Molecular Profile of Aminoglycoside, Fluoroquinolone, and Class 1 Integron Genes among Gentamicin-Resistant *Escherichia coli* in Najaf City, Iraq

Nabil Salim Saaid Tuwaij*
Faculty of Science, University of Kufa, Iraq

**Article History:**
Received on: 26 Mar 2020
Revised on: 27 Apr 2020
Accepted on: 28 Apr 2020

**Keywords:**
Aminoglycoside, Fluoroquinolone, Class 1 Integron, Gentamicin-Resistant *Escherichia coli*, UTIs

**ABSTRACT**
*Escherichia coli* has a major cause of women urinary tract infection, which it harbours various kinds of drug resistance-associated genes. So, the current study examined the prevalence and frequency of genes. These genes are responsible for the resistance of aminoglycoside and fluoroquinolone drugs in uropathogenic gentamicin-resistant *E. coli* isolated from urinary tract infections among women. Six hundred urine specimens were tested. The data revealed 348 (58%) and 70 (11.66%) had gram-positive and gram-negative, respectively. The other 182 (30.33%) were found without any growth. A total of 600 clinical specimens were 167(27.833%) identified as *E.coli* isolate according to biochemical tests and Vitek-2 System. The phenotypic gentamicin-resistant screening (MIC and disk diffusion) revealed out of 167(27.833%) *E.coli* isolates were 25(4.166%) gentamicin-resistance. Antibacterial agents susceptibility of 25 gentamicin-resistant *E.coli* isolates showed concern level of resistance among different categories of antibacterial agents, ranged from high resistance 25(100%) for nalidixic acid to less rate of resistance 4/25(16%) by imipenem drug. Molecular data have demonstrated the prevalence of associated resistance genes for both aminoglycosides and fluoroquinolones. Among 25 gentamicin-resistant *E.coli* isolates 24/25(96%) were harbours for the genes gyr-B, aac(6’)-Ib-cr, strA/B, and 23/25(92%) of isolates were harbouring for the genes gyrA, qnrS, and aacC-2. In contrast, qnr-B, aac(6’)/aph(2’), and aph(3)Ila were identified in 20/25(80%), 11/25(44%) and 8/25(32%) respectively. At the same respect, aacC-1, qnrA, and qnrC genes were no detect in the current study. However, 24/25(96%) of isolates were carrying the class 1 integron (intel-1) gene.

**INTRODUCTION**
Urinary tract infection (UTIs) is one of the infectious diseases that have a high frequency and prevalence among different groups of society. Uropathogenic *Escherichia coli* have a significant role in most hospital-acquired infections as well as it is the principal cause of various clinical specimens, especially urine (Abad *et al.*, 2019).

Antimicrobial resistance has been a cause of concern and alarm over the period time worldwide, especially in developed and developing countries (Rather *et al.*, 2017). Aminoglycosides drugs are significant antimicrobial agents for the treating
of several infections in humans. At the same respect, gentamicin has shown marked as perfect curative options for UTIs treatment (Goodlet et al., 2018).

Quinolones drugs are broadly applied to heal UTIs generated via E. coli. This expanded application of quinolones becomes led to enhanced resistance in E. coli. Target alteration and modifications in the permeability of the cell membrane can give quinolone resistance. Moreover, plasmid-mediated quinolones genes such as qnrA, qnrB, and qnrS can promote resistance to these drugs (Düzgün et al., 2019).

Recently, traditional drug resistances and increase of multi-drug resistance (MDR) organisms in UTIs are related to higher averages of unsuitable empirical medication due to weakening drug covering. E. coli, like other gram-negative bacteria, the aminoglycosides resistance usually are mediated mainly through enzyme production; it changes or modifies antibiotics either by adenylation, acetylation or phosphorylation and maybe by efflux pump mechanism. (Reygaert, 2018).

MATERIALS AND METHODS

Specimens collection

The contemporary study was associated with 600 non-duplicate women patients undergoing UTIs of both Medical Al-Sader City and Al-Hakim General Hospitals as well as leading clinical laboratories in Najaf City, from January to June of 2019. A clean catching midstream urine specimens collected in a sterile urine container and carried directly to the Advance Medical Bacteria Laboratory, Department of Biology, Faculty of Science, University of Kufa, Iraq.

All specimens were cultured and streaking on different media included blood agar (Oxoid, England), MacConkey agar (Oxoid, England), chromagar orientation (CHROMagar™, France) till reached a single colony. The petri-dish were incubated under aerobic conditions overnight at 37°C. Moreover, a negative growth incubated for two days. The present investigation depended on specimens that concentration bacteria at least 10^5 colony-forming unit (cfu) per ml. Isolates of E.coli were diagnosis based on the characters of microscopic, the colour of Chromagar Orientation media, IMViC tests, motility and oxidase tests (MacFaddin, 2000). Vitek-2 system (bioMérieux France) used to confirm the diagnosis.

