**DOİ:** http://doi.org/10.5281/zenodo.7361124 **Accepted:** 24.11.2022

# Investigation of Various Aminoglycoside Resistant Genes in Providencia Isolates

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## Abstract

Providencia species cause severe nosocomial infections with multi-drug resistance, especially in people who are hospitalized for a long time. This study aimed to determine the presence of aminoglycoside drug resistance genes that are part of multidrug resistance in Providencia species. The strains that reproduced Providencia spp from the biological material sent to Necmettin Erbakan University Meram Medical Faculty Medical Microbiology Laboratory and were resistant to aminoglycoside drugs were included in our study. PCR method was used to determine the genes causing drug resistance at the molecular level and the presence of 33 genes was investigated.
As a result of our research, seven aminoglycoside modifying enzyme genes (aac (2 ") - Ia, aac (3) -IIa, aac (3) -IIc, aac (6 ') - Ib, aac (6') - Ib-cr, ant (2 ') - Ia, ant (3 ") - Ia) and one 16S rRNA methylation gene (armA) a total of eight genes were found to be positive in combination. In addition, ESBL positivity was detected in our isolates.To the best of our knowledge, our study is the first study in Turkey in which the aminoglycoside resistance gene as well as the armA and aac (6') - Ib-cr gene were positively detected in Providencia species.



**Keywords**: Providencia Stuartii, Multiple Drug Resistance**,** 16S rRNA Methylransferase, Aminoglycoside Resistance

## 1. INTRODUCTION

Although the genus *Providencia* rarely occurs as an infectious agent, it can cause epidemics that are difficult to treat due to the long-term use of catheters, especially in intensive care units of hospitals and nursing homes. The species most commonly observed in hospitals are *Providencia stuartii* and *Providencia rettgeri*. They can cause urinary tract infections and rarely respiratory and skin infections, mostly in hospitalized patients (Wie 2015).

Long-term and inappropriate use of antibiotics in these patients leads to multi-drug resistance (MDR), especially in species such as *Providencia*, which are part of the normal flora and are naturally resistant to many antibiotics.

The aminoglycosides (AGs) are an important group of antibiotics, which are active against a broad spectrum of gram-negative bacteria and gram-positive bacteria. AGs bind to the 30S subunit of the bacterial ribosome and cause death by destroying the protein structure of the bacteria. Bacteria have developed various resistance mechanisms to this group of antibiotics over time. The emergence of resistant strains has reduced the potential efficacy of AGs in empirical treatments (Mingeot-Leclercq, Glupczynski, and Tulkens 1999).

The following three mechanisms are involved in bacterial resistance to AGs: alterations in ribosomal binding sites, decreased entry (reduced uptake) by altering the permeability of porin proteins in their outer membrane, and production of aminoglycoside-modifying enzymes (AMEs), and the most common mechanism of clinically occurring aminoglycoside resistance (Park 2009). These modifications reduce the binding affinity of the drug to its target and lead to loss of antibacterial activity (Krause et al. 2015) .

Enzymes modifying aminoglycosides are classified into three groups: N-acetyltransferase (AAC), O-adenylytransferase (ANT/ADT), and O-phosphotransferase (APH) (Vanhoof, R., Hannecart-Pokorni, E., & Content 1998).

Several variants of the plasmid-derived gene encoding the aac(6')-Ib enzyme have been found. One of these variants, the aac(6')-Ib-cr enzyme, was first reported in China in 2006 (Robicsek, A., Strahilevitz, J., Jacob, G. A., Macielag, M., Abbanat, D., Park 2006). The suffix -cr in the aac(6')-Ib-cr enzyme refers to resistance to ciprofloxacin (fluoroquinolone). Thereby, the aac(6')-Ib-cr variant gene can cause simultaneous resistance to aminoglycosides and fluoroquinolones (Kim et al. 2011).



Another mechanism of resistance that causes a high level of resistance against aminoglycosides is 16S-rRNA methylation. Genes encoding 16S-rRNA methyltransferases confer a high level of resistance to almost all aminoglycosides (Costello et al. 2019).

The enzymes ArmA and rmtA were first reported in *Klebsiella pneumoniae* and *Pseudomonas aeruginos*a by Galimand et al. in 2003 (15). Since then, additional plasmid-mediated 16S-RMTase genes (rmtB, rmtC, rmtD, rmtE, rmtF, rmtG, rmtH ve npmA) have been identified in clinical isolates (Galimand, Courvalin, and Lambert 2003; Lee et al. 2018; Wachino et al. 2007) (Galimand, Courvalin, and Lambert 2012).

All of the genes encoding these enzymes have been shown to be plasmid-mediated and associated with β-lactamases, which may facilitate their spread (Costello et al. 2019; Galimand, Courvalin, and Lambert 2003; Bercot, Poirel, and Nordmann 2011; Doi and Arakawa 2007).

In our study, we investigated the presence of different genes at the molecular level to understand the origin of the mechanism of resistance in isolates phenotypically resistant to aminoglycoside group drugs and to determine exactly which AME is present.

## 2. MATERIAL-METHOD

### 2.1. Selection of Isolates

Our study samples were cultured from biological material, which were sent to the Medical Microbiological Bacteriology Laboratory of the Necmettin Erbakan University Meram Faculty of Medicine between the dates of January 2017-January 2019. Among these samples, isolates showing resistance profile to aminoglycoside antibiotics (netilmicin, gentamicin, tobramycin, and amikacin) were included in the study. All samples were from inpatients in different clinical units (plastic surgery, n=2 and nephrology, n=3) and intensive care units (reanimation, n=30; neurology, n=3, and emergency N.B., n=2) and a total of 40 strains were isolated.

