

# Plasmid Profile Analysis of Aminoglycoside-Resistant *Escherichia coli* Isolated From Urinary Tract Infections

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## Abstract

**Background:** Uropathogenic *E. coli* (UPEC) is the primary cause of human urinary tract infections (UTIs) worldwide. Moreover, there has been renewed and growing interest in using older antibiotics for treatment, such as aminoglycosides.

**Objectives:** The goal of this study was to determine the plasmid profile patterns of UPEC isolates harboring the aminoglycoside resistance gene *aac(3)-IIa*.

**Patients and Methods:** A total of 276 uropathogenic *E. coli* (UPEC) samples were isolated from UTI patients at the Tehran heart center in Tehran, Iran. Antimicrobial susceptibility testing against five aminoglycosides was performed by the disk diffusion method, and the *aac(3)-IIa* gene was detected via PCR. Moreover, plasmid profiling was carried out on those UPEC isolates harboring the *aac(3)-IIa* gene. Finally, the similarities among these isolates were determined on the basis of their plasmid profiles.

**Results:** The highest level of resistance was seen for tobramycin (24.6%), and the *aac(3)-IIa* gene was found in 51 isolates. Twenty-seven different plasmid profiles were identified among the isolates harboring the *aac(3)-IIa* gene, with the 15 kb plasmid being the most common. Moreover, no significant correlation was found between the resistance patterns and the number of plasmids. The cluster analysis based on the plasmid profiles grouped the isolates into five different clusters, of which cluster one was the largest (containing 14 of 51 isolates).

**Conclusions:** Our data suggest the monitoring of aminoglycoside resistance, and its consideration in the empirical therapy of UPEC infections.

**Keywords:** Aminoglycoside Resistance, Plasmid, Uropathogenic *E. coli*, *aac(3)-IIa* gene

## 1. Background

Uropathogenic *Escherichia coli* (UPEC) is one of the main causes of both nosocomial and community-acquired urinary tract infections (UTIs) in humans. The organism is, therefore, of clinical importance, and accounts for substantial medical costs, morbidity, and mortality worldwide (1). UTIs are often treated with antibiotics such as ampicillin, aminoglycosides, nitrofurantoin, and trimethoprim-sulfamethoxazole (2); among which, aminoglycosides are highly potent, broad-spectrum, bactericidal antibiotics that are used to treat severe bacterial infections, often in combination with cell wall active antimicrobial agents (3). Aminoglycosides are excreted almost entirely via the kidneys by glomerular filtration; thus, the resulting urine contains very high concentrations of the drug (4). There are three mechanisms of resistance to aminoglycosides: reduced uptake and increased efflux, alteration of the target RNA, and enzymatic modification of the aminoglycoside (5). However, enzymatic

modification is the most common type of aminoglycoside resistance.

The genes encoding for aminoglycoside modifying enzymes are categorized into three classes: acetyltransferases (AAC), nucleotidyltransferases (ANT), and phosphotransferases (APH). Within each class, the enzymes are grouped according to the sites of the aminoglycoside modifications (6). Overall, the 3-N-aminoglycoside acetyltransferases [AAC(3) enzymes] and the 6'-N-aminoglycoside acetyltransferases [AAC(6') enzymes] are among the most commonly encountered modifying enzymes in gram negative pathogens. Moreover, the AAC(3)-II acetyltransferase is commonly seen in the various clinical isolates of gram-negative bacteria, such as *E. coli* (7). The *aac(3)-IIa* gene, which was originally identified in resistance plasmids, has been reported to be detected with a relatively high frequency in clinical isolates (8).

Antibiotic resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes. Moreover, antimicrobial ther-

apy can favor the selection of antibiotic resistant strains, and the presence of antibiotic resistant genes on plasmids can facilitate their transfer and spread among bacteria (9). Plasmid profiling is an epidemiological tool used to follow the spread of antibiotic resistance and to differentiate between bacterial isolates. It has been used successfully for tracing plasmids carrying different antibiotic resistance genes (10). In addition, the number and size of the plasmids present can be used as the basis for the differentiation between bacterial isolates. This technique has been successfully used for the analysis of outbreaks of nosocomial and community-acquired infections caused by a variety of gram negative bacteria (11).