Screening of gentamicin-resistant E.coli isolates using minimal inhibitory concentration (MIC) strip and disk diffusion

Phenotypically, all isolates of uropathogenic E.coli procedure were investigated and screened. These were to detect gentamicin-resistant E.coli. For detecting these E.coli isolates, both gentamicin MIC strip (Liofilchem®, MTS, Italy), covering of 0.016-266 μg/mL and gentamicin disk (10μg) (Bioanalyse, Turkey) were used. It was employed on sterile media of Mueller Hinton agar (England) The suspension of all tested isolates were achieved based on 0.5 McFarland standard. The MIC is recorded at the point where the edge of the inhibition ellipse touches with the Strip further the results were described via the instructions guide of the Clinical Laboratory Standards Institute (CLSI, 2018). At the same time, all these achieved at the same moment beside the procedure of disk diffusion; furthermore, all plates were incubated at the same conditions. The strain of E.coli ATCC 25922 employed as the negative control.

Antibacterial Agents susceptibility

The present study covered testing susceptibility profile of various commercial classes of antibacterial agents (Bioanalyse, Turkey) against all 25 isolates of gentamicin-resistant E.coli according to Kirby-Bauer procedure using sterile media of Mueller Hinton agar (Oxoid, England), (Bauer et al., 1966). The resistance, intermediate and sensitive of isolates were expressed according to guide instructions of the clinical and Laboratory Standards Institute (CLSI, 2018). Disks of antibacterial agents and their concentration marked in the Table 5.

Total DNA extraction

The entire genomic DNA of 25 isolates of gentamicin-resistant E.coli was extracted after 24 hours of liquid growth for these pathogens using a kit of the total genomic DNA extraction (iNtRoN, Biotech. Inc., Korea), wherever the extraction was completed according to the instructions of manufacture company. The DNA was saved below -20°C situation employing deep freezing apparatus, till executed PCR to the investigation of gyr-A, gyr-B, aac(6')-Ib-cr, qnrA, qnrB, qnrC, qnrS, aacC-1, aacC-2, strA/B, aac(6')/aph(2'), aph(3)Ila and Int-1 genes by specific primers and requirements listed in Table 1 and Table 2. The system of gel document (Cleaver, United Kindom), applied to examine and separate the migration of PCR products using 1% agarose (iNtRoN, Biotech. Inc., Korea), after staining the gel with 0.5 μg/ml ethidium bromide.

RESULTS

Specimens collection and E.coli identification

The present study was involved 600 no duplicate urine specimens taken from woman patients suffering from UTI, who attended to main hospitals and...
clinical laboratories in Najaf city-Iraq, through the period the time five months (from January to June of 2019). Results in Table 3 were demonstrated that the percentage of the bacterial growth was 348 (58%) and 70(11.66%) to both Gram-negative and gram-positive bacteria, while no bacterial growth was 182(30.33%).

According to microscopic features, culture growth on MacConkey Agar, Chromagar Orientation, biochemical tests and finally all suspected *E. coli* isolates were confirmed using the Vitek-2 system, the data showed the number and percentage of this pathogen reached to 167(27. 833%) isolates.

![Figure 1: PCR result of StrA/B gene of 25 gentamicin-resistant *E.coli* isolates](image1)

![Figure 2: PCR result of aacC-2 gene of 25 gentamicin-resistant *E.coli* isolates](image2)

![Figure 3: PCR result of aph(6)-(2) gene of 25 gentamicin-resistant *E.coli* isolates](image3)

Phenotypic detection of gentamicin-resistant *E.coli* isolates according to disc diffusion method and MIC strip

According to the results of phenotypic gentamicin susceptibility using gentamicin disk and MIC strip revealed thoroughly 167(27. 833%) isolates from

![Figure 4: PCR result of aph(3)lla gene of 25 gentamicin-resistant *E.coli* isolates](image4)

![Figure 5: PCR result of aac(6')-Ib-crgene of 25 gentamicin-resistant *E.coli* isolates](image5)

![Figure 6: PCR result of gyr-B gene of 25 gentamicin-resistant *E.coli* isolates](image6)

![Figure 7: PCR result of gyr-A gene of 25 gentamicin-resistant *E.coli* isolates](image7)