The sample types were as follows: bronchial lavage (n=27; 67%), tracheal aspirate (n=4; 10%), blood (n=3; 7.5%), urine (n=2; 5%), catheter (n=2, a total of 40 isolates were collected; 5%), wound (n=1; 2.5%) and drainage (n=1; 2.5%). (Table 1 shows the types of clinical samples from which strains are isolated, disc diffusion susceptibility (mm) and MIC values (mg/L) of aminoglycoside drugs).

**Table 1.** Clinical specimens in which strains isolated from patients were isolated, inhibition zone diameters (mm) and MIC values (mg / L) of aminoglycoside drug sensitivities (EUCAST 2019).

| İsolate | Clinical sample type | Gentamicin ZONE | MIC | Tobramycin ZONE | MIC | Netilmicin ZONE | MIC | Amikacin ZONE | MIC | Ciprofloxacin ZONE | MIC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <6 | ≥≥32 | <8 | ≥≥64 | <11 | ≥≥4 |
| 33 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <9 | ≥≥4 |
| 44 | Bronchial lavage | <7 | ≥≥16 | <6 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <9 | ≥≥4 |
| 55 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <8 | ≥≥4 |
| 66 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <7 | ≥≥64 | <11 | ≥≥4 |
| 77 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <7 | ≥≥64 | <10 | ≥≥4 |
| 78 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <7 | ≥≥64 | <9 | ≥≥4 |
| 99 | Bronchial lavage | <7 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <7 | ≥≥64 | <10 | ≥≥4 |
| 110 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <6 | ≥≥64 | <12 | ≥≥4 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 111 | Bronchial lavage | <7 | ≥≥16 | <8 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <10 | ≥≥4 |
| 112 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <8 | ≥≥64 | <9 | ≥≥4 |
| 113 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <7 | ≥≥32 | <8 | ≥≥64 | <12 | ≥≥4 |
| 114 | Bronchial lavage | <6 | ≥≥16 | <8 | >≥4 | <7 | ≥≥32 | <6 | ≥≥64 | <12 | ≥≥4 |
| 115 | Bronchial lavage | <7 | ≥≥16 | <6 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <12 | ≥≥4 |
| 116 | Bronchial lavage | <8 | ≥≥16 | <7 | >≥4 | <7 | ≥≥32 | <6 | ≥≥64 | <10 | ≥≥4 |
| 117 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <8 | ≥≥32 | <6 | ≥≥64 | <12 | ≥≥4 |
| 118 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <8 | ≥≥64 | <11 | ≥≥4 |
| 119 | Bronchial lavage | <7 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <6 | ≥≥64 | <15 | ≥≥4 |
| 220 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <7 | ≥≥32 | <9 | ≥≥64 | <13 | ≥≥4 |
| 221 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <13 | ≥≥4 |
| 222 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <6 | ≥≥32 | <8 | ≥≥64 | <10 | ≥≥4 |
| 223 | Bronchial lavage | <7 | ≥≥16 | <6 | >≥4 | <7 | ≥≥32 | <8 | ≥≥64 | <12 | ≥≥4 |
| 224 | Bronchial lavage | <7 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <10 | ≥≥4 |
| 225 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <9 | ≥≥64 | <9 | ≥≥4 |
| 226 | Bronchial lavage | <6 | ≥≥16 | <6 | >≥4 | <7 | ≥≥32 | <6 | ≥≥64 | <10 | ≥≥4 |
| 227 | Bronchial lavage | <6 | ≥≥16 | <7 | >≥4 | <6 | ≥≥32 | <6 | ≥≥64 | <10 | ≥≥4 |
| 228 | Tracheal aspirate | <7 | ≥≥16 | <8 | >≥4 | <6 | ≥≥32 | <9 | ≥≥64 | <11 | ≥≥4 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 229 | Tracheal aspirate | <7 | ≥≥16 | <7 | ≥≥4 | <6 | ≥≥32 | <10 | ≥≥64 | <11 | ≥≥4 |
| 330 | Tracheal aspirate | <8 | ≥≥16 | <6 | ≥≥4 | 77 | ≥≥32 | <9 | ≥≥64 | <12 | ≥≥4 |
| 331 | Tracheal aspirate | <7 | ≥≥16 | <6 | ≥≥4 | <7 | ≥≥32 | <9 | ≥≥64 | <12 | ≥≥4 |
| 332 | Blood | <8 | ≥≥16 | <8 | ≥≥4 | <6 | ≥≥32 | <8 | ≥≥64 | <10 | ≥≥4 |
| 333 | Blood | <8 | ≥≥16 | <7 | ≥≥4 | <6 | ≥≥32 | <8 | ≥≥64 | <12 | ≥≥4 |
| 334 | Blood | 88 | ≥≥16 | <7 | ≥≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <10 | ≥≥4 |
| 335 | Urine | <7 | ≥≥16 | <6 | ≥≥4 | <7 | ≥≥32 | <8 | ≥≥64 | <10 | ≥≥4 |
| 336 | Urine | <6 | ≥≥16 | <6 | ≥≥4 | <7 | ≥≥32 | <9 | ≥≥64 | <12 | ≥≥4 |
| 337 | Catheter | <6 | ≥≥16 | <6 | ≥≥4 | <6 | ≥≥32 | <6 | ≥≥64 | <12 | ≥≥4 |
| 38 | Catheter | <6 | ≥≥16 | <7 | ≥≥4 | <8 | ≥≥32 | <6 | ≥≥64 | <9 | ≥≥4 |
| 39 | Wound | <8 | ≥≥16 | <7 | ≥≥4 | <6 | ≥≥32 | <11 | ≥≥64 | <11 | ≥≥4 |
| 40 | Drainage | <8 | ≥≥16 | <6 | ≥≥4 | <6 | ≥≥32 | <7 | ≥≥64 | <15 | ≥≥4 |



More than one isolate from the same patient were not included in the study, i.e., only one isolate was used from each patient.

