Emergence of five kinds of aminoglycoside-modifying enzyme genes simultaneously in a strain of multidrug-resistant *Escherichia coli* in China

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Abstract

A strain of *Escherichia coli* was positive for 5 aminoglycoside modifying enzyme genes (*aac*(3)-I, *aac*(6′)-Ib-cr, *ant*(3′′′′)-I, *aad*A5, and *aph*(3′)-I) in PCR assays. And these positive genes confer resistance to aminoglycosides (gentamicin and tobramycin). This is the first report of emergence of five kinds of aminoglycoside-modifying enzymes genes simultaneously in *E. coli* worldwide.

Keywords: Aminoglycoside-modifying enzymes, *Escherichia coli*, multidrug resistance, *N*-acetyltransferases, *O*-nucleotidyltransferases, *O*-phosphotransferases

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A 31-year-old male patient suffering from a urinary tract infection was admitted to The First Affiliated Hospital of Nanjing Medical University in Nanjing, China, on 5 August 2006. A strain of *Escherichia coli* was isolated from the urine. The patient was treated with moxifloxacin, and was healed and discharged from hospital on 17 August 2006.

The isolate was identified with Vitek Gram-negative identification cards (bioMérieux-Vitek, Hazelwood, MO, USA), and the antimicrobial susceptibility profile was determined with the disk diffusion and agar dilution methods according to the CLSI. Modified three-dimensional tests were performed to confirm an extended-spectrum β-lactamase, and not an AmpC, phenotype, with the CLSI method [1]. Whole cell DNA from the strain of *E. coli*, prepared by proteinase K digestion, was used as a template in PCR assays. PCR was performed with the primers (Wuxi Clone Gen-Tech Institute, Wuxi, Jiangsu, China) for 14 aminoglycoside-modifying enzyme genes (*aac*(3)-I, *aac*(3)-II, *aac*(3)-III, *aac*(3)-IV, *aac*(6′)-Ib, *aac*(6′)-II, *ant*(2′′)-I, *ant*(3′′)-I, *aad*A4/5, *aad*A6/16, *ant*(4′)-I, *aph*(3′)-I, *aph*(3′)-IIa, and *aph*(3′)-IIb) and six 16S rRNA methylase genes (*arm*A, *rmt*A, *rmt*B, *rmt*C, *rmt*D, and *npm*A), which were designed on the basis of genotypes published in GenBank. PCR conditions were: 2 min at 93°C; 35 cycles of 30 s at 93°C, 30 s at 55°C, and 1 min at 72°C; and, finally, 5 min at 72°C. Positive and negative controls were performed in every PCR assay. The amplicons were purified with PCR Kits (Wuxi Clone Gen-Tech Institute), and were subsequently sequenced on an ABI PRISM377 sequencer analyser (Applied Biosystems, Foster City, CA, USA).

According to antibiotic susceptibility testing with disk diffusion methods and agar dilution methods, the *E. coli* strain was susceptible to amikacin, but resistant to gentamicin and tobramycin (Table 1).

Moreover, the *E. coli* strain was defined as a multidrug-resistant strain, as it was resistant to three or more unique antimicrobial classes. According to antibiotic susceptibility testing with disk diffusion methods, the *E. coli* strain was resistant to β-lactams, including cefuroxime, cefazolin, cefepime, cefotaxime, ceftazidime, and aztreonam, and it was resistant to quinolones, including ciprofloxacin and levofloxacin; it was also resistant to sulphamethoxazole–trimethoprim.

Aminoglycoside-modifying enzymes catalyse the covalent modification of specific amino or hydroxyl functions, leading to a chemically modified drug that binds poorly to ribosomes and for which the energy-dependent phase II of accelerated drug uptake also fails to occur, thereby resulting most often in high-level resistance. The enzymes modifying aminoglycosides are *N*-acetyltransferases (AACs), which use acetyl-coenzyme A as the donor and affect amino functions, and *O*-nucleotidyltransferases (ANTs) and *O*-phosphotransferases (APHs), which both use ATP as the donor and affect hydroxyl functions [2]. The *E. coli* strain was positive for *aac*(3)-I, *aac*(6′)-Ib, *ant*(3′′)-I, *aad*A4/5 and *aph*(3′)-I in PCR assays. However, it was negative for the other nine aminoglycoside-modifying enzyme genes. The presence of *aac*(6′)-Ib, confirmed as *aac*(6′)-I b-cr, and the presence of *aad*A4/5, confirmed as *aad*A5, were determined by sequencing and BLASTn algorithm. The *aac*(3)-I gene encodes aminoglycoside 3-*N*-acetyltransferase, the *ant*(3′′)-I gene encodes aminoglycoside 3′′-*O*-nucleotidyltransferase, the *aph*(3′)-I gene

TABLE 1. Antimicrobial susceptibilities of a strain of multidrug-resistant *Escherichia coli* related to aminoglysoside resistance linked to the genes detected

Antimicrobial agent	Disk diffusion (mm)	Agar dilution (mg/L)	Laboratory report	Genes detected
Gentamicin	7	128	R	*aac*(3)-I, *ant*(3″)-I, *aph*(3′)-I, *aad*A5, *aac*(6′)-Ib-cr
Tobramycin	7		R	
Amikacin	19	8	S	

R, resistant; S, sensitive.

encodes aminoglycoside 3-*O*-phosphotransferase, and the *aad*A5 gene encodes aminoglycoside 3″-adenyltransferease, so these four genes confer resistance to aminoglycosides. Moreover, the *aac*(6′)-Ib-cr genes encodes aminoglycoside *N*-acetyltransferase and confers resistance against aminoglycosides as well as fluoroquinolones simultaneously [3]. This is the first report of the emergence of five aminoglycoside-modifying enzyme genes simultaneously in *E. coli* worldwide. Sequencing of the whole genome of this strain is needed to better understand how this strain has accumulated these resistance-encoding genes.

Transparency Declarations

No declarations were made by the authors of this paper.

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