Recently, there has been renewed and growing attention towards using older antibiotics, such as aminoglycosides. Due to their relatively low level of usage, older antibiotics seem to remain active against many bacterial isolates that are becoming more resistant to the most widely used antibacterial drugs (12).

## 2. Objectives

The aims of this study were to determine the aminoglycoside resistance profiles of *E. coli* isolated from urinary tract infections (UTIs) in Tehran, Iran, and to determine the plasmid profiles of those aminoglycoside-resistant isolates.

## 3. Patients and Methods

### 3.1. Bacterial Isolates

A total of 276 uropathogenic *E. coli* samples were isolated from UTI patients at the Tehran heart center in Tehran, Iran. The Tehran heart center is one of the largest medical centers in Iran dedicated to the diagnosis and treatment of coronary and heart diseases, with 440 beds and more than 520,000 outpatient visits, and patients are referred to it from nearly all parts of the country.

Urine cultures showing a growth of  $\geq 10^5$  cfu/mL were considered to be positive, and the *E. coli* isolates were then identified using conventional microbiological and biochemical tests (13). Stock cultures of the isolates were stored frozen, at  $-70^\circ\text{C}$ , in trypticase soy broth (Merck, Darmstadt, Germany) containing 20% glycerol.

### 3.2. Susceptibility Testing

The susceptibility of the *E. coli* isolates to five aminoglycoside antibiotics was determined using the disk diffusion method on Mueller-Hinton agar plates (Merck, Darmstadt, Germany), as recommended by the clinical and laboratory standards institute (CLSI, 2011) (14). Disks containing the following antibiotics ( $\mu\text{g}$ ) were used (Mast, UK):

gentamicin (GM, 10), tobramycin (TN, 10), kanamycin (K, 30), amikacin (AK, 30), and netilmicin (NT, 30). *E. coli* ATCC25922, *Staphylococcus aureus* ATCC25923, and *Klebsiella pneumoniae* ATCC23823 (harboring the *aac(3)-IIa* gene) were used as control strains; the *K. pneumoniae* ATCC23823 was provided by the statens serum institute of Denmark.

### 3.3. Amplification of *aac(3)-IIa* Gene by PCR

The genomic DNA was prepared by the freeze-thaw method, and used as the template for the PCR reactions (15). The PCR amplification of the *aac(3)-IIa* gene was performed with the Gene Amp PCR System PTC-1148 (Bio-Rad, USA) using the published specific primers 5'-CGGAAGGCAATAACGGAG-3' and 5'-TCGAACAGGTAGCACTGAG-3' (amplicon size: 740 bp) (16). *E. coli* ATCC25922 and *K. pneumoniae* ATCC23823 [*aac(3)-IIa+*] were used as the negative and positive controls, respectively.

The cycling conditions were as follows: initial denaturation at  $95^\circ\text{C}$  for 5 minutes; 30 cycles of  $94^\circ\text{C}$  for 1 minute,  $62^\circ\text{C}$  for 1 minute, and  $72^\circ\text{C}$  for 1 minute; followed by a final elongation at  $72^\circ\text{C}$  for 10 minutes. The PCR products were analyzed via electrophoresis with 1% agarose gels in a 1X TAE (tris-acetate-EDTA) buffer, the gels were stained with ethidium bromide, and the PCR products were visualized under a UV light. A 100 bp Plus DNA Ladder (Fermentas, Germany) was used as a molecular size marker.

### 3.4. Plasmid Isolation

The plasmid DNA was extracted from the UPEC isolates harboring the *aac(3)-IIa* gene using a plasmid extraction kit (Bioneer, South Korea), according to the manufacturer's guidelines. The plasmids were electrophoresed with a 0.8% agarose gel in 1X TAE buffer at 80 V for 5 hours, and visualized under UV light following straining with ethidium bromide. A 1 kb DNA ladder (Fermentas, Germany) was used as a molecular size marker to estimate the molecular weight of the plasmids in the studied strains.

### 3.5. Cluster Analysis

The similarities between the strains, based on their plasmid profiles and cluster analyses, were determined using NTSYS-PC software (version 2.02). Moreover, the matrix of the similarity of coefficients was subjected to an unweighted pair-group method algorithm (UPGMA) to generate a dendrogram.