**2.2. Microbiological Diagnostic Methods**

Classical microbiological methods were used for identification. The results of the lactose negative, and methyl red, phenyl pyruvic acid, and phenylalanine deaminase tests were positive.

Lysine, and ornithine decarboxylase, and arginindihidrolase tests were negative.

Bacteria with gram-negative bacilli morphology capable of producing acid from D-mannose were identified using the VITEK 2 automated system (bioMérieux, Marcyl'Etoile, France).

As a result of the identification tests, all isolates were found to belong to the species *Providencia stuartii*.

### 2.3. Antibiotic Sensitivity Tests

The diameters of the zones of inhibition were determined according to the criteria of the European Committee on Antimicrobial Susceptibility (EUCAST) using the Kirby-Bauer disk diffusion test. Double-disk synergy test was used to determine the production of extended-spectrum beta-lactamase (ESBL) in the isolates (Jarlier et al. 1988). The minimum inhibitory concentration (MIC) was measured using the VITEK 2 automated system (bioMérieux, Marcyl'Etoile, France).

Finally, the polymerase chain reaction (PCR) was used to determine the phenotypically observed resistance profiles of the strains.

### 2.4. Determination of Genes by PCR Study

Bacterial DNA isolation was performed using the GF-1 Bacterial DNA Extraction Kit (Vivantis, Malaysia) according to the company's recommendations.

To identify 15 aminoglycoside-modified enzymes by real-time PCR method, we searched for the presence of 29 genes, three genes expressing 16S rRNA methylating enzymes, and a total of 19 enzymes and 33 genes, together with an aminoglycoside enzyme variant, the AAC (6')-Ib-cr enzyme gene (Table 2. List of AME genes included in the study).

**Table 2**. List of AME genes included in the study.

| **Acetyltransferases** | **Adenyltransferases** | **Phosphotransferases** | **Methyltransferases** |
|---|---|---|---|
| *aac (2')-Ia* | *ant (2'')-Ia* | *aph (2')-Ia* | *armA* |
| *aac (3)-Ia* | *ant (2'')-Ib* | *aph (3')-Ia* | *rmtA* |
| *aac (3)-Ib* | *ant (4')-Ia* | *aph (3')-Ib* | *rmtB* |
| *aac (3)-IIa* | *ant (4')-IIa* | *aph (3')-IIIa* | |
| *aac (3)-IIb* | *ant (3'')-Ia* | | |
| *aac (3)-IIc* | | | |
| *aac (3)-IIIa* | | | |
| *aac (3)-IIIb* | | | |
| *aac (3)-IVa* | | | |
| *aac (3)-VIa* | | | |
| *aac (6')-Ia* | | | |
| *aac (6')-Ib* | | | |
| *aac (6')-Ic* | | | |
| *aac (6')-If* | | | |
| *aac (6')-Ig* | | | |

*aac (6')-Ih*

*aac (6')-Ii*

*aac (6')-IIa*

*aac (6')-IIb*

*aac (6')-Ib-cr*

Primers were identified based on sequences in the GenBank database using IDTDNA's primer design program.

The primer sequences we designed were compared with the gene sequences in the National Center for Biotechnology Information (NCBI-BLAST www.ncbi.nih.gov) and the appropriate ones were selected (Table 3. Base sequence of genes included in the study).

**Table 3.** Base sequence of genes included in the study.

| Resistance gene | Primer | Primer Sequence | bp |
|---|---|---|---|
| *aac (2')-Ia* | Forward | 5'- CAT TCG GTT GGA TGG CAA ATC -3' | 89 |
| | Reverse | 5'- ACT CCG CCT TCT TCT TCA ATA G -3' | |
| *aac (3)-Ia* | Forward | 5'- GAG GGC TGC TCT TGA TCT TT -3' | 94 |
| | Reverse | 5'- GAG CAA GTT CCC GAG GTA ATC -3' | |
| *aac (3)-Ib* | Forward | 5'- CAG CCG ACC AAT GAG TAT CTT -3' | 124 |
| | Reverse | 5'- CTT GCT CGA ACT TGG GTA GAA -3' | |
| *aac (3)-IIa* | Forward | 5'- AAA CGA TGG GTG ACG TAT GAG -3' | 98 |
| | Reverse | 5'- CAA TCG AGA ATG CCG TTT GAA T-3' | |
| *aac (3)-IIb* | Forward | 5'- ACG ATC GCC AAG GCT TAT G -3' | 87 |
| | Reverse | 5'- TCC TGC GCT TCA AAC AGA TAG -3' | |
| *aac (3)-IIc* | Forward | 5'- CAT ACG CGG AAG GCA ATA AC -3' | 98 |
| | Reverse | 5'- ACC GGA CCA ATC GCT TTA -3' | |
| *aac (3)-IIIa* | Forward | 5'- GTG ATC CGC ACG ATT ATA G-3' | 93 |
| | Reverse | 5'- CAT CCG CAT CAC CGA CTT T -3' | |
| *aac (3)-IIIb* | Forward | 5'- ACC TAC AGG CAC GCA CTG -3' | 96 |
| | Reverse | 5'- GTC ACG ATT CCC GCG AAA TA -3' | |

