Dissemination of Genes Encoding Aminoglycoside-Modifying Enzymes and *armA* Among *Enterobacteriaceae* Isolates in Northwest Iran

Reza Ghotaslou,^{1,2} Fatemeh Yeganeh Sefidan,^{1,2} Mohammad Taghi Akhi,²
Mohammad Asgharzadeh,³ and Yalda Mohammadzadeh Asl²

Introduction: Enzymatic inactivation is one of the most important mechanisms of resistance to aminoglycosides. The aim of this study was to investigate the prevalence of *armA* and diversity of the genes encoding aminoglycoside-modifying enzymes (AMEs) and their associations with resistance phenotypes in *Enterobacteriaceae* isolates.

Methods: Three hundred and seven *Enterobacteriaceae* isolates were collected from five hospitals in northwest Iran. The disk diffusion method for amikacin, gentamicin, tobramycin, kanamycin, and streptomycin, as well as the minimum inhibitory concentration for amikacin, gentamicin, tobramycin, and kanamycin were done for susceptibility testing. Thirteen AME genes and *armA* methylase were screened using the PCR and sequencing assays.

Results: Two hundred and twenty (71.7%) of isolates were resistant to aminoglycosides and 155 (70.5%) of them were positive for aminoglycoside resistance genes. The most prevalent AME genes were *ant(3'')-Ia* and *aph(3'')-Ib* with the frequency 35.9% and 30.5%, respectively. Also, 21 (9.5%) of resistant isolates were positive for *armA* methylase gene.

Conclusions: The prevalence of resistance to aminoglycoside is high and AME genes frequently are disseminated in *Enterobacteriaceae* isolates. There is an association between phenotypic resistance and the presence of some aminoglycoside genes.

Keywords: aminoglycoside-modifying enzymes, *armA*, *Enterobacteriaceae*

Introduction

AMINOGLYCOSIDES ARE POTENT and broad-spectrum antibiotics. They have been used in clinical settings, especially for the treatment of life-threatening infections caused by Gram-negative bacteria.^{1,2} Furthermore, they act synergistically against certain Gram-positive organisms. Unfortunately, their efficacy has been reduced by the emergence and dissemination of resistance.³

Several resistance aminoglycoside mechanisms in *Enterobacteriaceae* and nonfermentative bacteria have been described.¹ The most common resistance mechanisms against aminoglycosides are mediated by enzymatic inactivation through the production of aminoglycoside-modifying enzymes (AMEs), classified in three families such as aminoglycoside acetyltransferases (AACs), aminoglycoside nucleotidyltransferases (ANTs), and aminoglycoside phosphoryltransferases (APHs).⁴ Furthermore, methylation of

16S rRNA by *armA*, the most prevalent methylase,⁵ confers a high level of resistance to almost all aminoglycosides.⁶ The *armA* is the most important class of plasmid-mediated methyltransferase enzymes.⁷ Until now, there are only some limited reports about the distribution of AME and *armA* genes in *Enterobacteriaceae* isolates from Iran.^{8,9}

In this study, we aimed to determine antimicrobial susceptibility patterns of *Enterobacteriaceae* against different aminoglycosides, specify the genetic determinants involved in enzymatic inactivation of this class of antibiotics, and characterize the correlation between phenotypic resistance and the presence of aminoglycoside genes.

Materials and Methods

Bacterial isolates

A total of 307 nonduplicate *Enterobacteriaceae* isolates were collected during February 2014 through August 2015

¹Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

²Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

³Department of Laboratory Sciences, Paramedical Faculty and Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

from five hospitals of two cities in northwest Iran; Tabriz (180 samples), and Orumieh (127 samples). The isolates obtained from various wards, including internal, ICU, surgery, burn, and the pediatric units were identified using conventional biochemical tests in the Department of Microbiology, Tabriz University of Medical Sciences, Iran. These isolates were cultured from urine ($n=219$), blood ($n=43$), burn ($n=13$), wound ($n=11$), trachea ($n=7$), sputum ($n=5$), feces ($n=4$), peritonea ($n=3$), and cerebrospinal fluid ($n=2$).