![Figure 8: PCR result of qnrS gene of 25 gentamicin-resistant *E.coli* isolates](image8)
Table 1: Oligonucleotides of primer used in present work

| Gene   | Name          | Sequence (5’ to 3’)                        | Product size (bp) | Reference               |
|--------|---------------|--------------------------------------------|-------------------|-------------------------|
| gyrA   | gyrA-F        | ATGGGCTGAAATTACCTCAATC                     | 398               | (Sierra et al., 2002)   |
|        | gyrA-R        | GTGTGATTTTAGCTACGACGC                     |                   |                         |
| gyrB   | gyrB-F        | CAAACTGGCGGACTGCGAGG                      | 345               | (Ling et al., 2003)     |
|        | gyrB-R        | TCCGGGCATCTGACGAGATAA                     |                   |                         |
| aac(6’)-Ib-cr | aac(6’)-Ib-cr-F | TTCCGGATGCTCTATGAGTAGGCTA               | 482               | (Kim et al., 2009)     |
|         | aac(6’)-Ib-cr-R | CTCGAATGCTGAGGGTGTTT               |                   |                         |
| qnr A  | qnr -F        | GATAAAGTTTTTCAGCAAGAGG                    | 593               | (Jacoby et al., 2003)   |
|         | qnr -R        | ATCCAGATCCGCAAAGGTGA                     |                   |                         |
| qnr B  | qnr B-F       | ATGACGCCATTACTGTGTTAA                    | 560               | (Jacoby et al., 2006)   |
|         | qnr B-R       | GATCGCAATGTGTAAGGTGA                     |                   |                         |
| qnr C  | qnr C-F       | GGTGTTGATCATTTATGGAATC                   | 447               | (Wang et al., 2009)     |
|         | qnr C-R       | TCCACTTTACGAGGTCT                        |                   |                         |
| qnr S  | qnrS-F        | ATGGAACCTACAATCATAC                      | 492               | (Afzal et al., 2013)    |
|         | qnrS-R        | AAAAACACCTCCAGCCTAA                      |                   |                         |
| aacC-1 | aacC-1-F      | ATGGGCTCATTTCCGACATGTAAGG                | 873               | (Hujer et al., 2006)    |
|         | aacC-1-R      | TTAGGTGGCTGTTACTGTTGGGT                 |                   |                         |
| aacC-2 | aacC-2-F      | ATGCAACGCGCGGAGCAATAAC                   | 861               | (Hujer et al., 2006)    |
|         | aacC-2-R      | CTAACCCGAAAGGCTCGCAAG                    |                   |                         |
| strA/B | strA-F        | ATGGTGACACCTAAAACCTCT                    | 893               | (Duran et al., 2012)    |
|         | strA-R        | CGTCTAGGATCGAGCAACAG                     |                   |                         |
| aac(6’)/aph(2’) | aac(6’)/aph(2’)-F | GAAGTACGCGAGAGAGA                  | 491               | (Duran et al., 2012)    |
|         | aac(6’)/aph(2’)-R | ACATGGCAAGCTCTAGGA                   |                   |                         |
| aph(3)lla | aph(3)lla-F   | GAACAAGATGGATTGCACGC                    | 510               | (Jaja et al., 2019)     |
|         | aph(3)lla-R   | GCTCTACGATGACATGAG                     |                   |                         |
| Intl-1 | Int1-F        | CAGTGGACATAAGCCTGTTG                   | 160               | (Xicohtencatl-Cortes et al., 2019) |

Figure 9: PCR result of qnrB gene of 25 gentamicin-resistant E.coli isolates

Figure 10: PCR result of Intl-1 gene of 25 gentamicin-resistant E.coli isolates

Uropathogen E.coli were 25(4.166%) gentamicin-resistant for both disk gentamicin disc at 10μg concentration and MIC strip with concentration beyond the breakpoint values depending on CLSI (2018). At the same respect, other E.coli isolates which gentamicin-sensitive were excluded from the current study (Table 4).

Antibacterial agents susceptibility of gentamicin-resistant E.coli isolates

Depending on the results of 20 antibacterial agents susceptibility in the Table 5, most gentamicin-resistant E.coli isolates were high resistance against
Table 2: Conditions of PCR which used in the present study

| PCR gene         | Initial denaturation | Temperature (°c) / Time | Cycling condition | Final extension | Cycle number |
|------------------|----------------------|-------------------------|-------------------|----------------|--------------|
|                  | Denaturation         | Annealing               | Extension         |                |              |
| gyra             | 94 °C/5 min          | 50 °C/45 sec            | 72 °C/1 min       | 72 °C/5 min    | 35           |
| gyrb             | 94 °C/5 min          | 62 °C/1 min             | 72 °C/2 min       | 72 °C/5 min    | 35           |
| aac(6’)-Ib-cr    | 94 °C/4 min          | 55 °C/45 sec            | 72 °C/1 min       | 72 °C/5 min    | 35           |
| qnrA             | 94 °C/5 min          | 57 °C/40 sec            | 72 °C/1 min       | 72 °C/7 min    | 35           |
| qnrB             | 94 °C/5 min          | 53 °C/45 sec            | 72 °C/1 min       | 72 °C/5 min    | 35           |
| qnrC             | 94 °C/5 min          | 50 °C/40 sec            | 72 °C/40 sec      | 72 °C/5 min    | 35           |
| qnrS             | 94 °C/5 min          | 55 °C/40 sec            | 72 °C/40 sec      | 72 °C/5 min    | 35           |
| aacC-1           | 94 °C/5 min          | 55 °C/1 min             | 72 °C/2 min       | 72 °C/5 min    | 35           |
| aacC-2           | 94 °C/5 min          | 55 °C/1 min             | 72 °C/2 min       | 72 °C/5 min    | 35           |
| strA/B           | 94 °C/10 min         | 54 °C/1 min             | 72 °C/1 min       | 72 °C/10 min   | 35           |
| aac(6′)/aph(2′)  | 94 °C/5 min          | 54 °C/1 min             | 72 °C/1 min       | 72 °C/7 min    | 35           |
| aph(3)lla        | 94 °C/5 min          | 50 °C/30 sec            | 72 °C/1.5 min     | 72 °C/5 min    | 30           |
| Intel-1          | 94 °C/5 min          | 55 °C/40 sec            | 72 °C/35 sec      | 72 °C/5 min    | 35           |