## 4. Results

An analysis of the antimicrobial susceptibility testing showed that, among the 276 clinical isolates of UPEC,

the resistances to tobramycin, kanamycin, gentamicin, netilmicin, and amikacin were 24.6% (n = 68), 23.1% (n = 64), 21% (n = 58), 6.1% (n = 17), and 3.6% (n = 10), respectively (Table 1).

**Table 1.** Aminoglycoside Susceptibility or Non-Susceptibility Patterns of *E. coli* Isolated From Urinary Tract Infections (n = 276)<sup>a</sup>

Antibiotic Tested	Isolates		
	Susceptible	Intermediate	Resistant
TN	208 (75.36)	0 (0.0)	68 (24.63)
K	210 (76.8)	2 (0.72)	64 (23.18)
GM	214 (77.53)	4 (1.44)	58 (21.01)
NT	252 (91.3)	7 (2.53)	17 (6.15)
AK	259 (93.84)	7 (2.53)	10 (3.62)

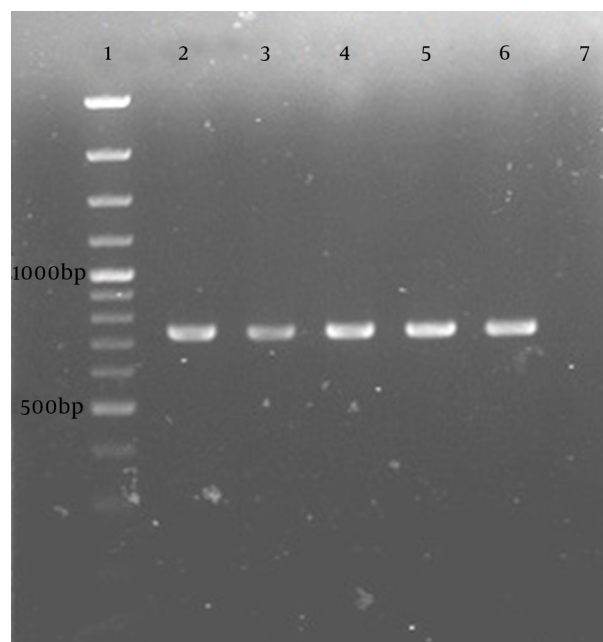
Abbreviations: AK, amikacin; GM, gentamicin; K, kanamycin; NT, netilmicin; TN, tobramycin.

<sup>a</sup>Values are expressed as No. (%).

Out of 276 UPEC isolates, 71 (25.7%) were resistant to at least one of the aminoglycoside antibiotics tested. Of those 71 isolates, 4 (5.6%) were resistant to all of the five aminoglycosides tested, 19 (26.7%) were resistant to four of the aminoglycosides (13 isolates showed resistance pattern TN, GM, K, NT and 6 showed resistance pattern TN, GM, K, AK), 28 (39.4%) were resistant to three (resistance pattern TN, GM, K), 17 (23.9%) were resistant to two (4 isolates showed resistance pattern TN, GM and 13 showed resistance pattern TN, K), and 3 (4.2%) of the isolates were resistant to one of the aminoglycosides tested.