Antimicrobial susceptibility testing

The disk diffusion method was performed to determine the susceptibility of isolates to streptomycin (10 µg), tobramycin (10 µg), gentamicin (10 µg), kanamycin (30 µg), and amikacin (30 µg) (Mast, Chemical Co). The minimum inhibitory concentrations (MICs) for tobramycin, gentamicin, kanamycin, and amikacin (Sigma-Aldrich) were determined using agar dilution method and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).¹⁰ Isolates with intermediate levels of susceptibility and resistant isolates were classified as nonsusceptible. According to CLSI guideline *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains for susceptibility testing.

Screening of AME and *armA* genes

All isolates that were phenotypically resistant to aminoglycosides were screened for the AMEs and *armA*-encoding genes. The DNA was extracted by boiling method as described previously.¹¹ PCR assays were done using primers specific for the *aac(2')-Ia*, *aac(3)-Ib*, *aac(3)-Ia*, *aac(3)-IIa*, *aac(6')-Ia*, *aac(6')-Ib*, *aac(6')-Ic*, *ant(2'')-Ia*, *ant(3'')-Ia*, *ant(4'')-IIa*, *aph(3')-Ia*, *aph(3'')-Ia*, *aph(3'')-Ib*, and *armA* genes as described previously (Table 1). Some of the PCR products were randomly selected and sequenced for confirmation of the presence of AME and *armA* genes.

Statistical methods

The results were analyzed using SPSS software for Windows (version 19 SPSS, Inc., Chicago, IL). The χ^2 test

was used to examine the relationship between aminoglycoside resistances and genes encoding AMEs. In this study, $p \leq 0.05$ was considered statistically significant.

Results

Phenotypic species identification and antimicrobial susceptibility patterns

Based on conventional biochemical tests, the isolates were identified as *E. coli* (219 isolates), *Klebsiella pneumonia* (57 isolates), *Enterobacter cloacae* (14 isolates), *Proteus mirabilis* (5 isolates), *Klebsiella oxytoca* (2 isolates), *Proteus vulgaris* (2 isolates), *Morganella morganii* (2 isolates), *Shigella sonnei* (2 isolates), *Shigella flexneri* (2 isolates), *Citrobacter freundii* (1 isolate), and *Serratia marcescens* (1 isolate).

Overall, 220 isolates (71.7%) were resistant to at least one aminoglycoside antibiotic. Twelve isolates (5.4%) showed resistance to all of the studied antibiotics. Amikacin showed the highest susceptibility rate (93.8%) followed by kanamycin (66.1%), gentamicin (63.5%), tobramycin (60.6%), and streptomycin (47.6%). The agar dilution results indicated that 27 and 7 of isolates were highly resistant (MIC ≥ 512 µg/ml) to kanamycin and amikacin, respectively. In addition, 2 and 3 of isolates had MIC ≥ 128 µg/ml to gentamicin and tobramycin, respectively.

AME and *armA* genes

One hundred and fifty-five of resistant isolates (70.5%, 155/220) were positive for AME genes. The most common AME gene was *ant(3'')-Ia* (35.9%) followed by *aph(3'')-Ib* (30.5%), *aac(3)-IIa* (23.6%), *aac(6')-Ib* (22.3%), and *aph(3')-Ia* (13.2%) (Table 2). The *aac(2')-Ia*, *aac(6')-Ia*, and *aac(6')-Ic* genes were not detected in any of the tested isolates. In total, *armA* gene was detected in 21 (9.5%) of the resistant isolates. Sixty-five (29.5%) of resistant isolates were negative for any type of the studied genes. Among the 155 isolates that were positive to at least one of the studied genes, 67 isolates had one AME gene, 49 isolates had two genes, 23 isolates had three genes, 10 isolates had four genes, 5 isolates had five genes, and 1 isolate had six genes. The *armA* and *aac(3)-Ia* were detected only in *E. coli* strains (Table 3). As shown in Table 2, 36 distinct combination patterns were recognized

