



# The Relationship Between Class I and II Integrations and Antibiotic Resistance Among *Escherichia coli* Isolates From Urinary Tract Infections

Farshad Nojoomi<sup>1</sup>, Mahtab Vafaei<sup>1</sup>, Hossein Rajabi Vardanjani<sup>2\*</sup>

<sup>1</sup>Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran

<sup>2</sup>Shahrekord University of Medical Sciences, Shahrekord, Iran

## \*Corresponding Author:

Hossein Rajabi Vardanjani,  
Tel: +98394514860,  
Email:  
[bacteriology94@gmail.com](mailto:bacteriology94@gmail.com)

Published Online February 27,  
2018

**Keywords:** Uropathogenic  
*Escherichia coli*, Antibiotic  
resistance, Integrations



## Abstract

**Background:** *Escherichia coli* develops drug resistance through several mechanisms.

**Objective:** The purpose of this study was the assessment of antibiotic susceptibility profile, and detection of class I and II integrations in *E. coli*, isolated from urinary tract infections (UTIs).

**Materials and Methods:** A total of 100 *E. coli* clinical isolates were collected from UTIs in a 1-year period. The antibiotic susceptibility of *E. coli* isolates was evaluated to 14 antibiotics as advised by CLSI 2016 guidelines. All the isolates were tested by polymerase chain reaction (PCR) for the presence of class I and II integrations.

**Results:** The highest resistance was observed to amoxicillin (72%), erythromycin (70%), ciprofloxacin (66%), nalidixic acid (57%), and tetracycline (55%). The class I and II integrations were detected in 32% and 21% of isolates, respectively. There were significant relationships between resistance to trimethoprim-sulfamethoxazole ( $P < 0.001$ ), ciprofloxacin ( $P < 0.01$ ), and tetracycline ( $P < 0.005$ ) and the class I integration. The class I integration gene was highly detected in the uropathogenic *E. coli*, possibly playing a role in the dissemination of drug resistance.

**Conclusion:** Because of the significant association between the presence of class I integration among multidrug-resistant isolates and antibiotic resistance, infection control, and establishment and implementation of appropriate strategies for suitable treatment in hospitals are essential for the prevention of dissemination of these isolates.

Received October 7, 2017; Revised November 27, 2017; Accepted January 16, 2018

## Background

Urinary tract infection (UTI) is one of the major bacterial infections among patients. Although *E. coli* is present in gut flora,<sup>1</sup> it can cause infection under some conditions as an opportunistic strain, when it spreads from gastrointestinal site. *E. coli* isolates are responsible for more than 80% of UTIs, but are more likely to cause infection in women.<sup>2</sup> Likewise, they cause infections in lower respiratory tract, skin, soft tissues, bloodstream, several other diseases such as pneumonia, and also lower rate of endocarditis, bacteremia, arthritis, and bone inflammation. Eye infections in the ICU wards have also been reported.<sup>3</sup> *E. coli* also leads to wound and surgical site infections. The development of antibiotic resistance by uropathogenic *E. coli* (UPEC) has led to severe problems in the treatment of infections.<sup>4,5</sup>

The uptake of genetic elements such as plasmids, integrations and transposons by *E. coli* is a key factor in the dissemination of drug-resistance.<sup>6</sup> Integrations which are transmitted horizontally include several genetic factors which can carry and spread drug resistance among

these bacteria. These elements are one of the major approaches for spread of these genes among multi-drug resistant (MDR) strains. More than 9 classes of integrations have been detected, among which class I integration is the predominant class among both gram-positive and gram-negative species.<sup>7</sup> According to the reports, approximately 70 various resistance genes are inserted into integrations which cause resistance to beta lactams, aminoglycosides, sulfonamides, macrolides and chloramphenicol which have been reported among *E. coli* and other species.<sup>8</sup> The aims of this study were determination of antibiotic resistance and investigation of the prevalence of class I and II integrations in UPEC.

## Materials and Methods

One hundred *E. coli* isolates were collected and identified with phenotypic or conventional tests from patients with UTIs in several hospital sections including urology, ICU, emergency and infectious settings. Antibiotic susceptibility pattern of UPEC was tested using the agar disk diffusion (Kirby-Bauer) method which followed the

CLSI 2016 advice. The disks were prepared from Roscoe and were as follows: gentamicin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), ceftazidime (30 µg), amoxicillin (30 µg), cephalothin (30 µg), trimethoprim-Sulfamethoxazole (25 µg), norfloxacin (10 µg), amikacin (30 µg), chloramphenicol (30 µg), nalidixic acid (NA) (30 µg), and nitrofurantoin (300 µg). The standard strain of *E. coli* ATCC25922 was employed as a quality control of antibiotic susceptibility.

