France in 2008 reported that the most prevalent species were *E. coli* (48.5%), *Enterobacter aerogenes* (23.7%), and *K. pneumoniae* (14.8%) (24). In our study, the contribution of *K. pneumonia* to the β-lactamase strains was higher (42.2%, 92/218), whereas that of *E. coli* was lower (22.9%, (50/218). Noteworthy, most of the isolates (72%) were obtained from urinary tract specimens, and they showed strong resistance to aminoglycosides (amikacin, tobramycin, and gentamicin), thus limiting the treatment options.

β-Lactamase-producing Enterobacteriaceae often harbor other genes (*ampC*, *MBL*, and 16S rRNA methylase genes) that confer resistance to other antibiotics, including carbapenams, cephems, and aminoglycosides (3, 4, 9). 16S rRNA methylase genes have been reported worldwide in Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter*

baumannii (4, 14, 16, 28). A previously published report suggested the spread of *armA* and *rmt*B in Enterobacteriaceae isolates globally (29), and these genes were often reported to coexist with other resistance determinants, such as *bla* and *MBL* genes (12, 13, 30, 31). Newer 16S ribosomal RNA methylases have been identified recently and added to the database of aminoglycoside resistance mechanisms (4, 14, 16).

Only a few studies are available on the prevalence of 16S rRNA methylase genes in clinical Enterobacteriaceae isolates in our region (4). Therefore, we investigated and characterized the association of 16S rRNA methylase genes with β-lactamase genes among clinical Enterobacteriaceae isolates in a Saudi hospital. We observed that the coexistence of the 16S rRNA methylase genes and β-lactamase was very common: 56% of isolates that were genotypically positive for β-lactamase possessed one of the 16S rRNA methylase genes, predominantly *armA*. This rate is higher than those reported in a Taiwanese

Table 3. Coexistence of *bla* genes with different 16S rRNA methylase genes in isolates phenotypically positive for β-lactamase

Positive for genes	*n*
β-Lactamase+*armA*	64
β-Lactamase+*armA*+*rmt B*	23
β-Lactamase+*rmt B*	10
β-Lactamase+*rmt C*	6
β-Lactamase+*armA*+*rmt C*	3
β-Lactamase+*npmA*	4
β-Lactamase+*armA*	4
β-Lactamase+*armA*+*rmt C*	1
β-Lactamase+*armA*+*rmt B*	1
armA only	4
armA+*rmt B* only	2
Positive for *bla* gene but no 16S rRNA detected but	66
No *bla* gene or 16S rRNA methylase gene detected*	30
Total	218

*Negative isolates may attributed to presence of *bla* or methylase genes that were not analyzed in this study.

Table 4. Coexistence of 16S rRNA genes with different ESBL genes in isolates phenotypically positive for β-lactamase

Positive for gene	*n*
TEM-1+SHV 12 like+CTX-M 14+16S rRNA methylases	64
TEM-1+CTX-M 14+16S rRNA methylases	23
TEM-1+SHV 12 like+CTX-M 14+16S rRNA methylases	10
TEM-1+SHV 12 like+CTX-M 14+16S rRNA methylases	6
TEM-1+16S rRNA methylases	4
TEM-1+SHV 12 like+CTX-M 14+16S rRNAmethylases	4
TEM-1+CTX-M 14+16S rRNA methylases	3
TEM-1+SHV 12 like	1
TEM-1+16S rRNA methylases	1
TEM-1+SHV 12 like+CTX-M 14+16S rRNA methylases	1
TEM-1 only	65
Positive for 16S rRNA but no *bla* gene detected	6
No *bla* or 16S rRNA methylase gene detected*	30
Total	218

*Negative isolates may be attributed to presence of *bla* or methylase genes that were not analyzed in this study.

study (21) and a Belgian study (28). But like previous reports (4, 16, 28), we also observed greater prevalence of *armA* than *rmtB*. We also report the presence of *rmtC* in four *E. coli* and four *Enterobacter* isolates. It is noteworthy that we identified *npmA* in two *K. pneumoniae* and two *Enterobacter* isolates. This is a novel finding in this region, because *rmtC* and *npmA* have been detected in *Proteus mirabilis* and *E. coli*, respectively, in Japan (13, 20). We did not detect any *rmtA* or *rmtD* gene in our isolates.

In agreement with previous reports (26, 32), β-lactamase positive isolates were more susceptible to colistin and tigecycline, which were the only available therapeutic options for them.

