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ORIGINAL ARTICLE

Phenotypic and genotypic evaluation of aminoglycoside resistance in *Escherichia coli* isolated from patients with blood stream infections in Tehran, Iran

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ABSTRACT

Background and Objectives: *Escherichia coli* is a significant causative agent of bloodstream infections (BSIs). Aminoglycoside antibiotics play a crucial role in treating severe infections such as sepsis and pneumonia. However, resistance to these antibiotics often occurs due to the production of aminoglycoside-modifying enzymes (AMEs). This study was conducted to assess antimicrobial susceptibility patterns against various aminoglycosides and to determine the prevalence of common AME genes in *E. coli* strains isolated from BSIs.

Materials and Methods: Sixty-five *E. coli* isolates were obtained from blood samples in a referral hospital in Tehran, Iran. The susceptibility patterns of aminoglycosides were determined using disk diffusion method and AMEs genes were investigated using PCR assay.

Results: Resistance to aminoglycosides was observed in 64.6% (42/65) of the isolates. The most frequent resistance rate was found for kanamycin (44.6%) and gentamicin (38.5%), followed by tobramycin (29.2%) and amikacin (4.6%). The most frequent AME gene was *aac(3)-IVa*, which detected in 49.2% isolates, followed by *aac(6)-Ib* (40%), *aac(3)-IIa* (32.3%), and *ant(2)-Ia* (30.8%), respectively.

Conclusion: Athough the findings of this survey are based on specimens collected from a single hospital, our study shows that the high prevalence of aminoglycoside resistance is primarily attributed to the presence of the *aac(3)-Iva*, *aac(6)-Ib* and *aac(3)-IIa* genes. The low rate of resistance to amikacin makes this antibiotic a good candidate for treatment of BSIs due to *E. coli*.

Keywords: Aminoglycoside resistance; *Escherichia coli*; Bacteremia; Aminoglycoside-modifying enzymes

INTRODUCTION

Escherichia coli is a significant cause of the bloodstream infections (BSIs) which impose a substantial disease burden in terms of mortality (1-4). Mortality rate due to *E. coli* BSI has been reported from 5% to 30% (5). One of the major concerns with this organism is its increasing resistance to antibiotics, resulting in treatment failures, prolonged length of stays, and increased medical costs (6). Aminoglycosides are used to treat severe Gram-negative infections such as sepsis, bacterial endocarditis, and pneumonia due to their bactericidal effect and broad-spectrum activity. Moreover, aminoglycosides often display synergistic effects when used in combination with other antibiotics. This synergism enhances the

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overall efficacy of the treatment, especially in cases of severe infections where a multi-pronged approach may be necessary (7). These antibiotics inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit, leading to bacterial death (8). In recent years, aminoglycoside-resistant *E. coli* isolates have been described which leading to global worries (9). Different mechanisms of resistance to aminoglycosides in *E. coli* and other Gram-negative bacteria have been reported such as decreased rate of drug entry into the bacterial cell, exportation of drug out of the cell by efflux pumps, methylation or mutation of the 16S rRNA (10), and the production of aminoglycoside-modifying enzymes (AMEs). Among the mentioned mechanisms, AMEs are the most common resistance mechanism to these antibiotics which are classified into three categories: aminoglycoside acetyltransferases (AACs), aminoglycoside phosphotransferases (APHs) and aminoglycoside nucleotidyltransferases (ANTs) (8). AMEs are generally carried on mobile gene elements, such as transposons, integrons or plasmids and can be moved horizontally between different bacteria (11). Identifying the prevalence of AMEs genes related to human infections could assist clinicians in selecting proper therapy. Given the significance of the resistance to aminoglycosides, the primary objective of the current study was to determine the susceptibility pattern of various aminoglycoside antibiotics and the frequency of AME genes in *E. coli* isolates causing BSIs in a referral hospital in Tehran, Iran.

MATERIALS AND METHODS

Sample collection. From January 2021 to December 2022, 65 non-duplicate isolates of *E. coli* were collected from blood specimens of patients who were admitted to a referral hospital in Tehran. At least two blood culture samples (simultaneously collected but from different sites) were obtained from each patient to ensure a comprehensive examination of bloodstream infections. Duplicate *E. coli* isolates from the same patient were omitted. Blood culture was performed using conventional method as described previously (12). All strains were identified as *E. coli* using colonial morphology, Gram- staining and conventional bio- chemical testing. Identified *E. coli* strains were grown in Trypticase soy broth (Merck, Germany), with glycerol 20% and kept at -20°C for additional tests.