Table 3: Number and percentage of growth and no growth among 600 urine specimens

| Status       | Number (%) |
|--------------|------------|
| Gram-negative| 348 (58%)  |
| Gram-positive| 70 (11.66%)|
| No growth    | 182 (30.33%)|
| Total        | 600 (100%)  |

Table 4: Phenotypic detection of gentamicin-resistant E. coli isolates according to disc diffusion method and MIC strip

| Number (%) of total specimens | Number (%) of E. coli | Number (%) of gentamicin-resistant E. coli | Number (%) of gentamicin-sensitive E. coli |
|-------------------------------|-----------------------|------------------------------------------|------------------------------------------|
| 600(100%)                     | 167(27.833%)          | 25(4.166%)                               | 142(23.666%)                             |
Table 5: Antibiogram profile of gentamicin-resistant *E.coli* isolates

| Antibacterial agent          | Symbol (µg) | Resistance | Intermediate | Sensitive |
|-----------------------------|------------|------------|--------------|-----------|
| Ampicillin                  | AM (10)    | 24(96%)    | 0(0%)        | 1(4%)     |
| Cefoxitin                   | FOX(30)    | 22(88%)    | 1(4%)        | 2(8%)     |
| Ceftriaxone                 | CRO(30)    | 23(92%)    | 1(4%)        | 1(4%)     |
| Aztreonam                   | ATM(30)    | 20(80%)    | 0(0%)        | 5(20%)    |
| Imipenem                    | IPM(10)    | 4(16%)     | 0(0%)        | 21(84%)   |
| Amikacin                    | AK(10)     | 24(96%)    | 0(0%)        | 1(4%)     |
| Netilmicin                  | NET(10)    | 17(68%)    | 2(8%)        | 6(24%)    |
| Tobramycin                  | TOB(10)    | 23(92%)    | 1(4%)        | 1(4%)     |
| Streptomycin                | S (10)     | 24(96%)    | 0(0%)        | 1(4%)     |
| Ofloxacin                   | OFX(10)    | 10(40%)    | 0(0%)        | 15(60%)   |
| Norfloxacin                 | NOR (10)   | 10(40%)    | 1(4%)        | 14(56%)   |
| Nalidixic acid              | NA(30)     | 25(100%)   | 0(0%)        | 0(0%)     |
| Ciprofloxacin               | CIP (10)   | 17(68%)    | 0(0%)        | 8(32%)    |
| Levofoxacin                 | LEV (5)    | 9(36%)     | 2(8%)        | 14(56%)   |
| Chloramphenicol             | C (30)     | 20(80%)    | 1(4%)        | 4(16%)    |
| Nitrofurantion              | F"(300)   | 23(92%)    | 0(0%)        | 2(8%)     |
| Trimethoprin                | TMP(5)     | 24(96%)    | 0(0%)        | 1(4%)     |
| Trimethoprin/ Sulphamethoxazole | SXT(25) | 21(84%)    | 2(8%)        | 2(8%)     |
| Tetracycline                | TE(30)     | 23(92%)    | 1(4%)        | 1(4%)     |
| Doxycycline                 | DO(30)     | 19(76%)    | 0(0%)        | 6(24%)    |

most various antibiotics categories used in the current study. At same respect, data of antibacterial agents susceptibility showed that uropathogenic gentamicin-resistant *E.coli* isolates regarded as multidrug resistance (MDR) through all isolates were resisted to three classes of antibacterial agents and above. However, these pathogens revealed a high effect on beta-lactam drugs (except imipenem) where resistance rates were 24(96%), 23(92%), 22(88%) and 20(80%) to Ampicillin, ceftriaxone, cefoxitin and aztreonam respectively. While imipenem drug was the best in inhibition the bacterial growth, the resistance rate was 4(16%) and this lowest rate among all antibacterial agents which used in current work.

Although all 25 *E.coli* isolates were resistant to gentamicin, the data in the Table 5 proved different resistance rates among aminoglycosides drugs. They were high resistance reached to 24(96%) in both amikacin and streptomycin, 23(92%) in tobramycin, while they were low resistance reached to 10(40%) in both ofloxacin and norfloxacin. At the same time, moderate resistance to netilmicin reached to 17(68%).