TABLE 1. PRIMERS USED FOR AMPLIFICATION OF AMES AND *armA* GENES

Gene	Forward sequence [5-3]	Reverse sequence [5-3]	Amplicon size (bp)	Reference
<i>aac(2')-Ia</i>	AGAAGCGCTTTACGATTTATTA	GACTCCGCCTTCTTCTTCAA	406	12
<i>aac(3)-Ib</i>	GCAGTCGCCCTAAAACAAA	CACTTCTTCCCGTATGCCCAACTT	563	13
<i>aac(3)-Ia</i>	GCAGTCGCCCTAAAACAAA	CACTTCTTCCCGTATGCCCAACTT	441	12, 13
<i>aac(3)-IIa</i>	GGCAATAACGGAGGCGCTTCAAAA	TTCCAGGCATCGGCATCTCATACG	563	12, 13
<i>aac(6')-Ia</i>	ATGAATTATCAAATTGTG	TTACTCTTTGATTTAAACT	558	12, 13
<i>aac(6')-Ib</i>	TATGAGTGGCTAAATCGAT	CCCGCTTTCTCGTAGCA	395	14, 15
<i>aac(6')-Ic</i>	CTACGATTACGTCAACGGCTGC	TTGCTTCGCCCACTCCTGCACC	130	12, 16
<i>ant(2'')-Ia</i>	ACGCCGTGGGTGCGATGTTTGATGT	CTTTTCCGCCCCGAGTGAGGTG	572	12, 13
<i>ant(3'')-Ia</i>	TCGACTCAACTATCAGAGG	ACAATCGTGACTTCTACAGCG	245	13, 16
<i>ant(4'')-IIa</i>	CCGGGGCGAGGCGAGTGC	TACGTGGGCGGATTGATGGGAACC	423	12
<i>aph(3')-Ia</i>	CGAGCATCAAATGAAACTGC	GCGTTGCCAATGATGTTACAG	625	12, 14
<i>aph(3'')-Ia</i>	CGGCGTGGGCGGCGACTG	CCGGATGGAGGACGATGTTGG	557	12, 13
<i>aph(3'')-Ib</i>	GTGGCTTGCCCCGAGGTCATCA	CCAAGTCAGAGGGTCCAATC	612	12, 13
<i>armA</i>	ATTTTAGATTTTGGTTGTGGC	ATCTCAGCTCTATCAATATCG	101	6

AMEs, aminoglycoside-modifying enzymes.

TABLE 2. THE SUSCEPTIBILITY TESTING RESULTS IN AME-POSITIVE ISOLATES

Genotype	Substrate	N(%)	MIC ($\mu\text{g/ml}$)											
			0.5	1	2	4	8	16	32	64	128	256	512	1024
<i>aac (3) Ib</i>	Gm, S	7 (3.2)	—	—	—	—	2	4	—	1	—	—	—	—
			—	—	1	1	1	1	—	—	—	1	—	2
			—	—	—	—	2	—	—	—	3	—	—	—
<i>aac (3) Ia</i>	Gm, S	9 (4.1)	—	—	—	—	—	—	2	1	—	1	—	—
			—	—	2	3	4	—	—	—	—	—	—	—
			—	—	4	2	3	—	—	—	—	—	—	—
<i>aac (3) IIa</i>	Gm, Tob, S	52 (23.6)	1	1	—	—	3	1	—	—	—	—	—	—
			—	—	—	4	1	—	2	1	1	—	—	—
			—	—	9	3	15	23	—	1	1	—	—	—
<i>aac (6') Ib</i>	Tob, Amk, Km, S	49 (22.3)	—	3	21	15	5	8	1	2	—	—	—	—
			5	—	3	2	24	6	1	1	—	2	1	—
			—	—	2	8	3	10	9	7	6	2	1	4
<i>ant(2'') Ia</i>	Gm, Tob, Km, S	3 (1.4)	—	—	5	9	14	17	1	2	—	1	—	—
			—	—	8	11	11	12	2	1	—	—	—	3
			4	1	3	1	18	6	2	1	3	5	1	5
<i>ant(3'') Ia</i>	S	79 (35.9)	—	—	2	—	1	3	10	12	7	—	—	—
			—	—	1	2	—	—	—	—	—	—	—	—
			1	1	1	—	—	—	—	—	—	—	—	—
<i>ant(4'') IIa</i>	—	14 (6.4)	—	—	—	—	—	—	—	—	—	—	—	—
			—	4	21	11	16	1	1	2	—	—	—	—
			13	—	34	18	11	10	—	1	—	1	—	4
<i>aph (3') Ia</i>	Gm, Km	29 (13.2)	—	9	3	1	27	7	1	1	3	5	3	10
			—	—	6	18	8	6	7	8	8	—	—	—
			—	1	2	—	1	9	—	1	—	1	—	1
<i>aph (3'') Ia</i>	S	1 (0.5)	2	2	—	3	—	1	—	—	2	1	—	—
			—	—	0	6	2	1	—	1	—	2	1	2
			1	1	13	4	3	7	—	—	—	—	—	—
			6	—	18	5	4	2	—	—	—	—	—	—
			—	—	4	1	6	2	—	—	—	1	3	4
			—	—	2	9	3	—	1	3	3	1	3	4
			—	—	—	—	—	1	—	—	—	—	—	—
			—	—	—	—	—	—	—	—	—	—	—	—
			—	—	—	—	—	—	—	—	1	—	—	1

