# Investigation of the Gene Expression of Class I Integron and Its Relationship to Antibiotic Resistance in Isolates of Escherichia Coli Causing Urinary Tract Diseases

Marwa Shaker Mahmood[1], Aida Hussain Ibrahim[2]
[1, 2]Dept. of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq
Email: marwa.Mahmood1106@sc.uobaghdad.edu.iq

**Abstract**
This study was aimed to determine the relationship between Integron class I and multidrug resistance in Uropathogenic Escherichia coli. In this investigation, 302 urine samples in total were used. 139 Uropathogenic E. coli were collected from patients in hospital. Then, using biochemical testing and the Vitek II compact system, all of the isolates were diagnosed and confirmed. All E. coli isolates were tested using a disc diffusion procedure to detect their susceptibility to 23 types of antibiotics from different classes, and the result showed that these bacteria were highly resistant to most used antibiotics especially, Cefotaxime, Ampicillin, Piperacillin, Trimethoprim- sulfamethoxazole, Cefepime and Ceftazidime, while the most effective antibiotics were Colistin and Tigecyclin. Also, the results show different resistance patterns in which 111 (79.86%) isolate showed multidrug resistance MDR, Extensively drug resistance XDR 18 isolate (12.94%), while Pandrug resistance PDR 3 (2.15%). PCR was used to detect the presence of integron class I in MDR isolates, which was found in 87 of isolates with a 483 bp amplification product. Using the broth dilution method, the minimum inhibitory concentration of UPEC isolates with pan drug resistance to Cefotaxime and Trimethoprim-Sulfamethoxazole was established. By measuring gene expression using real-time PCR, the relationship between Integron class I and resistance to these antibiotics was estimated. The results of gene expression revealed a close relationship between the presence of integron class 1 and the resistance of E. coli to cefotaxime and trimethprim.
Keywords: UPEC, Integrons, Multidrug resistance, Gene expression.

## 1. Introduction

An international public health problem is antibiotic resistance (AMR). that has grown to frightening proportions[1], [2]. widespread use of antibiotics in veterinary, medical, and agricultural treatments in recent years has considerably boosted the Enterobacteriaceae family's resistances, resulting in the selection and widespread spread of resistant clones worldwide [3], [4]. The vast bacterial family Enterobacteriaceae is led by Escherichia coli. *E. coli* strains are divided into intestinal and extraintestinal pathotypes based on the variety of pathogenicity and associated clinical symptoms. Enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* are intestinal pathogenic *E. coli* pathotypes that cause diarrheal disorders, or DEC (DAEC). In addition, extraintestinal *E. coli* pathotypes, such as uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and sepsis-associated *E. coli*, cause illnesses outside of the digestive system (SEPEC) [5]. Multi-drug resistance (MDR) in clinical isolates of bacteria like *E. coli* pathotypes is a significant healthcare issue today and is linked to higher morbidity and mortality rates globally [6]. Resistance genes can be transmitted via extrachromosomal elements obtained from other bacteria, despite the fact that they are often acquired and distributed through chromosomal changes. Plasmids, transposons, and integrons are a few examples of the several mobile DNA segments that fall under this category (1Integrons are genetic elements that can mobilize or integrate gene cassettes that encode antibiotic resistance determinants [7], [8]. The integrase gene (intI), the attachment site (attI), and the promoter (Pant), which encourages the production of any appropriately integrated gene, are the three main components of an integron (s). The basic integron structure consists of a 3'-Conserved Segment (CS) with qac E delta and sulI genes and a 5'-Conserved Segment (CS) with the integrase gene. Gene cassettes are DNA sequences that are located between these conserved sections and can vary in length and molecular complexity; several of these sequences have already been identified. Integrons contain gene cassettes that represent key classes of antibiotics and more than 70 distinct antibiotic resistance genes [9].