Results of resistance in fluoroquinolones drugs, *E.coli* were more effect by nalidixic acid 25(100%), while the resistance by levofoxacin was 9(36%) compared with ciprofloxacin 17(68%). Also, 25 gentamicin-resistant *E.coli* isolates were proved high resistance rates among other antimicrobial agents including chloramphenicol, nitrofurantoin, Trimethoprin, Trimethoprin/Sulphamethoxazole, Tetracycline and Doxycycline reached to 20(80%), 23(92%), 24(96%), 21(84%), 23(92%) and 19(76%) respectively.

Molecular assay

Detection of aminoglycoside, fluoroquinolones and Integron class 1 genes

One of the principal aim of this work was to detect the frequency of some aminoglycoside genes, using specific primers. The results of PCR revealed accurate positive bands at variable numbers, for strA/B, aacC-2, aac(6′)/aph(2′) and aph(3)Ila genes they were 24/25(96%), 23/25(92%), 11(44%) and 8(32%) respectively. There was no found aacC-1 gene in this work (Figure 1, Figure 2, Figure 3 and Figure 4).

This study also focused on investigation and distribution of genes which were responsible for the resistance of both quinolones and fluoroquinolones drugs among 25 isolates of gentamicin-resistant *E.coli* which isolated from the urine of non-duplicate women. The data in Figure 5and Figure 6 revealed...
that 24/25(96%) were harbours for the genes gyr-B, and aac(6')-Ib-cr, as well as 23/25(92%) of isolates, were harbours for the genes gyr-A, and qnrS, (Figure 7 and Figure 8), while qnrB, was identified in 20/25(80%), (Figure 9). At the same respect, qnrA and qnrC genes were not detect in the current study. However, 24/25(96%) of isolates were carrying the intel-1 gene (Figure 10).

**DISCUSSION**

**Specimens collection and E.coli identification**

UTIs are the common persistent infection in women usually generated by bacteria. The current study appeared E. coli isolates remained the most prevalent species isolated from UTIs. Data of this study was following the findings of numerous articles from various sections of the world (Kulkarni et al., 2017).

Domestically, several studies have been conducted on the bacterial causes of urinary tract infections, all of which showed that the frequency of E. coli is the highest between gram-negative and positive bacteria. These results were near to a study carries out in Iraq (Erbil) found the rate of E.coli isolates was 44.6 % and it is highest amongst other bacteria causes which isolated from a pregnant woman suffering from UTIs (Mohammad et al., 2018).

**Antibacterial agents susceptibility of gentamicin-resistant E.coli isolates**

MDR E. coli becomes expanded in the recent years reasonably because of the increasing and wrong application of antimicrobials agents(Kulkarni et al., 2017). The susceptibility results are shown in Table 5 revealed high resistance to E. coli isolates for different groups of antibiotics, especially those drugs used in UTIs (aminoglycosides, Fluoroquinolones, and sulfonamides drugs), which constitute a source of reduced human health, especially the elderly or those with weak immunity. Therefore, a realistic plan and solutions must be put in place to limit its spread. However, the results of this study are closely related and somewhat compatible with previous local studies (Almamoori et al., 2019). The study also showed the sensitivity of bacteria to imipenem. Despite its high efficacy, it is used only in critical illness cases which reduces the exposure of bacteria to this antagonist and thus reduces the chance of a mutation.

**Molecular assay**

**Detection of aminoglycoside, fluoroquinolones and Integron class 1 genes**

Aminoglycoside resistance is expanding year after year. It is significant and dangerous rate; this is so, not because of their capability to create chronic diseases but also because they are capable of building resistance to conventional drugs. globally and locally, Found an abundance of articles about resistance to aminoglycosides in E.coli isolates. (Chaudhary and Payasi, 2014; Fasugba et al., 2015; Tawfeeq et al., 2017). Previous articles have done through Ho et al. (2010) observes the appearance of aacC-2 gene were a rate of 84.1% and 75.5% from E.coli isolated from human and animal, respectively. A further article by Dias-Goncalves et al. (2015) manifested about 80% of the gentamicin-resistant E. coli isolates were carried out the aacC-2 gene. However, this gene was found and detected in a study that done in Iraq on E.coli isolates and other Enterobacteriaceae (Tawfeeq et al., 2017).

Sunde and Norstrom (2005) conducted the study and found the strA/B gene was high frequency among streptomycin-resistant E.coli strain. Locally, several reports achieved in Najaf City, Iraq that indicated the frequency of strA/B gene among gram-negative isolated from clinical specimens Locally, many reports delivered in Najaf City, Iraq stated the frequency of aacC-2 gene among gram-negative isolated from clinical and environmental samples (Hayder and Aljanaby, 2019; Tuwaij et al., 2019).