(continued)

TABLE 2. (CONTINUED)

Genotype	Substrate	N(%)	MIC (µg/ml)											
			0.5	1	2	4	8	16	32	64	128	256	512	1024
<i>aph</i> (3'') <i>Ib</i>	S	67 (30.5)	Gm	2	1	18	7	13	22	—	2	1	1	—
			Amk	—	—	29	16	7	8	1	2	—	1	—
			Tob	14	6	4	1	26	5	3	1	3	—	—
			Km	—	—	4	12	9	6	7	8	5	2	—
<i>ant</i> (3'')- <i>Ia</i> + <i>aph</i> (3'')- <i>Ib</i>	S	33 (15)	Gm	1	—	7	4	6	12	—	2	1	1	—
			Amk	—	—	13	6	6	3	—	1	—	1	—
			Tob	7	6	2	—	10	3	1	1	3	—	3
			Km	—	—	3	4	3	3	6	2	2	1	6
<i>aac</i> (3)- <i>IIa</i> + <i>aph</i> (3'')- <i>Ib</i>	Gm, S, Tob	28 (12.7)	Gm	—	—	6	1	4	15	—	—	—	—	—
			Amk	—	—	13	7	1	5	1	1	—	—	—
			Tob	3	3	3	1	13	3	1	1	—	—	—
			Km	—	—	1	5	2	5	4	3	1	2	4
<i>ant</i> (3'')- <i>Ia</i> + <i>aph</i> (3')- <i>Ia</i>	Gm, S	23 (10.5)	Gm	—	—	10	3	3	7	—	—	—	—	—
			Amk	—	—	15	3	3	2	—	—	—	—	—
			Tob	7	5	3	1	6	1	—	—	—	—	—
			Km	—	1	2	8	2	—	1	2	2	1	4
<i>ant</i> (3'')- <i>Ia</i> + <i>aac</i> (6')- <i>Ib</i>	Tob, Amk, Km, S	22 (10)	Gm	—	1	1	3	7	7	1	2	—	1	—
			Amk	—	—	4	3	6	4	—	1	—	—	—
			Tob	3	—	1	—	11	3	—	1	3	1	3
			Km	—	—	—	2	—	1	3	6	2	2	5
<i>aac</i> (6')- <i>Ib</i> + <i>aph</i> (3'')- <i>Ib</i>	Gm, Tob, Km, S	21 (9.5)	Gm	—	—	3	3	4	9	—	—	—	—	2
			Amk	—	—	3	4	5	4	1	1	1	1	—
			Tob	3	—	2	1	6	4	1	1	3	2	4
			Km	—	—	—	1	1	—	3	6	3	1	—
<i>aac</i> (3)- <i>IIa</i> + <i>ant</i> (3'')- <i>Ia</i>	S, Gm, Tob	20 (9.1)	Gm	—	—	4	1	5	9	—	1	—	—	—
			Amk	—	—	9	4	1	5	—	1	—	—	—
			Tob	2	5	2	—	6	4	—	1	—	—	—
			Km	—	1	1	3	1	3	3	2	1	1	2
<i>ant</i> (3'')- <i>Ia</i> + <i>ant</i> (4'')- <i>IIa</i>	S	11 (5)	Gm	—	1	2	—	1	6	—	1	—	—	1
			Amk	—	—	7	1	—	1	—	—	—	1	—
			Tob	2	4	—	—	2	1	—	—	2	—	—
			Km	—	—	—	4	—	1	—	—	—	1	2
<i>aac</i> (3)- <i>IIa</i> + <i>ant</i> (3'')- <i>Ia</i> + <i>aph</i> (3'')- <i>Ib</i>	Gm, Tob, S	14 (6.4)	Gm	—	—	4	—	2	7	—	1	—	—	—
			Amk	—	—	6	3	1	3	—	1	—	—	—
			Tob	2	3	2	—	4	2	—	1	—	—	—
			Km	—	—	1	1	—	2	3	—	1	1	2

(continued)

(continued)

TABLE 2. (CONTINUED)

[illegible]

The bold values show non-susceptible range.