Antimicrobial susceptibility testing. Aminoglycoside susceptibility patterns were determined by the disk agar diffusion (DAD) method for the following antimicrobials: gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg) and kanamycin (30 µg) (Mast, UK). The inoculum, containing a bacterial suspension equivalent to a 0.5 McFarland standard, was cultured on Mueller Hinton Agar plates (Merck, Germany) and incubated at 37°C for 18-24 hours. For all tested antibiotics, the susceptibility results were interpreted based on the breakpoints of the Clinical Laboratory Standards Institute (CLSI). *E. coli* ATCC 25922 was implemented as the quality control strain.

Detection of AME genes. For all *E. coli* strains, genomic DNA was obtained from purified colonies by boiling method (13). The genes encoding AMEs including *aac(6')-Ib, aac(3)-IIa, aac(3)-Ia, aac(3)-IVa, ant(2'')-Ia, ant(4')-IIa* and *aph(3')-Ia* were detected by PCR (Bio-Rad, USA) (14). The specific primers and PCR amplification conditions are detailed in Table 1. Each PCR reaction mixture contained: 12.5 µL PCR Master Mix (Ampliqon, Denmark), which contains Taq DNA polymerase, PCR Buffer, and dNTPs, 1 µL of 20 pM of each primer (Metabion, Germany) and 100 ng of genomic DNA. The total volume of the mixture was 25 µL. The amplified PCR products were analyzed by electrophoresis on 0.8% agarose gel. The DNA bands were visualized by stain- ing with a safe dye and photographed under UV light.

Statistical analysis. All data concerning the frequency of genes encoding AMEs and aminoglycoside resistance profiles were added to the statistical package IBM SPSS Version 24 (Armonk, NY, USA) and analysis was performed using descriptive statistical tests. The visualization of the phenotypic and genotypic aminoglycoside susceptibility profiles was done by heatmap, using GraphPad PRISM Version 9 software.

RESULTS

Antibiotic susceptibility. The phenotypic and genotypic features of *E. coli* isolates are presented in Fig. 1. Of the 65 *E. coli* isolates, 42 (64.6%) isolates exhibited resistance to at least one of the tested aminoglycosides. Resistance rates were 44.6% (29/65) for kanamycin, 38.5% (25/65) for gentamicin, 29.2%

Table 1. The primers and the conditions used for the amplification of genes encoding AMEs.

Gene	Primer sequence (5' to 3')	Amplicon size (bp)	PCR programme	Reference
ant(2")-Ia	ACGCCGTGGGTCGATGTTTGATGT	572	94°C, 60s; 67°C, 40s*; 72°C,	
	CTTTTCCGCCCCGAGTGAGGTG		35s: 30 cycles**	
ant(4")-IIa	CCGGGGCGAGGCGAGTGC	423	94°C, 60s; 68°C, 40s*; 72°C,	
	TACGTGGGCGGATTGATGGGAACC		30s: 30 cycles**	
aac(6')-Ib	TTGCGATGCTCTATGAGTGGCTA	482	94°C, 60s; 61°C, 40s*; 72°C,	
	CTCGAATGCCTGGCGTGTTT		30s: 30 cycles**	
aac(3)-Ia	GCAGTCGCCCCTAAAACAAA	464	94°C, 60s; 61°C, 40s*; 72°C,	(14)
	CACTTCTTCCCGTATGCCCAACTT		30s: 30 cycles**	
aph(3') Ia	CGAGCATCAAATGAAACTGC	624	94°C, 60s; 57°C, 40s*; 72°C,	
	GCGTTGCCAATGATGTTACAG		30s: 30 cycles**	
aac(3)-Iva	TCGGTCAGCTTCTCAACCTT	314	94°C, 60s; 61°C, 40s*; 72°C,	
	GATGATCTGCTCTGCCTGTG		30s: 30 cycles**	
aac(3)-IIa	GGCAATAACGGAGGCGCTTCAAAA	563	94°C, 60s; 66°C, 40s*; 72°C,	
	TTCCAGGCATCGGCATCTCATACG		35s: 30 cycles**	

*for amplification of all AMEs genes before the initial cycle, the sample was denatured at 94°C for 5 min.

**After the last cycle, the sample was extended at 72°C for 5 min.

(19/65) for tobramycin and 4.6% (3/65) for amikacin. Eight different resistance patterns to aminoglycosides were observed. The most frequent were K, TOB, GM (n=12, 28.5%), K, GM (n=6, 14.2%), K, TOB (n=3, 7.1%) and K, GM, TOB, AK (n=2, 4.7%). Twenty-three (35%) isolates were sensitive to all aminoglycoside drugs tested (Fig. 1).