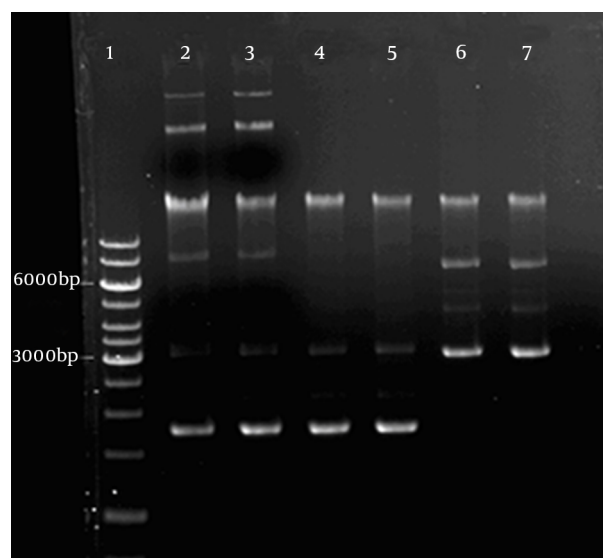
The PCR results showed that the *aac(3)-IIa* gene was present in 18.5% (51) of all isolates, and 71.8% of the 71 aminoglycoside resistant isolates (Figure 1). The analysis of the plasmid DNA of these isolates revealed the presence of plasmids in all of the isolates (Figure 2); some of the isolates possessed single sized plasmids, while others had multiple plasmids with different sizes ranging from 1 to 20 kb, as shown in Table 2. In total, 27 different plasmid profile groups for the isolates could be defined; the number of strains per plasmid profile group varied from 1 to 10 (Table 2), and the number of plasmids present in these isolates ranged from 1 to 6. A plasmid of 15 kb was the most frequently seen, and was found in 49 isolates (96.07%). A plasmid of 1.5 kb was detected in 52.9% (27/51) of the isolates, and plasmids of 1 kb, 2 kb, 3 kb, and 3.5 kb were detected in 29.4% (15/51), 23.5% (12/51), 21.5% (11/51), and 21.5% (11/51) of the isolates, respectively. A plasmid of 6 kb was the least frequently detected plasmid, and was observed in only 2 of the isolates (3.9%). The plasmid profiles and aminoglycoside resistance patterns of 51 of the UPEC isolates harboring the *aac(3)-IIa* gene are shown in Table 2.

**Figure 1.** PCR for the Detection of the *aac(3)-IIa* Gene From the Clinical Isolates of the UPEC



Lane 1, 100 bp plus DNA ladder; lane 2, *K. pneumoniae* ATCC23823 [*aac(3)-IIa*+] as a positive control; lanes 3 - 6, clinical isolates of UPEC; lane 7, *E. coli* ATCC25922 as a negative control.

**Figure 2.** Plasmid Profiles of the UPEC Isolates Harboring the *aac(3)-IIa* Gene



Lane 1, 1000 bp ladder; lanes 2 - 7, clinical isolates.

No significant correlation was found between the resistance patterns and the number of plasmids in the iso-

**Table 2.** Plasmid Profiles and Aminoglycoside Resistance Patterns of 51 UPEC Isolates

Number of Plasmid(s) and Plasmid Profile	Plasmid Sizes, kb	Number of Isolate(s)	Drug Resistance
<b>1</b>			
1	15	4	NT, K, GM, TN
1	15	1	K, GM, TN
1	15	1	GM, TN
2	18	1	K, GM, TN
<b>3</b>			
3	20, 15, 3.5	2	GM, TN
4	15, 3.5, 3	2	GM, TN
5	3.5, 1.5, 1	1	GM, TN
6	1.5, 2, 15	6	K, GM, TN
6	1.5, 2, 15	1	K, TN
6	1.5, 2, 15	3	NT, K, GM, TN
<b>2</b>			
7	15, 1.5	3	K, GM, TN
8	15, 5	2	K, GM, TN
9	1, 15	2	K, GM, TN
9	1, 15	1	K, TN
10	4, 15	2	K, GM, TN
11	3, 15	1	GM, TN
12	15, 20	1	GM, TN
<b>4</b>			
13	20, 15, 3.5, 3	1	NT, K, GM, AK, TN
13	20, 15, 3.5, 3	1	K, GM, TN
14	15, 10, 1.5, 1	1	K, TN
15	1.5, 6, 2, 15	1	TN, NT, K, GM
16	15, 10, 3.5, 1.5	1	K, GM, TN
17	15, 3.5, 1.5, 1	1	NT, K, GM, AK, TN
<b>5</b>			
18	20, 15, 3, 2, 1	1	NT, K, GM, TN
19	15, 6, 3, 2.5, 1.5	1	Not found
20	18, 15, 2.5, 1.5, 1	2	K, GM, TN
21	15, 3, 2.5, 1.5, 1	1	K, TN
22	18, 15, 10, 3.5, 1.5	1	NT, K, GM, AK, TN
23	18, 15, 10, 3.5, 1	1	NT, K, GM, AK, TN
24	15, 10, 3, 1.5, 1	1	TN, GM
<b>6</b>			
25	20, 18, 15, 3, 1.5, 1	1	K, GM, TN
26	20, 15, 10, 5, 1.5, 1	1	GM
27	15, 4, 3, 2.5, 1.5, 1	1	GM, TN

Abbreviations: AK, amikacin; K, kanamycin, GM, gentamicin; NT, netilmicin; TN, tobramycin.

lates. In several cases, two to four plasmid profiles were observed for the same resistance pattern; for instance, resistance pattern NT, K, GM, TN showed 4 different plasmid profiles (Table 3). However, some isolates with the same plasmid profile showed the same resistance pattern (e.g. for plasmid profiles 3, 4, 7, and 8).