[illegible]

TABLE 3. THE FREQUENCY OF AME GENOTYPES AND *armA* IN DIFFERENT *ENTEROBACTERIACEAE* ISOLATES

Genotype	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Proteus mirabilis</i>	<i>Shigella sonnei</i>	<i>Shigella flexneri</i>	<i>Proteus vulgaris</i>
<i>aac(3)-Ib</i>	2	4	1	0	0	0	0
<i>aac(3)-Ia</i>	9	0	0	0	0	0	0
<i>aac(3)-IIa</i>	37	10	4	1	0	0	0
<i>aac(6')-Ib</i>	32	13	4	0	0	0	0
<i>ant(2'')-Ia</i>	3	0	0	0	0	0	0
<i>ant(3'')-Ia</i>	53	18	4	2	0	1	1
<i>ant(4'')-IIa</i>	11	2	0	1	0	0	0
<i>aph(3')-Ia</i>	22	4	0	2	0	0	1
<i>aph(3'')-Ia</i>	0	1	0	0	0	0	0
<i>aph(3'')-Ib</i>	48	13	2	2	2	1	0
<i>armA</i>	21	0	0	0	0	0	0

among 155 PCR-positive isolates. The sequencing results confirmed the AME and *armA* genes.

Discussion

The level of aminoglycoside resistance in *Enterobacteriaceae* has changed throughout the past few decades. In the present study, nearly three-quarters of isolates (71.7%) were resistant to aminoglycosides and 70.5% of the resistant isolates exhibited a remarkable diversity of AMEs genes. In total, we identified 44 AME patterns (36 combination and 8 single gene forms), which associated with different levels of aminoglycoside resistance. We found *ant(3'')-Ia* and *aph(3'')-Ib* as the most prevalent AME genes, an observation similar to the previous report.¹² These genes occurred in 21.3% of the isolates in combination with each other. In comparison to other aminoglycosides, amikacin showed the least (6.2%) and streptomycin had the most (52.4%) resistance rates. This finding is in concordance with the study of Miró *et al.*¹² A total of 27 (12.3%) high-level aminoglycoside-resistant isolates were observed in our study, in comparison with a study conducted in Belgium indicating low resistance rates to gentamicin, tobramycin, and amikacin.¹¹ This may be due to the high use of aminoglycosides in these countries.

The genes responsible for streptomycin resistance were *aph(3'')-Ib* and *ant(3'')-Ia* with a frequency of 88.1% and 83.5%, respectively. It has previously been reported with low percentages by other authors.^{12,17,18} The possible explanations are that in some studies selection criteria is different and hybridization methods were used instead of the PCR.

The frequency of APH(3')-I in previous studies were 46% in 1993¹⁸ and 13.9% in 2006¹² that showed the decline in the frequency of this enzyme. It should be noted that kanamycin usage in 1993 was probably substantial. We detected the prevalence of *aph(3')-Ia* for kanamycin-resistant isolates as 48.3%, but this gene had no statistical association with kanamycin. High resistance to kanamycin in our country is perhaps due to its high consumption.

Previous studies demonstrated that *ant(2'')-Ia* is more frequent in countries that use gentamicin more frequently than amikacin.¹⁸ In our study, 36.5% of isolates were resistant to gentamicin and 33.3% of them were positive for *ant(2'')-Ia* gene, which is more than recorded in Spain (3.6%).¹² This may be explained by the fact that gentamicin is frequently used as a therapeutic option in Iran. Also, the isolates harboring *ant(2'')-Ia* were resistant to

streptomycin and tobramycin. In the current study, all of the isolates that were resistant to streptomycin and 33.3% of tobramycin-resistant isolates carried this enzyme.