## 2. Materials and Methods

### sample gathering, UPEC isolation, and identification

This research was authorized by the Baghdad University of Science's ethical committee (CSEC/0921/0047) From October 2020 to April

doi.org/10.31838/hiv23.01.119 Received: 15.09.22, Revised: 13.10.22, Accepted: 09.12.22

2021Approximately 302 urine samples from Those who have been enrolled at the medical city hospital were placed in sterile tubes and quickly sent to the laboratory for culture. *E. coli* was identified by cultivating on MacConky agar, Eosin methyl blue (EMB) agar, and biochemical assays (Indol test, Oxidase test, Catalase test, Methyl-Red, and Vogas-Broskaor tests), and the results of identification were validated by utilizing the API 20 E and Vitek 2 systems.

## Antibiotic susceptibility of Uropathogenic *E. coli*

In order to conduct this test, the disk diffusion method was used, as recommended by CLSI (2020) [9]. This study involved 23 antibiotics, with more than one antibiotic from each class being used (Ampicillin 10 µg, Piperacillin 1001 µg, Amoxicillin-clavulanate 301 µg, Aztreonam 30µg, Cefotaxime 301 µg, Cefoxitin 301 µg, Cefepime 30 µg, Ceftriaxone 30 µg, Ceftazidime 30 µg, Imipenem 10 µg, Meropenem 10 µg, Ciprofloxacin 5µg, Levofloxacin 5µg, Nalidixic acid 301 µg, Gentamicine 101 µg, Amikacin 301 µg, Azithromycin 15 µg, Tetracycline 30 µg, Trimethoprim-1 sulfamethoxazole 1.251\ 23.75 µg, Chloramphenicol 301 µg, Nitrofurantoin 300 µg, Colistin 10µg and Tigecyclin 15µg). The results were interpreted using CLSI guidelines (2020)[6].

## Minimum Inhibitory Concentration MIC

The MICS of Cefotaxime and Trimethoprim-sulfamethoxazole for Pan Drug Resistance UPEC Isolates were detected by the broth microdilution method, in which these antibiotics were dissolved in Muller Hinton Broth and then transferred to 96-well microdilution plates with starting concentrations for Cefotaxime of 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, and 8192 µg/100 µl, while for Trimethoprim-sulfamethoxazole of 0.78125\3.890625, 1.5625\7.78125, 3.125\15.5625, 6.25\31.125, 12.5\62.25, 25\125, 50\250, 100\500, 200\1000 and 400\2000 µg/100 µl. After 18 hours of growth, the MIC result was evaluated in accordance with CLSI (2020) guidelines [6].

## Extraction of DNA

Using a DNA extraction kit (WizPrep Company, Korea), genomic DNA was obtained from all bacterial isolates.

## PCR for *INTI 1* detection

The detection was carried out by amplifying the INTI 1 gene in a DNA template isolated from E. coli and using a reaction mixture with end volume of 25µl. The primer sequence utilized in this study is as in Table 1. The PCR reaction was carried out with mixing the components that was listed in Table 2. to make mixture 25 µl. In a Pioneer/Korea MyGenie 96/384 Gradient Thermal Block, the PCR reaction was carried out. The reaction of PCR was performed in number of cycles under thermal condition controlling as in Table 3.

**Table 1.Primer designs that were used in this investigation**

| Primer name | Primers sequence (5´ → 3´) | Target and Experiment | Product size(bp) | References |
|---|---|---|---|---|
| INTI 1 | F- GGTCAAGGATCTGGATTTCGR – ACATGCGTGTAAATCATCGTC | Integron class I genePCR | 483 | [10] |
| | F- AGGATGCGAACCACTTCATCR- CGGCCTTGCTGTTCTTCTAC | Gene expression | 127 | This study |
| RecA | F- GGCCGTATCGTCGAAATCTAR- ATATCGACGCCCAGTTTACG | Hose Keeping geneGene expression | 158 | This study |

**Table 2. Components and volumes of PCR reaction solutions**

| Component | Volume (µl) |
|---|---|
| Go Taq Green master mix | 12.5 |
| Forward primer | 1 |
| Reverse primer | 1 |
| DNA | 5 |
| Nuclease free water | 5.5 |
| Total volume | 25 |

**Table 3. The PCR program design**

| Steps | °C | Time | Cycle |
|---|---|---|---|
| Initial Denaturation | 95 | 5 min. | 1 |
| Denaturation | 95 | 30 sec. | 35 |
| Annealing | 62 | 30 sec. | |
| Extension | 72 | 1 min. | |
| Final extension | 72 | 5 min. | 1 |

## RNA extraction

To ascertain the gene expression for the Integron class I gene, RNA must be extracted from PDR E. coli isolates. RNA was extracted for this study utilizing a kit for RNA extraction (SV Total RNA Isolation system, Promega, USA).