The aac(6')/aph(2') and aph(3)lla genes give a high resistance to most agents of aminoglycosides drugs (Chow et al., 2001; Woegerbauer et al., 2015). Results of the current study appeared 11/25(44%) and 8/25(32%) of gentamicin-resistant E. coli isolates were carried out the aac(6')/aph(2') and aph(3)lla genes respectively. The rate 8/25(32%) of aph(3)lla gene was lower from a study achieved in the same City (Najaf, Iraq) found the frequency of aph(3)lla gene among E. coli isolates reach to 90% (Almamoori et al., 2019).

The horizontal transfer of genes by genetic elements like integron or plasmid among microorganisms or chromosomal mutation possesses an essential role in the acquisition of new genes to contribute drug resistance (Düzgün et al., 2019). This may be one of the reasons that explain the vast spreading of fluoroquinolones genes among E. coli isolates in the current study.

An earlier article by Abbasi and Ranjbar (2018), data of PCR revealed that among 100 clinical uropathogenic E.coli isolates obtained 0%, 25% and 36% for qnrA, qnrB, and qnrS respectively. A study achieved by Ranjbar et al. (2018) in Iran found a high frequency of qnrS 92/95 (96.84%) among clinical quinolone-resistant E.coli isolates. Data of current work about the qnrA gene similar to previ-
ous articles, which showed none of this contained qnrA gene (Vaz-Moreira et al., 2016; Conte et al., 2017). However, Among 25 gentamicin-resistant E.coli isolates 24/25(96%) were harbours for the genes aac(6')-Ib-cr, and this result was congruence with previous local article done by Almamoori et al. (2019) showed that 98.3% of clinical uropathogenic E.coli isolates contain aac(6')-Ib-cr gene. The primary goal for destroying quinolones drug in isolates of E coli usually by produce DNA gyrase that constituted from two subunits encoding through gyrA and gyrB (Jaktaji and Mohiti, 2010). The results of PCR of current work revealed a high rate of gyrA and gyrB among gentamicin-resistance E.coli isolates, however, these data trend to accordance with earlier surveillance (Bhatnagar and Wong, 2019; Hassan et al, 2019).

Integrons can be found inside plasmid or transposons and transport along with them, as well as it promotes the diffusion of antibacterial resistance genes among microorganisms causing dangerous public health effects (Khoramrooz et al, 2016). A high prevalence of intl1 in current study may be due to all isolates were clinical and isolated from the patient. A study achieved by Oliveira-Pinto et al. (2017) pointed that molecular examination of integrase gene (intl-1) exhibited a higher frequency of class 1 integrons in isolates of uropathogenic E coli reach to 65 % compared with 11.9 % in commensal isolates. However, numerous researches are examining the predominance of integrons in uropathogenic E. coli isolates have recorded a notable relationship between antibacterial resistance and integrin. (Khoramrooz et al, 2016).

CONCLUSION

The current research proved that gentamicin-resistant E.coli isolated from women infected with UTIs was a significant rate, as well as these isolates, were resistant to multiple commercial antimicrobials agents. In the same respect, imipenem drug was an effect on bacterial growth. A high frequency of aminoglycoside and fluoroquinolone genes is a concern in the country.

REFERENCES

Abassi, H., Ranjbar, R. 2018. The prevalence of quinolone resistance genes of A, B, S in Escherichia coli strains isolated from three major hospitals in Tehran. Iran. Cent. European J Urol, 71(1):129–133. Afzal, A., Sarwar, Y., Ali, A., Maqbool, A., Salman, M., Habeeb, M. A., Haque, A. 2013. Molecular evaluation of drug resistance in clinical isolates of Salmonella enterica serovar Typhi from Pakistan. The Journal of Infection in Developing Countries, 7(12):929–940.

Almamoori, K. O., Hadi, Z. J., Almohana, A. M. 2019. Molecular Investigation of Aminoglycoside Modifying Enzyme among Aminoglycoside-Resistant Uropathogenic Escherichia Coli Isolates from Najaf Hospitals, Iraq. Indian Journal of Public Health Research & Development, 10(10):2298–2303.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology, 45(4):493–496.

Bhatnagar, K., Wong, A. 2019. The mutational landscape of quinolone resistance in Escherichia coli. PLOS ONE, 14(11):e0224650–e0224650.

Chaudhary, M., Payasi, A. 2014. Resistance patterns and prevalence of the aminoglycoside modifying enzymes in clinical isolates of Gram negative pathogens. Global Pharmacology Journal, 8(1):73–79.

Chow, J. W., Kak, V., You, I., Kao, S. J., Petrin, J., Clewell, D. B., Lerner, S. A., Miller, G. H., Shaw, K. J. 2001. Aminoglycoside Resistance Genes aph(2")-lb and aac(6')-Iib Detected Together in Strains of both Escherichia coli and Enterococcus faecium. Antimicrobial Agents and Chemotherapy, 45(10):2691–2694.