The AAC(6')-Ib is the most prevalent among AAC(6') enzymes.^{15,17,18} In countries, where amikacin usage is very high, the incidence of AAC(6')-I as a single or combination aminoglycoside resistance mechanism is high. The prevalence of *aac(6')-Ib* was significantly higher (31.6%) than previous reports from Spain¹² and China³ that detected 4.2% and 19.6%, respectively. Moreover, a report from Poland showed that the highest prevalence (83.3%) of this enzyme was in *P. mirabilis* strains. This significant difference may be due to geographical factors. We found a significant association between the presence of AME and resistance to each of amikacin, kanamycin, and tobramycin as expected substrates.

The isolates producing 16S rRNA methylase, exhibit high-level resistance to all aminoglycosides through methylation of the aminoglycoside-binding site.¹⁹ Resistance rates in *armA*-positive isolates to amikacin, kanamycin, gentamicin, tobramycin, and streptomycin were 0.9%, 2.3%, 2.3%, 4.1%, and 7.3%, respectively. The *armA* gene has been found in many species of Gram-negative bacteria in Korea, Japan, China, and Taiwan.²⁰ The prevalence of this gene in the present study is higher than what was found in China surveys 0.9%²⁰ and 1.6%,²¹ Belgium 0.12%,¹¹ and France 1.3%,²² and lower than other reports from Iran 13.6%.⁹

Although we observed similar antibiotic resistance patterns in Tabriz and Orumieh cities, there were considerable differences in the distribution patterns of AME genes and *armA* between the two cities. This observation suggested that these distribution patterns had occurred as a result of various geographic regions.

Similar to previous studies,¹² we observed incompatibility (29.5%) between phenotype and genotype results that refer to other nonenzymatic resistance mechanisms against aminoglycosides such as efflux pumps, alteration in permeability, and rare types of AMEs. As well as in some cases, AME genotype was an inadequate predictor of the aminoglycoside phenotype, suggesting the contribution of multiple coincident aminoglycoside resistance mechanisms.

Conclusions

The results of this study indicate that the prevalence of resistance to aminoglycoside in Iran is high and AME genes

and *armA* frequently spread among *Enterobacteriaceae* isolates. Furthermore, there is a statistically significant association between phenotypic resistance and the presence of some aminoglycoside genes. It seems that increasing complexity of aminoglycoside resistance mechanisms was related to increasing complexity of aminoglycoside usage. Results of this study show that two geographic regions had the different distribution of AME and *armA* among *Enterobacteriaceae* isolates.

Acknowledgments

This project (number: 93–08) was financially supported by Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences. This article was written based on a dataset of PhD thesis (number: 93.5–4.8), registered in Tabriz University of Medical Sciences.

Disclosure Statement

No competing financial interests exist.