## One step quantitative real time PCR assay (qRT-PCR)

The expression of the INTI 1 gene and its connection to cefotaxime and trimethoprim-sulfamethoxazole resistance in PDR UPEC isolates were evaluated using real-time quantitative PCR.SYBR green dye, which can only bind with double-strand DNA and emits light after binding with DNA, was utilized in this method as the fluorescence dye. Real-time PCR can be used to measure fluorescence light. Corbett GoTaq 1-Step RT-qPCR System (Promega, USA) was used to carry out this technique. The reaction mixture of this experiment and thermocyclear conditions were described in table 1 and 2 respectively and the primers listed in table 1. In this experiment, Cefotaxime and Trimethoprim-Sulfamethoxazole were added to PDR UPEC isolates at sub-MIC levels.

| Table 4: GoTaq® 1-Step RT-qPCR reaction mix | |
|---|---|
| Master mix components | Volume (µl) |
| GoTaqR qPCR Master Mix, 2X | 10 |
| GoScript™ RT Mix (50X) | 0.4 |
| Forward primer | 1 |
| Reverse primer | 1 |
| Nuclease free water | 2.6 |
| RNA template | 5 |
| Total volume | 20 |

| Table 5: General thermocycler (RT-PCR) program used in the study | | | | |
|---|---|---|---|---|
| Steps | Temperature (°C) | Duration | Cycles | Reference |
| Reverse transcription | 37 | 15 min | 1 | Company instructions |
| Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation | 95 | 10 min | | |
| Denaturation | 95 | 10 sec | 40 | |
| Annealing | 57* | 30 sec | | This study |
| | 58** | 30 sec | | |
| Extension | 72 | 30 sec | | |
| * recA, ** *INTI 1*gene. | | | | |

## 3. Statistical Methods

A statistical analysis is carried out. SPSS is a statistical package for the social sciences (version 25 ). For independence and goodness of fit, the Chi-square ($\chi 2$) test was employed; P≤1 0.05 was regarded statistically significant, and P ≤ 10.01 was considered Statistically significant high [11].

## 4. Results and Discussion

In the current study, 87 urine samples showed no growth, while 215 showed positive growth, with 139 (64.65%) being identified as E. coli isolates by producing glossy and off-white or beige in color on Blood Agar, Pink to dark pink, dry, and donut-shaped on MacConkey agar; encircled by a dark pink area of precipitated bile salts, and metallic green sheen on Eosin methylene blue (EMB). Biochemical tests (IMVC), API 20 also confirmed the results.