| | | | |
|---|---|---|---|
| *aac (3)-IVa* | Forward | 5'- TTG ATG GCA AAG GTT CCC AT -3' | 101 |
| | Reverse | 5'- TCA CAG CAG TGG TCA TTC TC -3' | |
| *aac (3)-VIa* | Forward | 5'- GTC ACC GCG CTC CAT TAT -3' | 80 |
| | Reverse | 5'- CGC AGT AAG GGC ATC GAA TA -3' | |
| *aac (6')-Ia* | Forward | 5'- CCT GGG AAT TGC ATC CAT TG -3' | 100 |
| | Reverse | 5'- CCT TGC TCT CTA GCT CTG TTT -3' | |
| *aac (6')-Ib* | Forward | 5'- AGA GTC CGT CAC TCC ATA CA -3' | 119 |
| | Reverse | 5'- GTA CTC CTG GAT CGG TTT CTT C -3' | |
| *aac (6')-Ic* | Forward | 5'- CGG AAA TGC GCG AGA TAT TG -3' | 117 |
| | Reverse | 5'- AGC CGT TGA CGT AAT CGT AG -3' | |
| *aac (6')-If* | Forward | 5'- AAG CAG TCC GGT GGT TTA TC -3' | 125 |
| | Reverse | 5'- CCA TCT CGG TGC AAC CTT T -3' | |
| *aac (6')-Ig* | Forward | 5'- ATG TGA GGA CTG AGA CTT C -3' | 110 |
| | Reverse | 5'- CAC TTC GGC CTG TCG AAT AA -3' | |
| *aac (6')-Ih* | Forward | 5'- TGG CCT GAT CAT GAA GAT GTG -3' | 98 |
| | Reverse | 5'- GCT TGT TGG GTG TCG GTA TAA -3' | |
| *aac (6')-Ii* | Forward | 5'- GCG CTA GAC CAA GAT GAG TTA G-3' | 97 |
| | Reverse | 5'- TCG GGA GCT TTC TAC AAC TAA TG-3' | |
| *aac (6')-IIa* | Forward | 5'- GCT CTC GTT GAA CTA CTG TTC TC -3' | 108 |
| | Reverse | 5'- CAC GAA TCC TGC CTT CTC ATA G -3' | |
| *aac (6')-IIb* | Forward | 5'- GAC GAC ATC GGT ATG CTT CA -3' | 101 |
| | Reverse | 5'- CGA TAG TCC TCT TTC ACC TCT TC -3' | |
| *aac (6')-Ib-cr* | Forward | 5'- ATC CAG GAG TAC GCG GAA TA -3' | 103 |
| | Reverse | 5'- TGA ACA GCA ACT CAA CCA GAG-3' | |
| *ant (2")-Ia* | Forward | 5'- GGC GAT CAT CTG GGA TTA CTT -3' | 89 |
| | Reverse | 5'- CTG TAG GAC TCT ATG TGC TTT GT-3' | |
| *ant (2")-Ib* | Forward | 5'- TTC TCT CGT TGG TCG AAA CC -3' | 81 |
| | Reverse | 5'- ATG GAC CAT GAC CGC ATC -3' | |

| | | | |
|---|---|---|---|
| *ant (4')-Ia* | Forward | 5'- GAA GAT TTC CGC CAA GCT ATT C -3' | 87 |
| | Reverse | 5'- CAA GGA TGG CAA GTA GGT AGA A -3' | |
| *ant (4')-IIa* | Forward | 5'- TTC TCT CCG CTT CGT CTC TA -3' | 100 |
| | Reverse | 5'- CAT GAA TAG CTT GGC GGA AAT C -3' | |
| *ant (3")-Ia* | Forward | 5'- AGA ATG GCA GCG CAA TGA -3' | 90 |
| | Reverse | 5'- CTA CCA AGG CAA CGC TAT GT -3' | |
| *aph (2")-Ia* | Forward | 5'- GCC ACA AAT GTT AAG GCA ATG A -3' | 93 |
| | Reverse | 5'- GCC ACA CTA TCA TAA CCA CTA CC -3' | |
| *aph (3')-Ia* | Forward | 5'- TAT CGG CTG CAT AGC AAG TC -3' | 83 |
| | Reverse | 5'- CCA ACG CAA TCT CAC CAT TTC -3' | |
| *aph (3')-Ib* | Forward | 5'- TCT GCC ACG GTG ATC TCT -3' | 88 |
| | Reverse | 5'- CCG TCC CAG GTC GAT GAA -3' | |
| *aph (3')-IIIa* | Forward | 5'- GGA AGG AAT GTC TCC TGC TAA G -3' | 95 |
| | Reverse | 5'- CAT CAT AGG TGG TCC CTT TAT ACC -3' | |
| *armA* | Forward | 5'- GGG TCT TAC TAT TCT GCC TAT CC -3' | 101 |
| | Reverse | 5'- GTT GCG ACT CTT TCA TTC GTC -3' | |
| *rmtA* | Forward | 5'- GGC CCT CTT TAT ACG TGA CAT AA -3' | 95 |
| | Reverse | 5'- ATC CCT GAT GAT GGG CAA AG-3' | |
| *rmtB* | Forward | 5'- GCT GGA TAC CCT GTA CGA TTT -3' | 103 |
| | Reverse | 5'- AAT GCC GCG CTC GTA TAG -3' | |



## 3. RESULTS

As a result of our study, 22 of the 29 aminoglycoside-modifying enzyme genes were found to be negative in all our samples, whereas seven AME genes, including five acetyltransferase and two adenyltransferase genes, were found to be positive (Table 4. List of genes with positive PCR results). None of the phosphotransferase genes were found in our isolates.