References

1. Chaudhary, M., and A. Payasi. 2014. Resistance Patterns and Prevalence of the Aminoglycoside Modifying Enzymes in Clinical Isolates of Gram Negative Pathogens. *Global J. Pharmacol.* 8:73–79.
2. Jana, S., and JK. Deb. 2006. Molecular understanding of aminoglycoside action and resistance. *Appl. Microbiol. Biotechnol.* 70:140–150.
3. Hu, X., B. Xu, Y. Yang, D. Liu, M. Yang, J. Wang, H. Shen, X. Zhou, and X. Ma. 2013. A high throughput multiplex PCR assay for simultaneous detection of seven aminoglycoside-resistance genes in *Enterobacteriaceae*. *BMC Microbiol.* 13:1–9.
4. Yamane, K., JI. Wachino, Y. Doi, H. Kurokawa, and Y. Arakawa. 2005. Global spread of multiple aminoglycoside resistance genes. *Emerg. Infect. Dis.* 11:951–953.
5. Doi, Y., and Y. Arakawa. 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. *Clin. Infect. Dis.* 45:88–94.
6. Berçot, B., L. Poirel, and P. Nordmann. 2011. Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM-1 producers. *Diagn. Microbiol. Infect. Dis.* 71:442–445.
7. Fritsche, TR., M. Castanheira, G.H. Miller, R.N. Jones, and E.S. Armstrong. 2008. Detection of methyltransferases conferring high-level resistance to aminoglycosides in *Enterobacteriaceae* from Europe, North America, and Latin America. *Antimicrob. Agents Chemother.* 52:1843–1845.
8. Soleimani, N., M. Aganj, L. Ali, L. Shokoohizadeh, and T. Sakinc. 2014. Frequency distribution of genes encoding aminoglycoside modifying enzymes in uropathogenic *E. coli* isolated from Iranian hospital. *BMC Res. Notes.* 7:842.
9. Ashrafian, F., F. Fallah, A. Hashemi, S. Erfanimanesh, S. Amraei, and S. Tarashi. 2015. First Detection of 16S rRNA Methylase and *bla*_{CTX-M-15} Genes among *Klebsiella pneumoniae* Strains Isolated from Hospitalized Patients in Iran. *Res. Mol. Med.* 3:28–34.
10. Clinical and Laboratory Standards Institute. 2014. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S24. Vol 34. N 1. CLSI, Wayne, PA.
11. Bogaerts, P., M. Galimand, C. Bauraing, A. Deplano, R. Vanhoof, R. De Mendonca, H. Rodriguez-Villalobos, M. Struelens, and Y. Glupczynski. 2007. Emergence of ArmA and RmtB aminoglycoside resistance 16S rRNA methylases in Belgium. *J. Antimicrob. Chemother.* 59:459–464.
12. Miró, E., F. Grünbaum, L. Gómez, A. Rivera, B. Mirelis, P. Colland, and F. Navarro. 2013. Characterization of aminoglycoside-modifying enzymes in *Enterobacteriaceae* clinical strains and characterization of the plasmids implicated in their diffusion. *Microb. Drug Resist.* 19:94–99.
13. Grünbaum, F., F. Navarro Risueño, and B. Mirelis Otero. 2011. Resistència Aaminoglucósidos in *Enterobacteriaceae*. Universitat Autònoma de Barcelona, Barcelona.
14. Noppe-Leclercq, I., F. Wallet, S. Haentjens, R. Courcol, and M. Simonet. 1999. PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for *Acinetobacter baumannii*. *Res. Microbiol.* 150:317–322.
15. Wiczorek, P., P. Sacha, T. Hauschild, A. Ostas, W. Kłosowska, J. Ratajczak, and E. Tryniszewska. 2008. The *aac* (6') *Ib* gene in *Proteus mirabilis* strains resistant to aminoglycosides. *Folia Histochem. Cytobiol.* 46:531–533.
16. Hannecart-Pokorni, E., F. Depuydt, E. van Bossuyt, and R. Vanhoof. 1997. Characterization of the 6'-N-aminoglycoside acetyltransferase gene *aac* (6')-*Im* [corrected] associated with a *sulI*-type integron. *Antimicrob. Agents Chemother.* 41:314–318.
17. Shaw, KJ., P.N. Rather, R.S. Hare, and G.H. Miller. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol. Rev.* 57:138–163.
18. Vakulenko, SB., and S. Mobashery. 2003. Versatility of aminoglycosides and prospects for their future. *Clin. Microbiol. Rev.* 16:430–450.
19. Yang, J., L. Ye, W. Wang, Y. Luo, Y. Zhang, and L. Han. 2011. Diverse prevalence of 16S rRNA methylase genes *armA* and *rmtB* amongst clinical multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates. *Int. J. Antimicrob. Agents.* 38:348–351.
20. Yu, F., L. Wang, J. Pan, D. Yao, C. Chen, T. Zhu, Q. Lou, J. Hu, Y. Wu, X. Zhang, Z. Chen, and D. Qu. 2009. 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.
21. Wu, Q., Y. Zhang, L. Han, J. Sun, and Y. Ni. 2009. Plasmid-mediated 16S rRNA methylases in aminoglycoside-resistant *Enterobacteriaceae* isolates in Shanghai, China. *Antimicrob. Agents Chemother.* 53:271–272.
22. Berçot, B., L. Poirel, and P. Nordmann. 2008. Plasmid-mediated 16S rRNA methylases among extended-spectrum β -lactamase-producing *Enterobacteriaceae* isolates. *Antimicrob. Agents Chemother.* 52:4526–4527.

Address correspondence to:
 Fatemeh Yeganeh Sefidan, PhD
 Infectious and Tropical Diseases Research Center
 Tabriz University of Medical Sciences
 Tabriz 51664
 Iran

E-mail: ftm.yeganeh@yahoo.com