CLSI 2018. Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition. CLSI Supplement M100. In Clinical and Laboratory Standards Institute, volume 38 of 3, pages 1–15, Wayne, PA.

Conte, D., Palmeiro, J. K., da Silva Nogueira, K., de Lima, T. M. R., Cardoso, M. A., Pontarolo, R., Pontes, F. L. D., Dalla-Costa, L. M. 2017. Characterization of CTX-M enzymes, quinolone resistance determinants, and antimicrobial residues from hospital sewage, wastewater treatment plant, and river water. Ecotoxicology and Environmental Safety, 136:62–69.

Dias-Goncalves, V., Bohrer-Lengrub, F., Oliveira-Fonseca, B., Santos-Pereira, R. M., Melo, L. D. B. D., Gazos-Lopes, U., Ribeiro-Bello, A., Adler-Pereira, J. A. 2015. Detection and characteriza-
tion of multidrug-resistant enterobacteria bearing aminoglycoside-modifying gene in a university hospital at Rio de Janeiro, Brazil, along three decades. Biomedica, 35(1):117–124.

Duran, N., Ozer, B., Duran, G., Onlen, Y., Demir, C. 2012. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res, 135:389–396.

Düzgün, A. Ö., Okumuş, F., Saral, A., Çiçek, A. Ç., Cine, S. 2019. Determination of antibiotic resistance genes and virulence factors in Escherichia coli isolated from Turkish patients with urinary tract infection. Revista da Sociedade Brasileira de Medicina Tropical, 52(20180499–20180499).

Fasugba, O., Gardner, A., Mitchell, B. G., Mnatza, G. 2015. Ciprofloxacin resistance in community- and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. BMC Infectious Diseases, 15(1):545–545.

Goodlet, K. J., Benhalima, F. Z., Nailor, M. D. 2018. A Systematic Review of Single-Dose Aminoglycoside Therapy for Urinary Tract Infection: Is It Time To Resurrect an Old Strategy? Antimicrobial Agents and Chemotherapy, 63(1):2165–2183.

Hassan, J. S., Al-Safar, M. A., Rhman, T. R. A. 2019. The Role of DNA Gyrase (gyrA) Gene in Ciprofloxacin-Resistant Locally Isolates Pseudomonas aeruginosa in Al-Khadimiya Teaching Hospital Baghdad, Iraq. Journal of Pure and Applied Microbiology, 13(1):499–503.

Hayder, T., Aljanaby, A. A. J. 2019. Genotypic Characterization of Antimicrobial Resistance-Associated Genes in Citrobacter Freundii Isolated from Patients with Urinary Tract Infection in Al-Najaf Governorate-Iraq. Online Journal of Biological Sciences, 19(2):132–145.

Ho, P.-L., Wong, R. C., Lo, S. W., Chow, K.-H., Wong, S. S., Que, T.-L. 2010. Genetic identity of aminoglycoside-resistance genes in Escherichia coli isolates from human and animal sources. Journal of Medical Microbiology, 59(6):702–707.

Hujer, K. M., Hujer, A. M., Hulten, E. A., Bajaksouzian, S., Adams, J. M., Donskey, C. J., Ecker, D. J., Masure, C., Eshoo, M. W., Sampaith, R., Thomson, J. M., Rather, P. N., Cholit, D. W., Fishbain, J. T., Ewell, A. J., Jacobs, M. R., Paterson, D. L., Bonomo, R. A. 2006. Analysis of Antibiotic Resistance Genes in Multidrug-Resistant Acinetobacter sp. Isolates from Military and Civilian Patients Treated at the Walter Reed Army Medical Center. Antimicrobial Agents and Chemotherapy, 50(12):4114–4123.

Jacoby, G. A., Chow, N., Waite, K. B. 2003. Prevalence of Plasmid-Mediated Quinolone Resistance. Antimicrobial Agents and Chemotherapy, 47(2):559–562.

Jacoby, G. A., Walsh, K. E., Mills, D. M., Walker, V. J., Oh, H., Robicsek, A., Hooper, D. C. 2006. Qnr B, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother, 50(4):1178–1182.

Jaja, I. F., Bhemne, N. L., Green, E., Oguttu, J., Muchenje, V. 2019. Molecular characterisation of antibiotic-resistant Salmonella enterica isolates recovered from meat in South Africa. Acta Tropica, 190:129–136.

Jaktaji, R. P., Mohiti, E. 2010. Study of Mutations in the DNA gyrase gyrA Gene of Escherichia coli. Iranian Journal of Pharmaceutical Research, 9(1):43–48.

Khoramrooz, S. S., Sharifi, A., Yazdanpanah, M., Hosseini, S. A. A. M., Emaneini, M., Gharibpour, F., Parhizgari, N., Mirzaei, M., Zoladl, M., Koshravani, S. A. 2016. High Frequency of Class 1 Integrons in Escherichia coli Isolated From Patients With Urinary Tract Infections in Yasuj, Iran. Iranian Red Crescent Medical Journal, 18(1):26399–26399.