**Table 4.** List of genes with positive PCR results

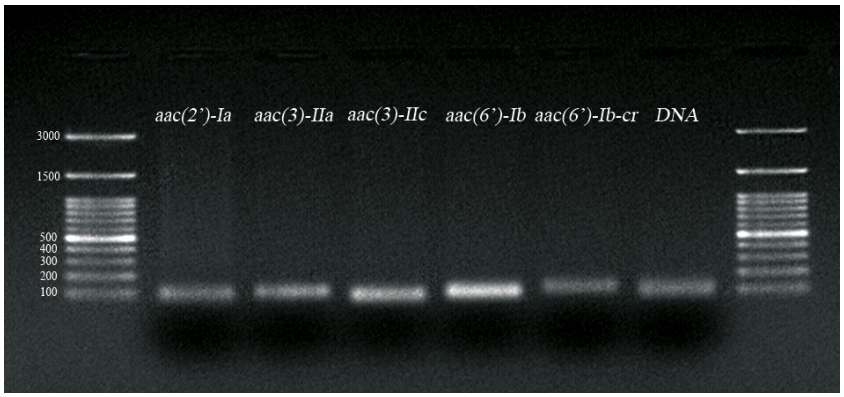
| Acetyltransferases | Adenyltransferases | Phosphotransfera | Methyltransferases |
|---|---|---|---|
| *aac (2')-Ia* | *ant (2'')-Ia* | | *armA* |
| *aac (3)-IIa* | *ant (3'')-Ia* | | |
| *aac (3)-IIc* | | | |
| *aac (6')-Ib* | | | |
| *aac (6')-Ib-cr* | | | |

The armA gene, one of the 16S-rRNA methyltransferase genes, was found to be positive in all patients, whereas the rmtA and rmtB methylase genes were negative. ESBL was also positive in the isolates.

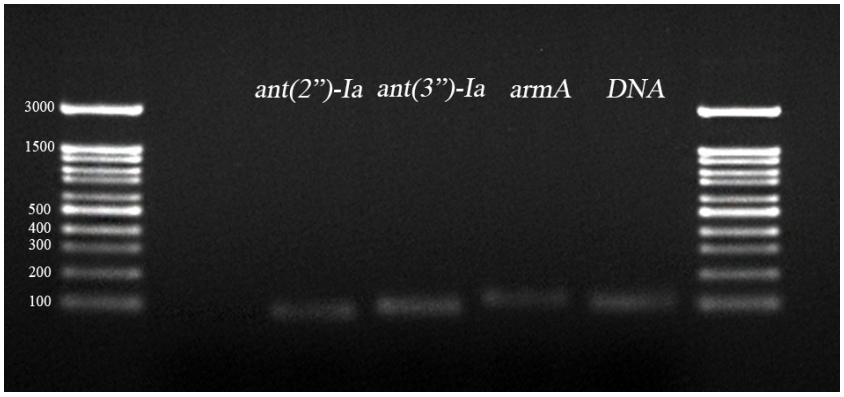
Genes that had a positive result in the PCR study were confirmed by performing gel electrophoresis. Figure 1 shows the gel electrophoresis images of the positive genes.

**Figure 1.** Gel electrophoresis images of genes that were found positive in the study.

a)




b)




a. Gel electrophoresis image showing positivity of *aac (2 ')- Ia, aac (3) -IIa, aac (6')- Ib, aac (6 ')- Ib-cr genes*
b. Gel electrophoresis image showing positivity of *ant (2 '')- Ia, ant (3'') -Ia, armA* genes

## 4. DISCUSSION

*Providencia* may cause nosocomial infections and epidemics, particularly in intensive care patients living in nursing homes with a history of long-term hospitalization (Wie 2015).

Almost all *Providencia* species can produce inducible Ambler class C cephalosporinase (AmpC) β-lactamases, and many isolates have been reported to acquire broad-spectrum β-lactamases (ESBLs) in nosocomial environments (Aubert et al. 2005). *P. stuartii* is naturally resistant to aminopenicillins and narrow-spectrum cephalosporins because AmpC is expressed as a chromosome (Jacoby 2009). Overexpression of AmpC generally confers low resistance to broad-spectrum cephalosporins such as ceftazidime to this strain. Higher levels of ceftazidime resistance are mainly associated with the acquisition of ESBL (Arpin et al. 2012). ESBL positivity in our isolates is associated with ceftazidime resistance (MIC > 64) and is supported by studies in the literature.

This breed is naturally resistant to many antibiotics, including ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefazolin, cephalothin, cephalexin, cefadroxil, cefuroxime, tetracyclines, tigecycline, polymyxin B, colistin, and nitrofurantoin (EUCAST n.d.).

Some studies have suggested a correlation between increased colistin consumption and increased prevalence of *P. stuartii* infections. Because of the proliferation of carbapenem-resistant *Enterobacteriales* species, particularly in intensive care units, the use of colistin and tigecycline as last-resort antibiotics to treat these infections has increased. This has exerted significant pressure on the patient flora and led to the selection of *P. stuartii*. Therefore, the *Providencia* infection rate has increased due to selective pressure (Hayakawa et al. 2012). In parallel with these data, we hypothesize that *P. stuartii* became dominant and multi-drug resistance (MDR) emerged because our patients, most of whom were ICU patients, were exposed to high levels of colistin during treatment.