**Table 3.** Diversity of Plasmid Sizes in the UPEC Isolates With the Same Resistance Pattern

Drug Resistance	Plasmid Sizes, kb
NT, K, GM, AM, TN	18, 15, 10, 3.5, 1; 15, 3.5, 1.5, 1; 18, 15, 10, 3.5, 1.5; 20, 15, 3.5, 3
GM, TN	20, 15, 3.5; 15, 3.5, 3; 3.5, 1.5, 1; 3, 15; 15, 10, 3, 1.5, 1; 15, 4, 3, 2.5, 1.5, 1; 15, 20; 15
K, GM, TN	15, 1.5; 15, 5; 15, 4; 15, 1; 15, 2, 1.5; 18, 15, 2.5, 1.5, 1; 20, 18, 15, 3, 1.5, 1; 15, 10, 3.5, 1.5; 15; 18; 20, 15, 3.5, 3
K, TN	15, 1; 15, 2, 1.5; 15, 3, 2.5, 1.5, 1; 15, 10, 1.5, 1
NT, K, GM, TN	20, 15, 3, 2, 1; 15, 2, 1.5; 15; 1.5, 6, 2, 15
-	15, 6, 3, 2.5, 1.5
GM	20, 15, 10, 5, 1.5, 1

To better characterize the 51 isolates harboring the *aac(3)-IIa* gene in the current investigation, the isolates were subjected to cluster analyses based on their plasmid patterns. These isolates were grouped into five clusters at a cutoff value of 50% similarity (Figure 3). Fourteen isolates forming the major cluster possessed plasmids of 15 kb and 1.5 kb (cluster 1), while the plasmid profiles 15; 15, 5; 15, 1; 15, 4 (cluster 2) and 20, 15, 3.5; 20, 15, 3.5, 3; 20, 15; 15, 3, 3.5; 15, 3 (cluster 3) formed the other close clusters (Figure 3). The isolates in clusters 1 and 3 showed a common resistance pattern to at least two of the five drugs tested. Overall, the similarity of the strains based on their plasmid patterns are represented using a dendrogram in Figure 3.

## 5. Discussion

*E. coli* isolates are among the most common causes of urinary tract infections worldwide. In recent years, an increase in the occurrence of anti-microbial resistant UPEC isolates has been observed in several countries, especially in underdeveloped ones (1). Aminoglycoside antibiotics are older drugs that, due to their relatively low level of usage, seem to have remained active against some of the infectious diseases that are more difficult to treat (4). Moreover, an adjustment in the aminoglycoside administration dosage results in effective serum and renal parenchymal levels and adequate urine concentrations, minimizing the severity and frequency of their side-effects (such as ototoxicity and nephrotoxicity) while still preserving the antibacterial properties (12). The difficulties in the development

of newer antibiotics which can inhibit resistant bacteria have prompted physicians to recycle these older antibacterial agents (4).

The results of this study have shown that the level of resistance to aminoglycoside antibiotics is relatively high, and amikacin was the most active aminoglycoside against the UPEC isolates. As shown in Table 1, resistance to older aminoglycosides, such as kanamycin, is generally higher than resistance to newer aminoglycosides, such as gentamicin, amikacin, and netilmicin. This suggests that the implementation of newer aminoglycosides could still be considered the gold standard in treating the more resistant pathogens. The different resistance patterns seen in the different geographical regions may be related to the differences in the aminoglycoside treatment regimens (17-19).