In our study, 35.2% (64/182), 5.5% (10/182), 3.3% (6/182), and 2.2% (4/182) of the isolates harboring $bla_{\text{TEM-1}}$ also contained *armA, rmtB, rmtC, and npmA*, respectively, in association with $bla_{\text{TEM-1}}$ + SHV 12 like + CTX-M 14. One isolate produced *armA* and *rmtB* along with $bla_{\text{TEM-1}}$ + SHV 12 like + CTX-M 14. This points to horizontal as well as clonal dissemination of these genes in our hospital. We also found strains harboring two different 16S rRNA methylase genes in combinations, including two strains carrying *armA* + *rmtB* and one strain carrying *armA* + *rmtC*.

In conclusion, β-lactamase-producing isolates of Enterobacteriaceae were common in our hospital and they usually harbored 16S rRNA methylase genes, predominantly a*rmA* and *rmtB*. The major co-producers of β-lactamase and 16S rRNA methylases were *K. pneumonia*, followed by *E. coli*. Our findings underline the emerging threat of pan-drug resistant pathogens that produce both β-lactamase and 16S rRNA methylase disseminating in this region. Further work to evaluate other β-lactamase types and novel antibiotic resistance mechanisms among Enterobacteriaceae and other nosocomial pathogens is needed.

Acknowledgements

Conflict of interest and funding

The authors certify that there is no financial or other conflict of interest regarding the material discussed in the manuscript.

References

1. Carlet J, Jarlier V, Harbarth S, Voss A, Goossens H, Pittet D, et al. Ready for a world without antibiotics? The Pensières Antibiotic Resistance Call to Action. Antimicrob Resist Infect Control. 2012; 1: 11.

2. 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. 2012; 61: 778–83.

3. Kashanian J, Hakimian P, Blute M, Jr, Wong J, Khanna H, Wise G, et al. Nitrofurantoin: the return of an old friend in the wake of growing resistance. BJU Int. 2008; 102: 1634–7.

4. Krishnappa LG, John J, Marie MAM, Gopalkrishnan S, Pradeep CS, Rani SRB. Detection of pan-amino glycoside-resistant Gram negative bacteria using a molecular method. South Asian J Exp Biol. 2012; 2: 256–8.

5. Sahni RD, Balaji V, Varghese R, John J, Tansarli GS, Falagas ME. Evaluation of fosfomycin activity against uropathogens in a fosfomycin-naive population in South India: a prospective study. Future Microbiol. 2013; 8: 675–80.

6. Mammina C, Di Carlo P, Cipolla D, Giuffrè M, Casuccio A, Di Gaetano V, et al. Surveillance of multidrug-resistant gram-negative bacilli in a neonatal intensive care unit: prominent role of cross transmission. Am J Infect Control. 2007; 35: 222–30.

7. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrob Agents Chemother. 2000; 44: 3249–56.

8. Lee H, Yong D, Yum JH, Roh KH, Lee K, Yamane K, et al. Dissemination of 16S rRNA methylase-mediated highly amikacin-resistant isolates of Klebsiella pneumoniae and Acinetobacter baumannii in Korea. Diagn Microbiol Infect Dis. 2006; 56: 305–12.

9. Doi Y, Yokoyama K, Yamane K, Wachino J-I, Shibata N, Yagi T, et al. Plasmid-mediated 16S rRNA methylase in Serratia marcescens conferring high-level resistance to aminoglycosides. Antimicrob Agents Chemother. 2004; 48: 491–6.

10. Galimand M, Courvalin P, Lambert T. Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. Antimicrob Agents Chemother. 2003; 47: 2565–71.

11. Galimand M, Courvalin P, Lambert T. *Rmt*F, a new member of the aminoglycoside resistance 16S rRNA N7 G1405 methyl-transferase family. Antimicrob Agents Chemother. 2012; 56: 3960–2.

12. Hidalgo L, Hopkins KL, Gutierrez B, Ovejero CM, Shukla S, Douthwaite S, et al. Association of the novel aminoglycoside resistance determinant *Rmt*F with NDM carbapenemase in Enterobacteriaceae isolated in India and the UK. J Antimicrob Chemother. 2013; 68: 1543–50.

13. Doi Y, de Oliveira Garcia D, Adams J, Paterson DL. Coproduction of novel 16S rRNA methylase *Rmt*D and metallo-β-lactamase SPM-1 in a panresistant pseudomonas aeruginosa isolate from Brazil. Antimicrob Agents Chemother. 2007; 51: 852–6.