Kim, E. S., Jeong, J., Jun, J., Choi, S., Lee, S., Kim, M., Woo, J., Kim, Y. 2009. Prevalence of aac(6’)-lb-cr Encoding a Ciprofloxacin-Modifying Enzyme among Enterobacteriaceae Blood Isolates in Korea. Antimicrob Agents Chemother, 53(6):2643–2645.

Kulkarni, S., Peerpurap, B., Saini, K. 2017. Isolation and antibiotic susceptibility pattern of Escherichia coli from urinary tract infections in a tertiary care hospital of North Eastern Karnataka. Journal of Natural Science, Biology and Medicine, 8(2):176–180.

Ling, J. M., Chan, E. W., Lam, A. W., Cheng, A. F. 2003. Mutations in Topoisomerase Genes of Fluoroquinolone-Resistant Salmonellae in Hong Kong. Antimicrobial Agents and Chemotherapy, 47(11):3567–3573.

MacFaddin, J. F. 2000. Biochemical tests for identification of medical bacteria. 3rd edition. The Williams and Wilkins, Baltimore, USA.

Mohammad, K., Ahmed, Z., Mohammed, B., R. S. 2018. Determination the site of antibiotic resistance genes in Escherichia coli isolated From Urinary Tract Infection. Kurdistan Journal of Applied Research, 3(2):6–12.

Oliveira-Pinto, C., Diamantino, C., Oliveira, P. L., Reis, M. P., Costa, P. S., Paiva, M. C., Nardi, R. M. D., Magalhães, P. P., Charte-onia-Souza, E., Nascimento, A. M. A. 2017. Occurrence and characterization of class 1
integrons in Escherichia coli from healthy individuals and those with urinary infection. *Journal of Medical Microbiology*, 66(5):577–583.

Ranjbar, R., Tolon, S. S., Sami, M., Golmohammadi, R. 2018. Detection of Plasmid-Mediated qnr Genes Among the Clinical Quinolone-Resistant Escherichia coli Strains Isolated in Tehran, Iran. *The Open Microbiology Journal*, 12(1):248–253.

Rather, I. A., Kim, B.-C., Bajpai, V. K., Park, Y.-H. 2017. Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. *Saudi Journal of Biological Sciences*, 24(4):808–812.

Reygaert, W. C. 2018. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3):482–501.

Sierra, J. M., Marco, F., Ruiz, J., de Anta, M. T. J., Vila, J. 2002. Correlation between the activity of different fluoroquinolones and the presence of mechanisms of quinolone resistance in epidemiologically related and unrelated strains of methicillin-susceptible and -resistant Staphylococcus aureus. *Clinical Microbiology and Infection*, 8(12):781–790.

Sunde, M., Norstrom, M. 2005. The genetic background for streptomycin resistance in Escherichia coli influences the distribution of MICs. *Journal of Antimicrobial Chemotherapy*, 56(1):87–90.

Tawfeeq, H. M., Tofiq, A. M., Ali, K. M. 2017. Incidence and Molecular Identification of Escherichia Coli Harbouring Gentamicin Resistant Gene among Pregnant Women. *Eurasian Journal of Science and Engineering*, 3(1):117–127.

Tuwaij, S. S. N., Al-Khilkhali, G. H., Alghazaly, F. N. 2019. Genetic detection of aminoglycoside resistance genes among Klebsiella pneumoniae isolated from environmental of Al-Najaf Hospitals. *Iraq. International Journal of Advances in Science Engineering and Technology*, 7(3):55–60.

Vaz-Moreira, I., Varela, A. R., Pereira, T. V., Fochat, R. C., Manaia, C. M. 2016. Multidrug Resistance in Quinolone-Resistant Gram-Negative Bacteria Isolated from Hospital Effluent and the Municipal Wastewater Treatment Plant. *Microbial Drug Resistance*, 22(2):155–163.

Wang, M., Guo, Q., Xu, X., Wang, X., Ye, X., Wu, S., Hooper, D., Wang, M. 2009. New Plasmid-Mediated Quinolone Resistance Gene, qnrC, Found in a Clinical Isolate of Proteus mirabilis. *Antimicrob Agents Chemother*, 53(5):1892–1897.

Woegerbauer, M., Kuffner, M., Domingues, S., Nielsen, K. M. 2015. Involvement of aph (3')-IIa in the formation of mosaic aminoglycoside resistance genes in natural environments. *Frontiers in Microbiology*, 6:442–442.

Xicohtencatl-Cortes, J., Cruz-Córdova, A., Cázares-Domínguez, V., Escalona-Venegas, G., Zavala-Vega, S., Arellano-Galindo, J., Romo-Castillo, M., Hernández-Castro, R., et al. 2019. Uropathogenic Escherichia coli strains harboring tosA gene were associated to high virulence genes and a multidrug-resistant profile. *Microbial Pathogenesis*, 134:103593–103593.