### Results of antibiotics susceptibility

The resistance to routinely used antimicrobials against urine isolates acquired from Medical City Hospital in Iraq is shows in Figure 1. For each antimicrobial agent, Table 4 displays the percentage of susceptible, intermediate, and resistance isolates, while Table 5 shows multidrug resistance. E. coli demonstrated a significant level of resistance to Cefotaxime, Ampicillin, Piperacillin, Trimethoprim-sulfamethoxazole, Cefepime, Ceftazidime at percentage (100, 98.6, 96.4, 95.7, 94.2, 91.4%) respectively. Followed by Ceftriaxone, Aztreonam, Amoxicillin-clavulanate, Azithromycin, Cefoxitin, Nalidixic acid, Levofloxacin, Nitrofurantoin, Tetracycline, Gentamicin, Ciprofloxacin and Chloramphenical at (89.9, 88.4, 84.9, 77.7, 73.4, 72.7, 71.2, 63.3, 61.2, 53.2, 51.1 and 41%) respectively. Tigecyclin, Colistin, Amikacin, Meropenem, and Imipenem were the most effective medications against these isolates that showed a high sensitivity to it at (3.6, 12.9, 25.2, 25.2 and 30.9) respectively. Out of 139 UPEC isolates, 111 ( 79.85%) isolate were multidrug resistance MDR which have at least one antimicrobial drug resistance in three or more antimicrobial categories, and 18 (12.94 %) were In all but two or fewer antimicrobial groups, XDR isolates were resistant to at least one agent in all but two or fewer antimicrobial catigories (i.e. bacterial isolates remain susceptible to only one or two categories), while 5 (2.780%) were PDR in which isolates exhibited non-susceptibility to all antimicrobial drugs in all categories [12]. Some of antibiotics assessment results in the current research were quite similar to those obtained by Manal, et al., (2018) in which the goal of their study was to investigate the frequency of integron class 2 and resistance among 301 fecal E. coli isolates from healthy individuals (1-80 years), also to determine the association of Integron class 2 with antibiotic resistance [13], as well as a study conducted by Zainab Jaber et al. [14]. There are a number of reasons why these isolates are extremely resistant to most antibiotics, but the presence of mobile genetic elements (Integrons) may be the most important [15,16].

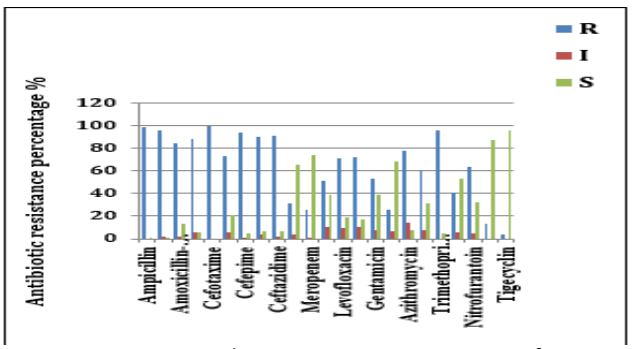


*Figure 1: Antibiotic resistance patterns of Uropathogenic E. coli*

### Result of Integron class I and its association with antimicrobial resistance

Class 1 integron was found in 87 (78.4%) of the 111 multidrug resistance isolates, with an amplification

product of 483 bp (Figure 6). The prevalence of antibiotic resistance for class I integron isolates revealed a substantial correlation between1 the existence of mobile1 genetic element ( integron) and resistance to the majority of antibiotics studied, as shown in Table 6; Ampicillin 78.4%, Piperacillin 78.4%, Amoxicillin-clavulanate 70.3%, Aztreonam 73.9%, Cefotaxime 78.4%, Cefoxitin 58.6%, Cefepime 78.4%, Ceftriaxone 75.7%, Ceftazidime 76.6%, Imipenem 34.2%, Meropenem 27.9%, Ciprofloxacin 55.9%, Levofloxacin75.7%, Nalidixic acid 72.1%, Gentamicin 53.2 %, Amikacin 27.9%, Azithromycin 76.6%, Tetracycline 59.5%, Trimethoprim- sulfamethoxazole 78.4%, Chloramphenicol 43.2% and Nitrofurantoin 64%. While very low association of Integron class I with resistance to Colistin and Tigecycline at percentage 1 % respectively. The presence of Integron class I at this frequency explains why E. coli isolates are able to have great resistance to diverse antibiotic classes. MDR is encoded by resistance genes clustered in integrons, which are potentially mobile genetic components that are thought to be involved in MDR transmission [16]. The current Integron class I results were similar to those reported by Mohammad Kargar et al. [10] who discovered Integron class I in 78.26% of clinical E. coli isolates While, Prior studies found a lower prevalence of class I integron than the current study [17,18].