The *aac(3)-IIa* gene has been reported to be detected in gram-negative bacteria in clinical settings with a relatively higher frequency than the other aminoglycoside modifying genes (8). In our study, a high prevalence of the *aac(3)-IIa* gene was observed in the aminoglycoside-resistant strains (71.83%), with several other studies also reporting a high prevalence of this gene (20, 21).

Originally identified in resistance plasmids, the *aac(3)-IIa* gene accounts for 85% of the AAC(3)-II phenotype. Recent studies, however, have also detected the *aac(3)-IIa* gene surrounded by integron elements. The presence of these genes on mobile molecular elements can facilitate the transfer and spread of resistant genes between bacteria (8). In the present study, out of the 51 isolates investigated, plasmids were detected in all of them. In the majority of the isolates, two to six plasmids were detected, while only a few strains carried one plasmid, and the most common plasmid encountered was 15 kb in size. Overall, the number of plasmids ranged from 1 to 6, which is different from the reports in Iran and other countries (18, 22-24). Daini and Adesemowo reported that 65.7% of the *E. coli* isolates possessed plasmids with sizes ranging from 0.12 kb to 23.1 kb (25). Various factors are involved in these differences, including the origin of the strains (i.e. inpatient or outpatient), the geographical differences across the world, etc.

Our study revealed 27 different plasmid profiles among the 51 isolates, with five clusters ranging from 1 to 14 isolates each. The clustered isolates were resistant to at least two out of the five drugs tested. This multiplicity of profiles may be due to the clonal spread of the isolates, but we did not investigate that in this study.

In conclusion, this study highlighted the significance of aminoglycoside-resistant *E. coli* isolates as a cause of UTI infections in Iran. Moreover, it was shown that plasmid profiling analysis distinguished more strains than the antimicrobial susceptibility patterns. Our study has demon-

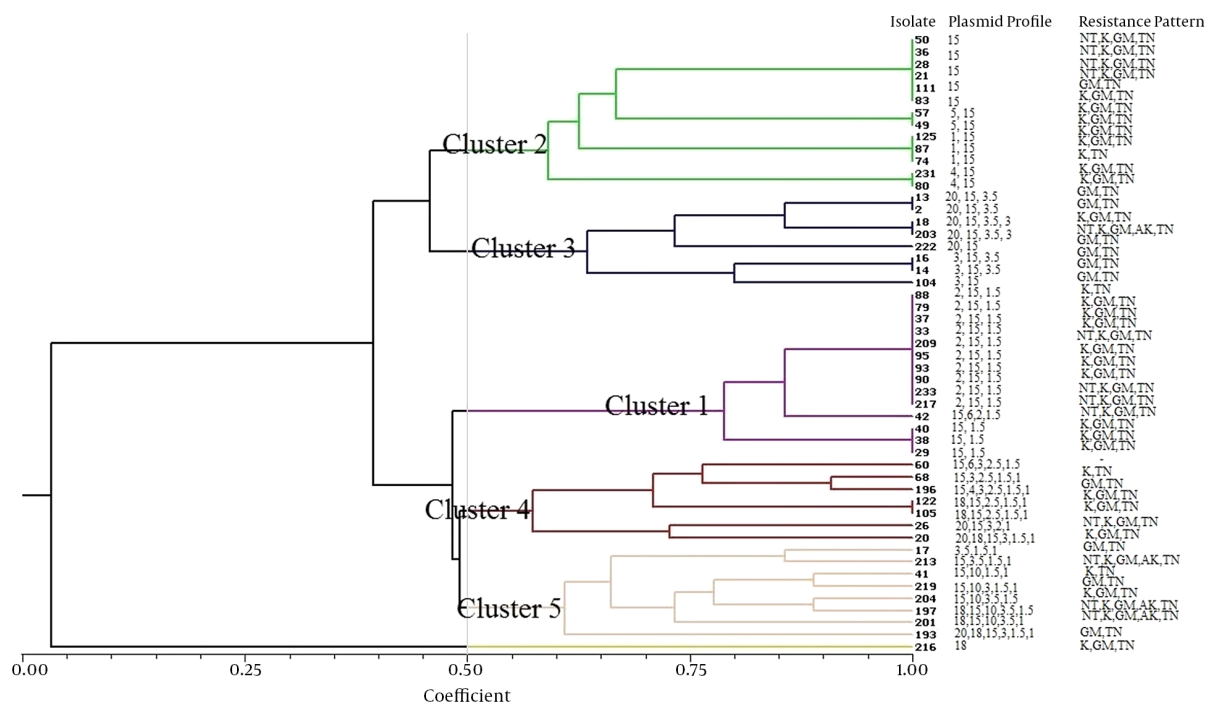


Figure 3. Dendrogram Based on the Plasmid Profiles of the UPEC Isolates Harboring the *aac(3)-IIa* Gene (n = 51)

strated the usefulness of plasmid profiles in local epidemiological studies.