*P. stuartii* contains an intrinsic and chromosomal acetyltransferase, aac(2')-1a, and confers a high aminoglycoside resistance when overexpressed (Macinga and Rather 1999). The existence of this gene was positively detected in all of our isolates. Because our strains were derived from intensive care patients, they were exposed to many antibiotics. As a result of this, the gene was overexpressed and we believe it contributes to aminoglycoside resistance.

In many studies on aminoglycoside resistance both in Turkey and worldwide, the most common AME enzymes were found to be aac (3)- II, aac (6')-I, ant (3'')-I, aph (3')- II, and ant (2'') )-I. The first studies on Gram-negative bacteria in Turkey aimed to understand aminoglycoside resistance mechanisms (Akalin, Torun, and Alacam 1988). In their large-scale study carried out particularly in South America and Greece in the years 1988-1993, Över et al. drew attention to the significant increase in the combined incidence of resistance mechanisms in Turkey (ÖVER et al. 2000).

The aac (6')-Ib-cr gene in Turkey was first reported by Nazik et al. (Nazic, Poirel, and Nordmann 2005). Since its initial identification, this enzyme has been reported in numerous geographical

locations and genetic settings (Strahilevitz et al. 2009). Recent reports have shown that quinolone resistance has reached a high level in Turkey and in the world (de Kraker et al. 2013). Surveillance studies of HITIT-1 (32.4%) and HITIT-2 (69.5%) conducted in Turkey show that the aac (6')-Ib-cr variant has increased over the years (Gur et al. 2009). Gur et al. measured the correlation between ESBL positivity rate and this gene and showed a high correlation between the two genes (Gur et al. 2009).

Another mechanism leading to aminoglycoside drug resistance is the methylation of 16S-rRNA. Genes encoding 16S-rRNA methyltransferases result in a high level of resistance to almost all aminoglycosides (Costello et al. 2019). There are 11 enzymes that provide resistance in this way, namely rmtA, rmtB, rmtC, rmtD, rmtD2, rmtE, rmtF, rmtG, rmtH, armA, and npmA. Although the prevalence of these enzymes is low, their occurrence is increasing day by day because they are carried by mobile genetic elements such as plasmids and transposons. Wachino et al. explained that armA and rmtB are the most common enzymes worldwide (Wachino and Arakawa 2012).

There are few studies on the 16S rRNA methyltransferase enzyme in Turkey. Although its incidence is very low, it is widely distributed among gram-negative bacteria. None of these genes were found in the first published studies in 2008 and 2012, which examined the armA, rmtA, and rmtB gene regions in ESBL-positive gram-negative isolates (Ermertcan et al. 2013; & Gazi 2008). Bercot et al. examined armA, RmtA, RmtB, RmtC, RmtD, and NpmA genes in ESBL-producing Enterobacteriaceae species in Kocaeli in 2010 and found the rmtB gene in a *Klebsiella pneumoniae* strain (Bercot, Poirel, and Nordmann 2011). In 2016, Gokmen et al. examined the RMTase genes (rmtA, B, C, D, E, F, G, H, and npmA) in *Klebsiella pneumoniae* isolates and found the rmtC gene positive in four ESBL-positive *Klebsiella pneumoniae* (Gokmen et al. 2016). In a study comparing the aminoglycoside resistance profiles of Syrian and Turkish patients in 2019, the most frequent AME genes were found to be aac (6')-Ib, aac (3)-IIa, aph (3')-Ia, and ant (2'')-Ia. In the same study, an ESBL-positive carbapenem-resistant *Klebsiella pneumoniae* strain was found to be rmC-positive (Cirit et al. 2019).



In a multi-center study conducted in 2020, 14 isolates were found to be positive for the armA (n=14), rmtC (n=8), rmtB (n=1), and rmtF (n=1) genes. According to the results of these studies, the most common RMTase gene in Turkey was found to be armA, followed by the enzymes rmtC and rmtB (Gür et al. 2020).

As a result of our research, similar to previous studies in Turkey, aac (2 ') - Ia, aac (3) -IIa, aac (3) -IIc, aac (6 ') - Ib, aac (6 ') - Ib-cr, ant (2'')-Ia, ant (3'')-Ia, and armA genes were found to be positive in all our isolates (n = 40). It was seen that the multi-drug resistance observed in our strains was due to the positivity of these genes.

The ESBL, armA and aac (6')-Ib-cr genes spread horizontally via plasmids in nosocomial settings, carrying various resistance genes and contributing to the formation of multi-drug resistant strains. ESBL, aminoglycoside modified enzyme armA and aac (6')-Ib-cr gene positivity were observed in all our patients. A positive correlation was observed between these genes. Our results confirm the findings in our literature review. To the best of our knowledge, our study have the feature of being

the first report of armA and aac(6')-Ib-cr positive genes in *Providencia* genera in Turkey. In addition, our study is the second report in Turkey that the armA gene is positive in gram-negative bacteria, following the study of Gür et al. (Gür et al. 2020).

We concluded that although *Providencia* species are members of the normal flora and rarely occur as infectious agents, they can become dominant with selective pressure, particularly in nosocomial settings, and lead to difficult-to-treat epidemics. To prevent this situation, more care should be taken in the use of antibiotics during treatment.

**REFERENCE**

& Gazi, H. Ermertcan Ş. Hoşgör-Limoncu M. Taşlı H. Eraç B. 2008. "Gram-Negatif Bakterilerde Plazmit Aracılı Y{ü}ksek D{ü}zey Aminoglikozit Direncinin Araştırılması." İnfeksiyon Dergisi (Turkish Journal of Infection). 2008. https://avesis.ege.edu.tr/yayin/2cbf7b83-3d8b-4ee2-a4b4-560f64f5d36b/gram-negatif-bakterilerde-plazmit-aracili-yuksek-duzey-aminoglikozit-direncinin-arastirilmasi.