PCR amplification of genes encoding AMEs. All 65 *E. coli* strains were examined for presence of selected AME genes. The most predominant AME gene was *aac(3)-IVa*, which observed in 49.2% (32/65) isolates, followed by *aac(6)-Ib* 40% (26/65), *aac(3)-IIa* 32.3% (21/65), *ant(2)-Ia* 30.8% (20/65), *aph(3)-Ia* 23.1% (15/65), *aac(3)-Ia* 15.4% (10/65) and *ant(4)-IIa* 7.7% (5/65), of these isolates, respectively. In addition, 40 (61.5%) isolates simultaneously harbored more than one AME genes of which the combination of *aac(6)-Ib* and *aac(3)-IVa* in 5 (12.5%) isolates and *aac(3)-IIa* plus *aac(3)-Iva* in 4 (10%) isolates were the most prevalent (Fig. 1).

DISCUSSION

In this work, 64.6% (42/65) of the isolates were resistant to at least one of the aminoglycoside drugs tested, of which 44.6%, 38.5%, 29.2% and 4.6% of *E. coli* isolates were resistant to kanamycin, gentamicin, tobramycin and amikacin, respectively. In a study from Iran, the percentages of strains resistant to kanamycin, gentamicin, tobramycin and amikacin in 276 samples of *E. coli* collected from urine were 23.18%, 21%, 24.6% and 3.6%, respectively (15). In Spain, Martinez et al. showed that 32.3%, 25.7%, 21.4% and 0.4% of *E. coli* isolates were resistant to kanamycin, tobramycin, gentamicin and amikacin, respectively (16). In India, Mir et al. stated that the percentages of strains resistant to gentamicin, tobramycin and amikacin were 73.4%, 57.4% and 8.16%, respectively (17). In Poland, Ojdana et al. observed that 70.5%, 59% and 11.4% of *E. coli* isolates were resistant to tobramycin, gentamicin and amikacin, respectively (18). In Egypt, Gad et al. studied 50 *E. coli* isolates from various clinical specimens and observed that 44%, 36%, 30% and 16% of isolates were resistant to kanamycin, gentamicin, tobramycin and amikacin, respectively (19). In this study, amikacin was the most active agent against *E. coli* than other tested aminoglysides, which is in agreement with previous studies (16, 18, 19). The high activity of amikacin can be attributed to the presence of the aminohydroxybutyryl group, by blocking the enzymatic modification of amikacin at multiple positions (20). Regrettably, one of the primary causes of antibiotic resistance in Iran is the extensive and often unnecessary use of antibiotics which provides powerful selective pressure for antibiotic resistance. In the current study, the