#### Footnotes

**Authors' Contribution:** Neda Soleimani performed the microbiological and molecular studies, while Safoura Derakhshan and Mojtaba Memariani prepared the manuscript.

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#### References

- Bien J, Sokolova O, Bozko P. Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *Int J Nephrol Renovasc Dis.* 2012;**2012**.
- Jan N, Meshram SU, Kulkarni A. Plasmid profile analysis of multidrug resistant *E. coli* isolated from UTI patients of Nagpur City, India. *Rom Biotechnol Lett.* 2009;**14**(5):4635-40.
- Zacharczuk K, Piekarska K, Szych J, Jagielski M, Hidalgo L, San Millan A, et al. Plasmid-borne 16S rRNA methylase *ArmA* in aminoglycoside-resistant *Klebsiella pneumoniae* in Poland. *J Med Microbiol.* 2011;**60**(Pt 9):1306-11. doi: [10.1099/jmm.0.024026-0](https://doi.org/10.1099/jmm.0.024026-0). [PubMed: 21252274].
- Gilbert DN. Urinary Tract Infections in Patients with Chronic Renal Insufficiency. *J Nephrol Nurs.* 2006;**1**(2):327-31. doi: [10.2215/cjn.01931105](https://doi.org/10.2215/cjn.01931105).
- Houghton JL, Green KD, Chen W, Garneau-Tsodikova S. The future of aminoglycosides: the end or renaissance?. *ChemBiochem.* 2010;**11**(7):880-902. doi: [10.1002/cbic.200900779](https://doi.org/10.1002/cbic.200900779). [PubMed: 20397253].
- Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. *Drug Resist Updat.* 2010;**13**(6):151-71. doi: [10.1016/j.drug.2010.08.003](https://doi.org/10.1016/j.drug.2010.08.003). [PubMed: 20833577].
- Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. *Int J Antimicrob Agents.* 2010;**35**(4):316-21. doi: [10.1016/j.ijantimicag.2009.11.003](https://doi.org/10.1016/j.ijantimicag.2009.11.003). [PubMed: 20060273].
- Diaz MA, Cooper RK, Cloeckaert A, Siebeling RJ. Plasmid-mediated high-level gentamicin resistance among enteric bacteria isolated from pet turtles in Louisiana. *Appl Environ Microbiol.* 2006;**72**(1):306-12. doi: [10.1128/AEM.72.1.306-312.2006](https://doi.org/10.1128/AEM.72.1.306-312.2006). [PubMed: 16391058].
- Palmer KL, Kos VN, Gilmore MS. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr Opin Microbiol.* 2010;**13**(5):632-9. doi: [10.1016/j.mib.2010.08.004](https://doi.org/10.1016/j.mib.2010.08.004). [PubMed: 20837397].
- Christopher A, Hora S, Ali Z. Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Ann Trop Med PH.* 2013;**6**(3):285. doi: [10.4103/1755-6783.120985](https://doi.org/10.4103/1755-6783.120985).
- Farshad S, Sheikh R, Japoni A, Basiri E, Alborzi A. Characterization of *Shigella* strains in Iran by plasmid profile analysis and PCR amplification of *ipa* genes. *J Clin Microbiol.* 2006;**44**(8):2879-83. doi: [10.1128/JCM.00310-06](https://doi.org/10.1128/JCM.00310-06). [PubMed: 16891506].
- Gad GF, Mohamed HA, Ashour HM. Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. *PLoS One.* 2011;**6**(2):e217224. doi: [10.1371/journal.pone.0017224](https://doi.org/10.1371/journal.pone.0017224). [PubMed: 21359143].