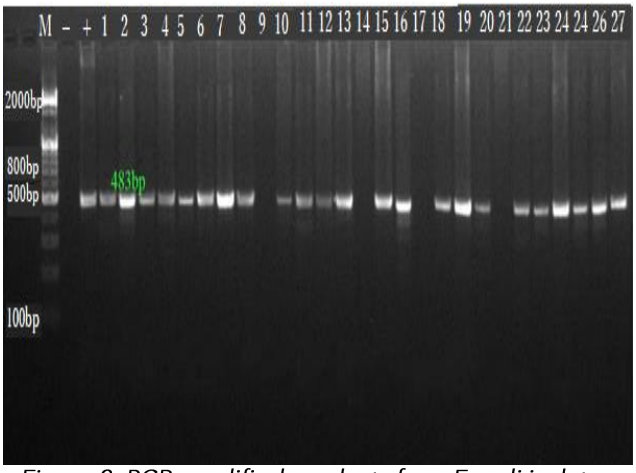


*Figure 2: PCR amplified products from E. coli isolates extracted DNA on Agarose gel, M: Marker DNA (100-2000 bp), Positive control in Lane 2 and negative control in Lane 1, Lane 1 to 8, 10 to 13, 14 and 15, 18,'19 and 20, 22 to 27 reflect positive results.*

Table 6. Distribution of antibiotics resistance in clinical *E coli* isolates, based on the existence or absence of Integron class I

| Antibiotics | Int. class I | | | | *P-value* |
|---|---|---|---|---|---|
| | Present | | Absent | | |
| | No. | % | No. | % | |
| Ampicillin | 87 | 8.4 | 4 | 1.6 | <0.0001** |
| Piperacillin | 87 | 8.4 | 4 | 21.6 | <0.0001** |
| Amoxicillin-clavulanate | 78 | 70.3 | 12 | 10.8 | <0.0001** |
| Aztreonam | 82 | 73.9 | 19 | 17.1 | <0.0001** |
| Cefotaxime | 87 | 78.4 | 24 | 21.6 | <0.0001** |
| Cefoxitin | 65 | 58.6 | 11 | 9.9 | <0.0001** |
| Cefepime | 87 | 78.4 | 22 | 19.8 | <0.0001** |
| Ceftriaxone | 84 | 75.7 | 22 | 19.8 | <0.0001** |
| Ceftazidime | 85 | 76.6 | 20 | 18 | <0.0001** |
| Imipenem | 38 | 34.2 | 5 | 4.5 | <0.0001** |
| Meropenem | 31 | 27.9 | 4 | 3.6 | <0.0001** |
| Ciprofloxacin | 62 | 55.9 | 9 | 8.1 | <0.0001** |
| Levofloxacin | 84 | 75.7 | 15 | 13.5 | <0.0001** |
| Nalidixic acid | 80 | 72.1 | 21 | 18.9 | <0.0001** |
| Gentamicin | 59 | 53.2 | 15 | 13.5 | <0.0001** |
| Amikacin | 31 | 27.9 | 4 | 3.6 | <0.0001** |
| Azithromycin | 85 | 76.6 | 23 | 20.7 | <0.0001** |
| Tetracycline | 66 | 59.5 | 19 | 17.1 | <0.0001** |
| Tigecyclin | 4 | 3.6 | 1 | 0.9 | $0.18^{NS}$ |
| Trimethopri sulfamethoxazole | 87 | 78.4 | 20 | 18 | <0.0001** |
| Chloramphenicol | 48 | 43.2 | 9 | 8.1 | <0.0001** |
| Nitrofurantoin | 71 | 64 | 17 | 15.3 | <0.0001** |
| Colistin | 17 | 15.3 | 1 | 0.9 | 0.0001** |

Chi-square (2) goodness of fit is used to present the data. NS Non-significant. ** Significant at P < 0.01; * significant at P < 0.05.

## the minimal inhibitory concentration's outcome

The minimum inhibitory concentration of only cefotaxime and Trimethoprim- sulfamethoxazole because it is the most resistant antibiotics by the bacteria under study, and because it belongs to different groups of antibiotics was determined for Pan drug resistance Uropathogenic E coli isolates as these isolates were resistance to all tested antibiotics. the MICs of cefotaxime against clinical isolates of PDR E. coli were as followed: isolate number 1 and 4 was 2048 µg/100 µl while isolate number 2,3 and 5 was 4096 µg/100 µl. As for the values of the MICs for Trimethoprim-sulfamethoxazole, they were as follows 6.25\31.125

µg/100 µl for isolate number 2, 12.5\ 62.25 µg/100 µl for isolate number 1 and 4 and 25\125 µg/100 µl for number 3 and 5 isolates as showen in figure 2 and 3 respectively. In order to determine Integron class I gene expression and how it relates to this antibiotic resistance, the minimum inhibitory concentrations of these antibiotics must be exposed to certain resistant E. coli isolates under these antibiotics' pressure.