Akalin, H. Erdal, Mustafa Torun, and Ruhi Alacam. 1988. "Aminoglycoside Resistance Patterns in Turkey." *Scandinavian Journal of Infectious Diseases* 20 (2): 199–203. https://doi.org/10.3109/00365548809032438.

Arpin, C., L. Thabet, H. Yassine, A. A. Messadi, J. Boukadida, V. Dubois, L. Coulange-Mayonnove, C. Andre, and C. Quentina. 2012. "Evolution of an Incompatibility Group IncA/C Plasmid Harboring Bla CMY-16 and QnrA6 Genes and Its Transfer through Three Clones of Providencia Stuartii during a Two-Year Outbreak in a Tunisian Burn Unit." *Antimicrobial Agents and Chemotherapy* 56 (3): 1342–49. https://doi.org/10.1128/AAC.05267-11.

Aubert, Daniel, Thierry Naas, Marie Frédérique Lartigue, and Patrice Nordmann. 2005. "Novel Genetic Structure Associated with an Extended-Spectrum β-Lactamase BlaVEB Gene in a Providencia Stuartii Clinical Isolate from Algeria." *Antimicrobial Agents and Chemotherapy* 49 (8): 3590–92. https://doi.org/10.1128/AAC.49.8.3590-3592.2005.

Bercot, Béatrice, Laurent Poirel, and Patrice Nordmann. 2011. "Updated Multiplex Polymerase Chain Reaction for Detection of 16S RRNA Methylases: High Prevalence among NDM-1 Producers." *Diagnostic Microbiology and Infectious Disease* 71 (4): 442–45. https://doi.org/10.1016/j.diagmicrobio.2011.08.016.

Cirit, Osman Sezer, Marta Fernández-Martínez, Buket Yayla, and Luis Martínez-Martínez. 2019. "Aminoglycoside Resistance Determinants in Multiresistant Escherichia Coli and Klebsiella Pneumoniae Clinical Isolates from Turkish and Syrian Patients." *Acta Microbiologica et Immunologica Hungarica* 66 (3): 327–35. https://doi.org/10.1556/030.66.2019.005.

Costello, Sarah E., Lalitagauri M. Deshpande, Andrew P. Davis, Rodrigo E. Mendes, and Mariana Castanheira. 2019. "Aminoglycoside-Modifying Enzyme and 16S Ribosomal RNA Methyltransferase Genes among a Global Collection of Gram-Negative Isolates." *Journal of Global Antimicrobial Resistance* 16 (March): 278–85. https://doi.org/10.1016/j.jgar.2018.10.020.

Doi, Yohei, and Yoshichika Arakawa. 2007. "16S Ribosomal RNA Methylation: Emerging Resistance Mechanism against Aminoglycosides." *Clinical Infectious Diseases*. Clin Infect Dis. https://doi.org/10.1086/518605.

Ermertcan, Şafak, Fethiye Ferda Yılmaz, Hüseyin Taşlı, and Ayşe Nur Yurtma. 2013. "Aminoglikozit Dirençli Gram Negatif Bakterilerde Plazmid Aracılı Metilaz Genlerinin Araştırılması." *Türk*

*Mikrobiyol Cem Derg* 43 (1): 12–16.

EUCAST. n.d. "Natural Resistance and Unexpected Phenotypes Expert Rules Release." EUCAST. Accessed May 23, 2021.

https://www.tmc-online.org/userfiles/file/EUCAST_Uzman_Kurallari_Surum_3_1.pdf.

Galimand, Marc, Patrice Courvalin, and Thierry Lambert. 2003. "Plasmid-Mediated High-Level Resistance to Aminoglycosides in Enterobacteriaceae Due to 16S RRNA Methylation." *Antımıcrobıal Agents And Chemotherapy* 47 (8): 2565–71. https://doi.org/10.1128/AAC.47.8.2565-2571.2003.

Galimand. 2012. "RmtF, a New Member of the Aminoglycoside Resistance 16S RRNA N7 G1405 Methyltransferase Family." https://doi.org/10.1128/AAC.00660-12.

Galimand. 2012. "RmtF, a New Member of the Aminoglycoside Resistance 16S RRNA N7 G1405 Methyltransferase Family." https://doi.org/10.1128/AAC.00660-12.

Gokmen, Tulin Guven, Togrul Nagiyev, Melda Meral, Cansu Onlen, Farzad Heydari, and Fatih Koksal. 2016. "NDM-1 and Rmtc-Producing Klebsiella Pneumoniae Isolates in Turkey." *Jundishapur Journal of Microbiology* 9 (10). https://doi.org/10.5812/jjm.33990.

Gur, Deniz, G. Hascelik, N. Aydin, M. Telli, M. Gültekin, D. Ögünç, O. A. Arikan, et al. 2009. "Antimicrobial Resistance in Gram-Negative Hospital Isolates: Results of the Turkish HITIT-2 Surveillance Study of 2007." *Journal of Chemotherapy* 21 (4): 383–89. https://doi.org/10.1179/joc.2009.21.4.383.

Gür, Deniz, Ufuk Hasdemir, Aslı Çakar, İffet Çavuşoğlu, Tuğçe Çelik, and Burak Aksu. 2020. "Comparative in Vitro Activity of Plazomicin and Older Aminoglyosides against Enterobacterales Isolates; Prevalence of Aminoglycoside Modifying Enzymes and 16S RRNA Methyltransferases." *Diagnostic Microbiology and Infectious Disease* 97 (4). https://doi.org/10.1016/j.diagmicrobio.2020.115092.