### Detection of Integrons by Polymerase Chain Reaction

Polymerase chain reaction (PCR) with specific primers (listed in Table 1) was used to detect the class 1 and class 2 integrons among UPEC.

### Results

Sixty-three percent of *E. coli* isolates were collected from females and 37% from the male patients. Most of isolates were resistant to the amoxicillin (72%), erythromycin (70%), ciprofloxacin (66%), nalidixic acid (57%), and tetracycline (55%). In addition, the highest sensitivity was observed to the nitrofurantoin disc among the isolates. The resistance pattern of isolates has been depicted in Figure 1.

The class I and II integrons were detected in 32% (26 female and 6 male patients) and 21% (18 female and 3 male patients) of isolates, respectively. There were significant relationships between resistance to trimethoprim-sulfamethoxazole ( $P < 0.001$ ), ciprofloxacin ( $P < 0.01$ ), and tetracycline ( $P < 0.005$ ), and the class I integron. The class I integron gene was highly detected in the UPEC, possibly playing a role in the dissemination of drug resistance.

### Discussion

In the present survey, among 100 *E. coli* isolates from UTIs, the prevalence of infection was higher in the females compared to the male patients. The majority of isolates were resistant to amoxicillin (72%), followed by erythromycin (69%), ciprofloxacin, ceftazidime, nalidixic acid, and tetracycline; while most of them indicated sensitivity to trimethoprim-sulfamethoxazole, norfloxacin, chloramphenicol, gentamicin, imipenem, amikacin, and nitrofurantoin (99%).

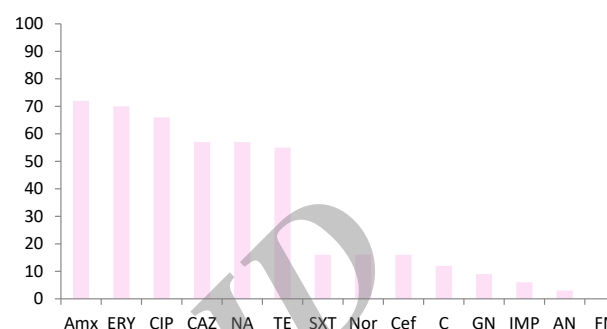
The results of previous studies have demonstrated a higher percentage of resistance<sup>10-12</sup>; and likewise, the rate of nalidixic acid resistance was significant supposing the existence of the NA-encoding gene, integrated into the integron classes.<sup>13</sup>

Resistance to the third and fourth generation cephalosporins and carbapenems has spread worldwide and in the country level and therefore has caused concerns. The role of inter-hospital or patient-to-patient transmission of resistance genes, especially those carried by integrons must be controlled and considered with surveillance programs.

*Escherichia coli* isolates mostly carried class I integrons

**Table 1.** The Specific Primers Used in This Study

Target Gene	Primer/sequence(5 to 3)	Amplicon Size	Ref.
Int1	F: 5'-CCTCCCGCACGATGA-3' R: 5'-TCCACGCATCGTCAG-3'	281	9
Int2	F: 5'-TTATTGCTGGGATTAGGC-3' R: 5'-ACGGCTACCCTCTGTTATC-3'	233	9



**Figure 1.** The Antibiotic Susceptibility Profile of the Isolates.

Abbreviations: Amx, amoxicillin; ERY, erythromycin; CIP, ciprofloxacin; Cef, cephalothin; NA, nalidixic acid; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; Nor, norfloxacin; CAZ, ceftazidime; C, chloramphenicol; GN, gentamicin; IMP, imipenem; AN, amikacin; FN, nitrofurantoin.

(31%) as was reported in other studies; thereby playing a critical role in the spread of drug-resistance.<sup>14</sup>

In this study, class II integron was detected in 21% of the isolates. Previous data confirmed that this class had a lower prevalence.<sup>15-17</sup>

In addition, the class I and II integrons were detected in 26 females and 6 male patients (32%) and 18 females and 3 male patients (21%), respectively. There were significant relationships between resistance to trimethoprim-sulfamethoxazole ( $P < 0.001$ ), ciprofloxacin ( $P < 0.01$ ), and tetracycline ( $P < 0.005$ ), and the class I integron. The class I integron gene was highly detected in the UPEC, possibly playing a role in the dissemination of drug resistance.

In Ranjbaran and colleagues' study, the prevalence of class I and II integrons in 100 isolates of *E. coli* was 86% and 8%, respectively.<sup>18</sup> The higher existence of class I integron among drug-resistant isolates and a possible association with multiple drug resistance is a phenomenon that requires more investigations.

### Conclusion

Because of the significant association found between the presence of class I integron among multidrug-resistant isolates and antibiotic resistance, infection control, and establishment and implementation of appropriate strategies for suitable treatment in hospitals are essential for the prevention of dissemination of these isolates.