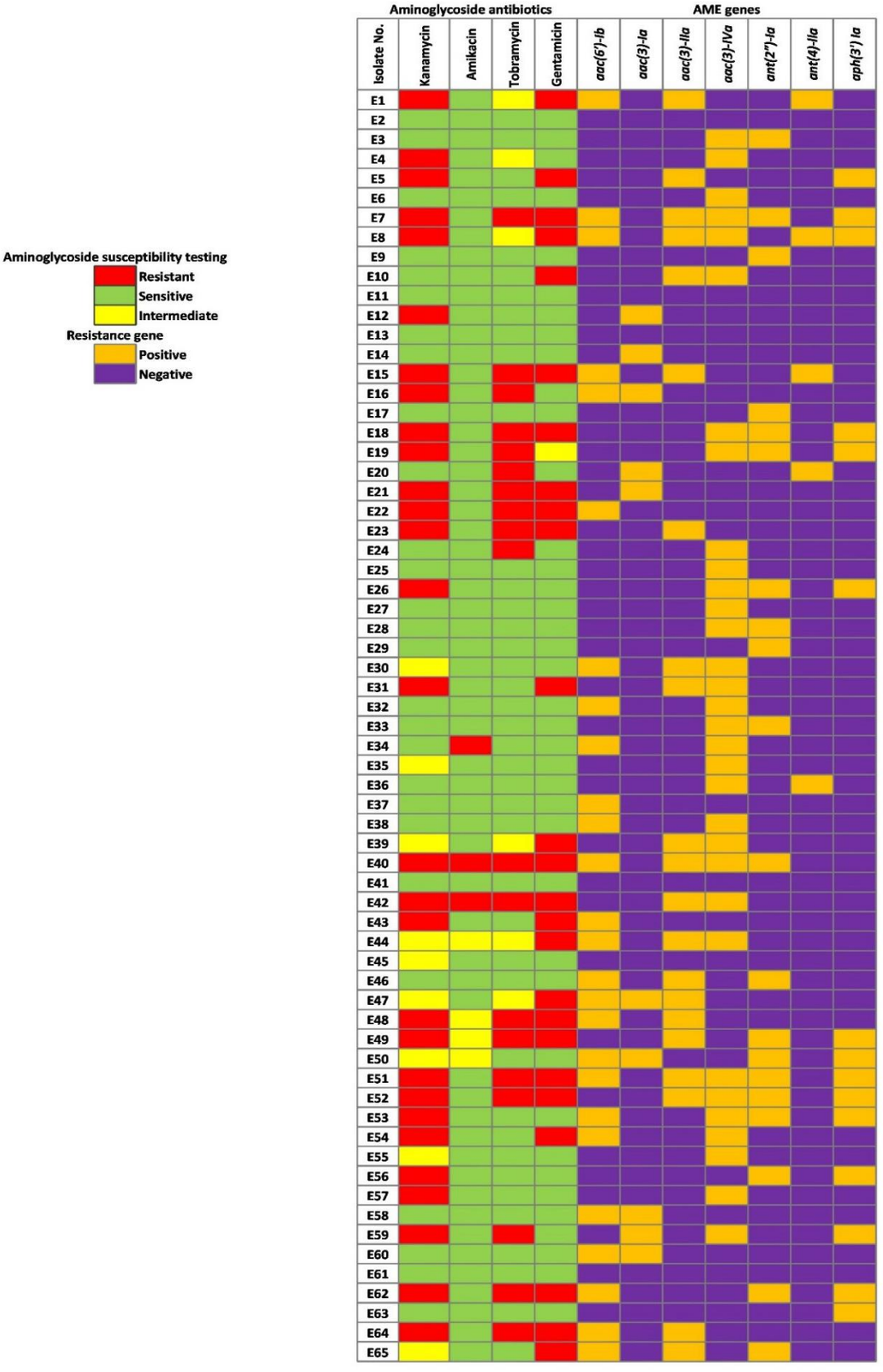

Fig. 1. The phenotypic and genotypic features of 65 of *Escherichia coli* strains isolated from bloodstream infections.

most common AME gene identified was *aac(3)-IVa* (49.2%), followed by *aac(6)-Ib* (40%) and *aac(3)-IIa* (32.3%). A survey in Iran by Sadeghi et al. revealed that the most common AME genes were *aac(6)-Ib* (49.4%) and *aac(3)-IIa* (39.8%), respectively (21). In another study from Iran in 2017, Ghotaslou et al. observed that the most prevalent types of AME genes in Enterobacteriaceae isolated from clinical samples were *ant(3'')-Ia* (36%) and *aph(3'')-Ib* (30.5%), respectively (22). In Spain, Martinez et al. reported that 34.3%, 29.5% and 27.6% of clinical isolates of *E. coli* carried the *aac(6)-Ib, aph(3)-Ia* and *ant(2)-la* genes, respectively (16). In Poland, the most common AME genes in *E. coli* isolates were *aac(6)-Ib* (59.2%) and *aac(3)-Ia* (15.9%) (18). In Egypt, *aph(3)-Ia* (32%) and *ant(2)-la* (8%) identified as the most common AME genes among clinical isolates of *E. coli* (19). As mentioned earlier, the rate of aminoglycoside resistance and the frequency of AME genes differs from country to country (15, 17-19, 22). These differences might be related to different usage patterns of aminoglycosides, horizontal transmission of resistance determinants, spread of specific clones containing AME genes and the number of studied isolates. We found the concomitance of AME genes among our isolates, which was in agreement with previous investigations (15, 17-19, 22). The *aac(3)-Iva, aac(6)-Ib,* and *aac(3)-IIa* genes encode enzymes that function as acetyltransferases. These enzymes are capable of acetylating aminoglycoside antibiotics. Acetylation alters the chemical structure of aminoglycosides, reducing their binding affinity to bacterial ribosomes. As a result, acetylation can confer resistance to the bactericidal effects of aminoglycosides. The presence of *aac(3)-Iva, aac(6)-Ib,* and *aac(3)-IIa* suggests a potential broadening of the spectrum of aminoglycoside resistance in the bacterial population. These enzymes can confer resistance to multiple aminoglycoside drugs, limiting the effectiveness of this class of antibiotics in treating infections caused by bacteria carrying these genes (7). Our study has several limitations, including the small sample size, the absence of demographic and clinical data of patients, and the collection of clinical specimens from only one hospital.

CONCLUSION

Based upon samples collected from one hospital, this study shows that the high prevalence of aminoglycoside resistance is primarily attributed to the presence of the *aac(3)-Iva, aac(6)-Ib* and *aac(3)-IIa* genes. The low rate of resistance to amikacin makes this antibiotic a good candidate for treatment of BSIs due to *E. coli*.

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