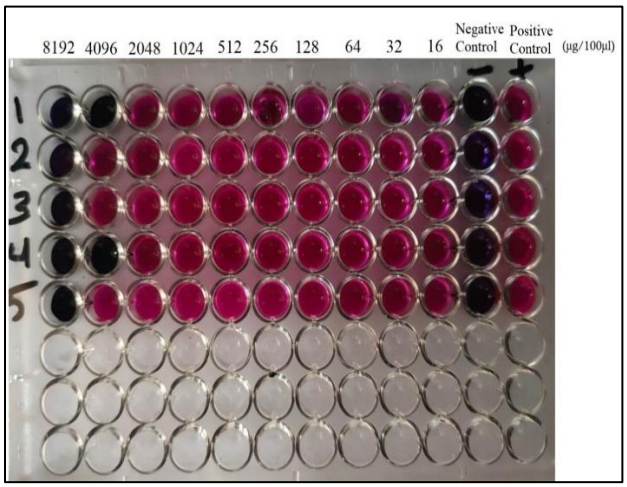


*Figure 2: Microdilution plate indicates the MICs values of Cefotaxime against Uropathogenic E. coli isolates.*

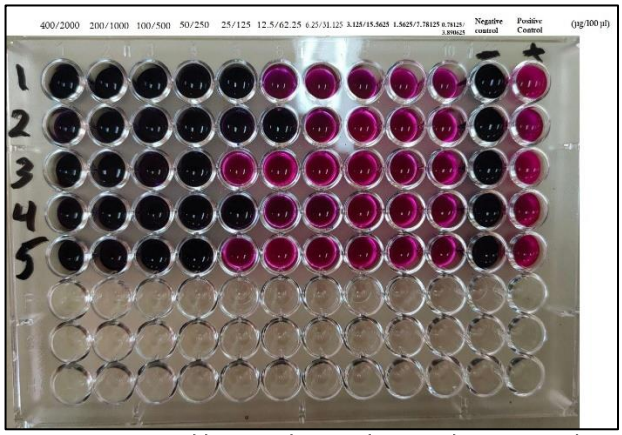


*Figure 3: Microdilution plate indicates the MICs values of Trimethoprim- sulfamethoxazole against Uropathogenic E. coli isolates.*

## Results of gene expression of Integron class I under antibiotics pressure

SYBR green was used in real-time quantitative PCR to assess the Integron class I gene expression. Gene amplification was measured using the cycle threshold (CT) parameter, where lower CT values indicate more expression of the gene and higher CT values indicate lower expression. The Integron class I gene's relative mean of gene expression before exposure to cefotaxime and Trimethoprim-sulfamethoxazole were 0.999 and 0.872 respectively while the relative mean of gene expression of INTI 1 gene after exposure to cefotaxime and Trimethoprim- sulfamethoxazole were 3.286 and 2.542 respectively as shown in figure 4 and 5. The phenomenon of MDR is a significant healthcare issue in pathogenic bacteria like Escherichia coli, where it is connected to higher mortality and morbidity [19], [20]. The rapid evolution of resistance among various bacteria to a number of unrelated antibiotics has been largely attributed to the ease with which resistance genes can be spread, despite the fact that utilizing antibiotics obviously plays a significant influence in the selection of bacterial resistance. Antibiotic resistance in bacteria spreads by a variety of methods, which is a complicated process. Mutations or the transmission of resistance genes found on mobile DNA elements like integrons are two ways that susceptible bacteria can develop resistance [21], [22]. Integrons are genetic components that help Gram-negative bacteria spread antibiotic resistance [23]. The stimulation of the SOS response by sub-MIC fluoroquinolones or B-lactams has previously been linked to the promotion of antibiotic resistance in E. coli through the integration and/or rearrangement of gene cassettes by class 1 integrons integrase. In current study the results showed increase in gene expression of Integron class I gene when the bacterial isolates undergo stress condition of Sub-MIC of Cefotaxime and Trimethoprim- sulfamethoxazole thus explain that resistance of UPEC isolates to these antibiotics was closely related to the presence of mobile genetic element Integron class I.