Hayakawa, Kayoko, Dror Marchaim, George W. Divine, Jason M. Pogue, Sarwan Kumar, Paul Lephart, Ken Risko, Jack D. Sobel, and Keith S. Kaye. 2012. "Growing Prevalence of Providencia Stuartii Associated with the Increased Usage of Colistin at a Tertiary Health Care Center." *International Journal of Infectious Diseases*. Int J Infect Dis. https://doi.org/10.1016/j.ijid.2012.05.1029.

Jacoby, George A. 2009. "AmpC B-Lactamases." *Clinical Microbiology Reviews*. Clin Microbiol Rev. https://doi.org/10.1128/CMR.00036-08.

Jarlier, Vincent, Marie Helene Nicolas, Genevieve Fournier, and Alain Philippon. 1988. "Extended Broad-Spectrum β-Lactamases Conferring Transferable Resistance to Newer β-Lactam Agents in Enterobacteriaceae: Hospital Prevalence and Susceptibility Patterns." *Clinical Infectious Diseases* 10 (4): 867–78. https://doi.org/10.1093/clinids/10.4.867.

Kim, Yun Tae, Ji Hyun Jang, Hyun Chul Kim, Hyogyeong Kim, Kyoung Ryul Lee, Kyung Sun Park, Hee Joo Lee, and Young Jin Kim. 2011. "Identification of Strain Harboring Both Aac(6')-Ib and Aac(6')-Ib-Cr Variant Simultaneously in Escherichia Coli and Klebsiella Pneumoniae." *BMB Reports* 44 (4): 262–66. https://doi.org/10.5483/BMBRep.2011.44.4.262.

Kraker, M. E.A. de, V. Jarlier, J. C.M. Monen, O. E. Heuer, N. van de Sande, and H. Grundmann. 2013. "The Changing Epidemiology of Bacteraemias in Europe: Trends from the European Antimicrobial Resistance Surveillance System." *Clinical Microbiology and Infection* 19 (9): 860–68. https://doi.org/10.1111/1469-0691.12028.

Krause, Kevin M, Alisa W Serio, Timothy R Kane, and Lynn E Connolly. n.d. "Aminoglycosides: An Overview." https://doi.org/10.1101/cshperspect.a027029.

Macinga, D. R., and P. N. Rather. 1999. "The Chromosomal 2'-N-Acetyltransferase of Providencia Stuartii: Physiological Functions and Genetic Regulation." *Frontiers in Bioscience : A Journal and Virtual Library*. Front Biosci. https://doi.org/10.2741/macinga.

Mingeot-Leclercq, Marie-Paule, Youri Glupczynski, and Paul M Tulkens. 1999. "Aminoglycosides: Activity and Resistance." Vol. 43. http://aac.asm.org/.

Nazic, Hasan, Laurent Poirel, and Patrice Nordmann. 2005. "Further Identification of Plasmid-Mediated Quinolone Resistance Determinant in Enterobacteriaceae in Turkey [4]." *Antimicrobial Agents and Chemotherapy*. American Society for Microbiology (ASM). https://doi.org/10.1128/AAC.49.5.2146-2147.2005.

Över, Ufuk, Deniz Gür, Serhat ÜnaL, and George H. Mıller. 2000. "Gram-Negatif Bakterilerde Aminoglikozid Antibiyotiklere Karşı Direnç Mekanizmaları: Son Gelişmeler ve Türkiye Sonuçları." *Flora İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Dergisi* 5 (3): 168–78. https://app.trdizin.gov.tr/makale/TVRBMk1URXg/gram-negatif-bakterilerde-aminoglikozid-antibiyotiklere-karsi-direnc-mekanizmalari-son-gelismeler-ve-turkiye-sonuclari.

Park, Yeon-Joon. 2009. "Aminoglycoside Resistance in Gram-Negative Bacilli." *Korean Journal of Clinical Microbiology* 12 (2): 57. https://doi.org/10.5145/kjcm.2009.12.2.57.

Robicsek, A., Strahilevitz, J., Jacob, G. A., Macielag, M., Abbanat, D., Park, C. H. et. al. 2006. "Fluoroquinolone-Modifying Enzyme: A New Adaptation of a Common Aminoglycoside Acetyltransferase." *Nat Med* 12 (1): 83–88.

Strahilevitz, Jacob, George A. Jacoby, David C. Hooper, and Ari Robicsek. 2009. "Plasmid-Mediated Quinolone Resistance: A Multifaceted Threat." *Clinical Microbiology Reviews*. Clin Microbiol Rev. https://doi.org/10.1128/CMR.00016-09.



Vanhoof, R., Hannecart-Pokorni, E., & Content, J. 1998. "Nomenclature of Genes Encoding Aminoglycoside-Modifying Enzymes." *Antimicrob Agents Chemother* 42 (2): 483.

Wachino, Jun Ichi, and Yoshichika Arakawa. 2012. "Exogenously Acquired 16S RRNA Methyltransferases Found in Aminoglycoside-Resistant Pathogenic Gram-Negative Bacteria: An Update." *Drug Resistance Updates* 15 (3): 133–48.

https://doi.org/10.1016/j.drup.2012.05.001.

Wie, Seong-Heon. 2015. "Clinical Significance of Providencia Bacteremia or Bacteriuria." *Korean J Intern Med* 30: 167–69. https://doi.org/10.3904/kjim.2015.30.2.167.