### Authors' Contributions

All the authors contributed equally to this study.

**Ethical Approval**

This article is a part of results of the approved proposal by AJA University of Medical Sciences.

**Conflict of Interest Disclosures**

The authors declare that they have no conflict of interests.

**Financial Support**

This study was supported by AJA University of Medical Sciences, Tehran, Iran.

**References**

- Nojoomi F, Ghasemian A. Effect of Overgrowth or Decrease in Gut Microbiota on Health and Disease. *Arch Pediatr Infect Dis*. 2016;4(2):e34558. doi:10.5812/pedinf.34558
- Sheerin NS. Urinary tract infection. *Medicine*. 2011;39(7):384-389.
- Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin Microbiol Rev*. 2013;26(4):822-880. doi:10.1128/cmr.00022-13
- Bush K. Extended-spectrum beta-lactamases in North America, 1987-2006. *Clin Microbiol Infect*. 2008;14 Suppl 1:134-143. doi:10.1111/j.1469-0691.2007.01848.x
- El-Badawy MF, Tawakol WM, Maghrabi IA, Mansy MS, Shohayeb MM, Ashour MS. Iodometric and Molecular Detection of ESBL Production Among Clinical Isolates of *E. coli* Fingerprinted by ERIC-PCR: The First Egyptian Report Declares the Emergence of *E. coli* O25b-ST131 clone Harboring blaGES. *Microb Drug Resist*. 2017;23(6):703-717. doi:10.1089/mdr.2016.0181
- Hudson CM, Bent ZW, Meagher RJ, Williams KP. Resistance determinants and mobile genetic elements of an NDM-1-encoding *Klebsiella pneumoniae* strain. *PLoS One*. 2014;9(6):e99209. doi:10.1371/journal.pone.0099209
- Gillings MR. Integrons: past, present, and future. *Microbiol Mol Biol Rev*. 2014;78(2):257-277. doi:10.1128/mmbr.00056-13
- Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol Rev*. 2009;33(4):757-784. doi:10.1111/j.1574-6976.2009.00175.x
- Yao F, Qian Y, Chen S, Wang P, Huang Y. Incidence of extended-spectrum beta-lactamases and characterization of integrons in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated in Shantou, China. *Acta Biochim Biophys Sin (Shanghai)*. 2007;39(7):527-532.
- Shayanfar N, Rezaei M, Ahmadi M. Evaluation of Extended Spectrum Betalactamase(ESBL) Positive Strains of *Klebsiella pneumoniae* And *Escherichia coli* in Bacterial Cultures. *Iran J Pathol*. 2010;5(1):34-39.
- Jazayeri Moghadas A, Irajian G, Ranjbar R. Detection of Extended Spectrum Beta Lactamase Producing *Salmonella* spp. and Multidrug Resistance Pattern. *Iran J Pathol*. 2009;4(3):128-132.
- Alheib O, Al Kayali R, Abajy MY. Prevalence of Plasmid-Mediated Quinolone Resistance (PMQR) Determinants Among Extended Spectrum Beta-Lactamases (ESBL)-Producing Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Aleppo, Syria. *Arch Clin Infect Dis*. 2015;10(3):e20631. doi:10.5812/archcid.20631
- Kargar M, Kargar M, Jahromi MZ, Najafi A, Ghorbani-Dalini S. Molecular detection of ESBLs production and antibiotic resistance patterns in Gram negative bacilli isolated from urinary tract infections. *Indian J Pathol Microbiol*. 2014;57(2):244-248. doi:10.4103/0377-4929.134688
- Zakharyan MK, Sedrakyan AM, Arakelova KA, et al. Antibiotic Resistance and Occurrence of Class 1 Integrins in Clinical Isolates of *Salmonella enterica*. *Glob J Immunol Allerg Dis*. 2013;1(2):44-48.
- Izadi N, Naderi Nasab M, Harifi Mood E, Meshkat Z. Prevalence of TEM and SHV Genes in Clinical Isolates of *Klebsiella Pneumonia* From Mashhad, North- East Iran. *Iran J Pathol*. 2014;9(3):199-205.
- Gillings MR. Class 1 integrins as invasive species. *Curr Opin Microbiol*. 2017;38:10-15. doi:10.1016/j.mib.2017.03.002
- Cury J, Jove T. Identification and analysis of integrons and cassette arrays in bacterial genomes. 2016;44(10):4539-4550. doi:10.1093/nar/gkw319
- Ranjbaran M, Zolfaghari M, Japoni-Nejad A, et al. Molecular Investigation of Integrins in *Escherichia Coli* and *Klebsiella Pneumoniae* Isolated from Urinary Tract Infections. *J Mazandaran Univ Med Sci*. 2013;23(105):20-27.