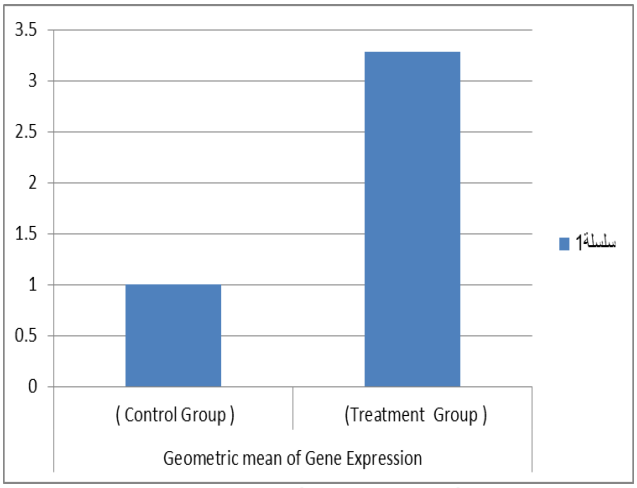


*Figure 3: The degree of expression of INTI 1 gene before and after exposure to sub-MIC of cefotaxime antibiotic*

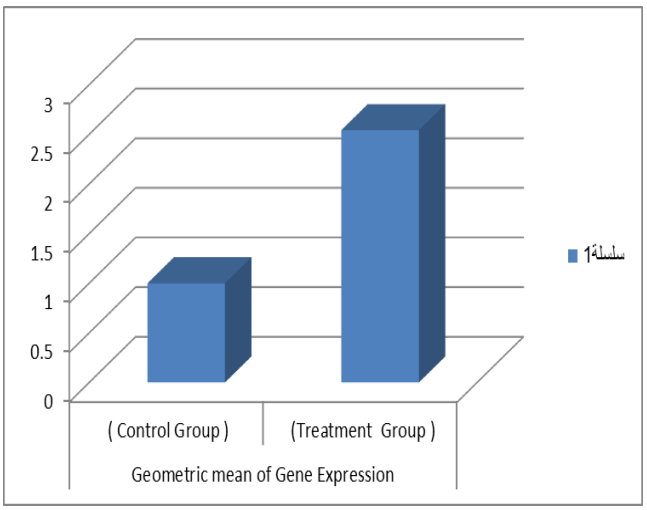


*Figure 4: The degree of expression of INTI 1 gene before and after exposure to sub-MIC of Trimethoprim-sulfamethoxazole antibiotic*

## 5. Conclusion

It was concluded that multiple drug resistance is very common in local isolates of uropathogenic E. coli and in most cases integron class I gene was also present which can be utilized as a marker for the detection of MDR isolates.