14. Yamane K, Rossi F, Barberino MGMA, Adams-Haduch JM, Doi Y, Paterson DL. 16S ribosomal RNA methylase *Rmt*D produced by Klebsiella pneumoniae in Brazil. J Antimicrob Chemother. 2008; 61: 746–7.

15. Castanheira M, Fritsche TR, Sader HS, Jones RN. *Rmt*D 16S RNA methylase in epidemiologically unrelated spm-1-producing Pseudomonas aeruginosa isolates from Brazil. Antimicrob Agents Chemother. 2008; 52: 1587–8.

16. Yamane K, Wachino J, Suzuki S, Shibata N, Kato H, Shibayama K, et al. 16S rRNA methylase-producing, gram-negative pathogens, Japan. Emerg Infect Dis. 2007; 13: 642–6.

17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-18. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

18. Barguigua A, El Otmani F, Talmi M, Zerouali K, Timinouni M. Prevalence and types of extended spectrum β-lactamases among urinary Escherichia coli isolates in Moroccan community. Microb Pathog. 2013; 61–62: 16–22.

19. Wachino J, Yamane K, Kimura K, Shibata N, Suzuki S, Ike Y, et al. Mode of transposition and expression of 16S rRNA methyltransferase gene *rmt*C accompanied by ISEcp1. Antimicrob Agents Chemother. 2006; 50: 3212–5.

20. Wachino J, Shibayama K, Kurokawa H, Kimura K, Yamane K, Suzuki S, et al. Novel plasmid-mediated 16S rRNA m1A1408 methyltransferase, *NpmA*, found in a clinically isolated Escherichia coli strain resistant to structurally diverse aminoglycosides. Antimicrob Agents Chemother. 2007; 51: 4401–9.

21. Yan J-J, Wu J-J, Ko W-C, Tsai S-H, Chuang C-L, Wu H-M, et al. Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in Escherichia coli and Klebsiella pneumoniae isolates from two Taiwanese hospitals. J Antimicrob Chemother. 2004; 54: 1007–12.

22. Al-Mayahie SM. Phenotypic and genotypic comparison of β-lactamase production by Vaginal Escherichia coli isolates from pregnant and non-pregnant women. Ann Clin Microbiol Antimicrob. 2013; 12: 7.

23. Ostholm-Balkhed A, Tärnberg M, Nilsson M, Johansson AV, Hanberger H, Monstein HJ, et al. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae and trends in antibiotic consumption in a county of Sweden. Scand J Infect Dis. 2010; 42: 831–8.

24. Galas M, Decousser JW, Breton N, Godard T, Allouch PY, Pina P, et al. Nationwide study of the prevalence, characteristics, and molecular epidemiology of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in France. Antimicrob Agents Chemother 2008; 52: 786–9.

25. Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, et al. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect. 2008; 14: 144–53.

26. Marie MAM, John J, Krishnappa LG, Gopalkrishnan S. Molecular characterization of the β-lactamases in Escherichia coli and Klebsiella pneumoniae from a tertiary care hospital in Riyadh, Saudi Arabia. Microbiol Immunol. 2013; 57: 805–10.

27. Aly M, Balkhy HH. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. Antimicrob Resist Infect Control. 2012; 1: 26. doi: 10.1186/2047-2994-1-26.

28. Bogaerts P, Galimand M, Bauraing C, Deplano A, Vanhoof R, De Mendonca R, et al. Emergence of *ArmA* and *Rmt*B aminoglycoside resistance 16S rRNA methylases in Belgium. J Antimicrob Chemother. 2007; 59: 459–64.

29. Zhou Y, Yu H, Guo Q, Xu X, Ye X, Wu S, et al. Distribution of 16S rRNA methylases among different species of gram-negative bacilli with high-level resistance to aminoglycosides. Eur J Clin Microbiol Infect Dis. 2010; 29: 1349–53.

30. Berçot B, Poirel L, Nordmann P. Plasmid-mediated 16S rRNA methylases among extended-spectrum beta-lactamase-producing Enterobacteriaceae isolates. Antimicrob Agents Chemother. 2008; 52: 4526–7.

31. Naas T, Bentchouala C, Lima S, Lezzar A, Smati F, Scheftel J-M, et al. Plasmid-mediated 16S rRNA methylases among extended-spectrum-beta-lactamase-producing Salmonella enterica Senftenberg isolates from Algeria. J Antimicrob Chemother. 2009; 64: 866–8.

32. Li J1, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant gram-negative bacteria. Int J Antimicrob Agents. 2005; 25: 11–25.