13. Turcovsky I, Kunikova K, Drahovska H, Kaclikova E. Biochemical and molecular characterization of Cronobacter spp. (formerly Enterobacter sakazakii) isolated from foods. *Antonie Van Leeuwenhoek*. 2011;**99**(2):257-69. doi: [10.1007/s10482-010-9484-7](https://doi.org/10.1007/s10482-010-9484-7). [PubMed: 20640509].
14. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20. CLSI, Wayne, PA, USA. 2011 Available at: [http://vchmedical.ajums.ac.ir/\\_vchmedical/documents/CLSI%202011.pdf](http://vchmedical.ajums.ac.ir/_vchmedical/documents/CLSI%202011.pdf).
15. Soleimani N, Aganj M, Ali L, Shokoohizadeh L, Sakinc T. Frequency distribution of genes encoding aminoglycoside modifying enzymes in uropathogenic E. coli isolated from Iranian hospital. *BMC research notes*. 2014;**7**(1):842-847.
16. Gow SP, Waldner CL, Harel J, Boerlin P. Associations between antimicrobial resistance genes in fecal generic Escherichia coli isolates from cow-calf herds in western Canada. *Appl Environ Microbiol*. 2008;**74**(12):3658-66. doi: [10.1128/AEM.02505-07](https://doi.org/10.1128/AEM.02505-07). [PubMed: 18424533].
17. Bashir S, Sarwar Y, Ali A, Mohsin M, Saeed MA, Tariq A, et al. Multiple drug resistance patterns in various phylogenetic groups of uropathogenic E.coli isolated from Faisalabad region of Pakistan. *Braz J Microbiol*. 2011;**42**(4):1278-83. doi: [10.1590/S1517-83822011000400005](https://doi.org/10.1590/S1517-83822011000400005). [PubMed: 24031752].
18. Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohamadzadegan R. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. *Arch Iran Med*. 2012;**15**(5):312-6. [PubMed: 22519382].
19. El-Najjar NG, Farah MJ, Hashwa FA, Tokajian ST. Antibiotic resistance patterns and sequencing of class I integron from uropathogenic Escherichia coli in Lebanon. *Lett Appl Microbiol*. 2010;**51**(4):456-61. doi: [10.1111/j.1472-765X.2010.02926.x](https://doi.org/10.1111/j.1472-765X.2010.02926.x). [PubMed: 20840552].
20. Jakobsen L, Sandvang D, Hansen LH, Bagger-Skjot L, Westh H, Jorgensen C, et al. Characterisation, dissemination and persistence of gentamicin resistant Escherichia coli from a Danish university hospital to the waste water environment. *Environ Int*. 2008;**34**(1):108-15. doi: [10.1016/j.envint.2007.07.011](https://doi.org/10.1016/j.envint.2007.07.011). [PubMed: 17804070].
21. Ho PL, Wong RC, Lo SW, Chow KH, Wong SS, Que TL. Genetic identity of aminoglycoside-resistance genes in Escherichia coli isolates from human and animal sources. *J Med Microbiol*. 2010;**59**(Pt 6):702-7. doi: [10.1099/jmm.0.015032-0](https://doi.org/10.1099/jmm.0.015032-0). [PubMed: 20185552].
22. Tsen H, Chi W. Plasmid Profile Analysis for Enterotoxigenic Escherichia coli and Detection of Heat Stable Enterotoxin I (STI) Gene by Polymerase Chain Reaction. *J Food Drug*. 1996;**4**(3).
23. Uma B, Prabhakar K, Rajendran S, Kavitha K, Sarayu YL. Antibiotic sensitivity and plasmid profiles of Escherichia coli isolated from pediatric diarrhea. *J Glob Infect Dis*. 2009;**1**(2):107-10.
24. Kalantar E, Soheili F, Salimi H, Soltan Dallal MM. Frequency, antimicrobial susceptibility and plasmid profiles of Escherichia coli pathotypes obtained from children with acute diarrhea. *Jundishapur J Microbiol*. 2011;**4**(1):23-8.
25. Daini OA, Adesemowo A. Antimicrobial susceptibility pattern and r-plasmids of clinical strains of Escherichia coli. *J basic appl sci*. 2008;**2**:397-400.