## References

[1] Organization W. H., Antimicrobial resistance: global report on surveillance. World Health Organization, 2014.
[2] Roca et al., I. "The global threat of antimicrobial resistance: science for intervention," New Microbes New Infect, vol. 6, pp. 22–29, 2015.
[3] Liu X.-J., Lyu Y., Li Y., Xue F., and Liu J., "Trends in antimicrobial resistance against enterobacteriaceae strains isolated from blood: A 10-year epidemiological study in mainland China (2004–2014)," Chin Med J (Engl), vol. 130, no. 17, pp. 2050–2055, 2016.
[4] Shaikh S., Fatima J., Shakil S., Rizvi S. M. D., and Kamal M. A., "Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment," Saudi J Biol Sci, vol. 22, no. 1, pp. 90–101, 2015.
[5] Friedrich T. et al., "High-throughput microarray technology in diagnostics of enterobacteria based on genome-wide probe selection and regression analysis," BMC Genomics, vol. 11, no. 1, pp. 1–22, 2010.
[6] Clinical Laboratory Standard Institute, "Performance Standard for Antimicrobial Susceptibility testing," M100-S30 ed., M100-S30, 2020.
[7] Grape M., Farra A., Kronvall G., and Sundström L., "Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant Gram-negative bacteria," Clinical microbiology and infection, vol. 11, no. 3, pp. 185–192, 2005.
[8] Carattoli A., "Importance of integrons in the diffusion of resistance," Vet Res, vol. 32, no. 3–4, pp. 243–259, 2001.
[9] Rowe-Magnus D. A., J. Davies, and D. Mazel, "Impact of integrons and transposons on the evolution of resistance and virulence," Curr Top Microbiol Immunol, vol. 264, pp. 167–188, 2002.
[10] Kargar M., Mohammadalipour Z., Doosti A., Lorzadeh S., and Japoni-Nejad A., "High prevalence of class 1 to 3 integrons among multidrug-resistant diarrheagenic Escherichia coli in southwest of Iran," Osong Public Health Res Perspect, vol. 5, no. 4, pp. 193–198, 2014.
[11] Lu Y., J.- Fang Q., Tian L., and Jin H., Advanced medical statistics, vol. 5. World Scientific, 2015.
[12] Magiorakos A.-P. et al., "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance," Clinical microbiology and infection, vol. 18, no. 3, pp. 268–281, 2012.
[13] Mobley H. L. T., "Measuring Escherichia coli gene expression during human urinary tract infections." MDPI, 2016.
[14] Jaber Hadi Z. et al., "Prevalence of Integrons and Antibiotic Resistance among Escherichia coli isolated from patient suspected with urinary tract infection from Al-Najaf hospitals," Systematic Reviews in Pharmacy, vol. 11, no. 7, pp. 258–264, 2020, doi: 10.13140/RG.2.2.31854.84805.
[15] Fasugba O., Gardner A., Mitchell B. G., and Mnatzaganian G., "Ciprofloxacin resistance in community-and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies," BMC Infect Dis, vol. 15, no. 1, pp. 1–16, 2015.
[16] Fluit A. C. and Schmitz F.-J., "Resistance integrons and super-integrons," Clinical microbiology and infection, vol. 10, no. 4, pp. 272–288, 2004.
[17] Japoni A. et al., "Assay for integrons and pattern of antibiotic resistance in clinical Escherichia coli strains by PCR-RFLP in Southern Iran," Jpn J Infect Dis, vol. 61, no. 1, p. 85, 2008.
[18] Rezaee M. A., Sheikhalizadeh V., and Hasani A., "Detection of integrons among multi-drug resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran," Brazilian Journal of Microbiology, vol. 42, pp. 1308–1313, 2011.
[19] Rezaee M. A., Langarizadeh N., and M. Aghazadeh, "First report of class 1 and class 2 integrons in multidrug-resistant Klebsiella pneumoniae isolates from northwest Iran," Jpn J Infect Dis, vol. 65, no. 3, pp. 256–259, 2012.
[20] Phongpaichit S., Wuttananupan K., and Samasanti W., "Class 1 integrons and multidrug resistance among Escherichia coli isolates from human stools," Southeast Asian journal of tropical medicine and public health, vol. 39, no. 2, p. 279, 2008.
[21] Barlow R. S., Pemberton J. M., Desmarchelier P. M., and Gobius K. S., "Isolation and characterization of integron-containing bacteria without antibiotic selection," Antimicrob Agents Chemother, vol. 48, no. 3, pp. 838–842, 2004.
[22] Normark B. H. and Normark S., "Evolution and spread of antibiotic resistance," J Intern Med, vol. 252, no. 2, pp. 91–106, 2002.
[23] Sütterlin S., Bray J. E., Maiden M. C. J., and Tano E., "Distribution of class 1 integrons in historic and contemporary collections of human pathogenic Escherichia coli," PLoS One, vol. 15, no. 6, p. e0233315, 2020