

# Genotyping Diversity of *Escherichia coli* isolated from UTI in Iraqi Patients

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## Abstract

**Background** *Escherichia coli* (*E. coli*) is the predominant uropathogen isolated in acute, community-acquired uncomplicated UTIs in adults and children. **Pateints and Methods:** A total of (150) clinical samples (Urine) were collected from the patients at admission to the hospital in a sterile container. A loopful of uncentrifuged urine samples was cultured onto blood agar, MacConkey agar, HiCrome UTI Agar and EMB media, the diagnosis of *E.coli* bacteria was confirmed by using VITEK 2 system. Antimicrobial susceptibility testing of *E. coli* isolates was carried out against the antibiotics by Disc Diffusion Method ,ERIC PCR used for determined genotyping diversity. **Results:** *E.coli* showed antibiotic resistant to Garamycine (86%), Ciproflaxcin (66%), Ceftriaxone (46%) and Rifampicine (100%). The biofilm formation result showed out of 50 *E. coli* isolate (12%) were weak biofilm formation, (72%) were moderate biofilm formation and (16%) were strong biofilm formation. Genotyping by ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus) of 50 *E.coli* isolates subjected to ERIC-PCR, 47 isolates have amplications ranging from 700 to < 3000bp The generated banding profile showed highly polymorphic DNA fragments in these 47 isolates **Conclusion** : : *E.coli* resistance to Garamycine (86%), Ciproflaxcin (66%), Ceftriaxone (46%) and Rifampicine (100%). The biofilm formation result 50 *E. coli* isolate (12%) were weak biofilm formation, (72%) were moderate biofilm formation. Genotyping by ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus) of *E.coli* isolates subjected to ERIC-PCR, 47 isolates have amplications ranging from 700 to < 3000bp The generated banding profile was highly polymorphic DNA fragments in these isolates.

**Key Words:** *E.coli* ,UTI , ERIC PCR, Biofilm, genotype

## Introduction

*E. coli* is a gram negative bacilli of the Enterobacteriaceae family, and it is commonly found in the intestines of warm-blooded animals. Most strains of *E. coli* are harmless, but some strains cause food poisoning, diarrhea, and urinary tract infections . Many bacteria can cause infection in the urinary tract. In fact, 80 to 90% of urinary tract infections in the world are caused by *E. coli* Extraintestinal pathogenic strains of *E. coli* that cause urinary tract infections in humans are known as uropathogenic *E.coli*<sup>1</sup>. Urinary tract infections (UTI) are the most common bacterial infections which are characterized by bacterial colonization of the urinary system and often occur in women of all age groups. The strains of uropathogenic *E. coli* are the most common pathogens isolated from UTI patients and often originate from the intestinal flora. The most common UTI symptom is dysuria which may be moderate or severe . In addition to common symptoms of the lower UTI, patients with the

upper UTI or pyelonephritis may experience flank pain, fever, or nausea and vomiting . *E. coli* is responsible for 80–90% of UTIs; the remaining UTIs are caused by *Staphylococcus saprophyticus*, *Citrobacter*, *Klebsiella*, and *Enterobacter*<sup>2</sup>. In various human diseases induced by *E. coli*, pathogenic *E. coli* is generally divided into two main categories: diarrheagenic *E. coli* and extraintestinal pathogenic *E. coli* which have two major pathotypes, uropathogenic *E. coli* and neonatal meningitis *E. coli* Extraintestinal bacteria can colonize different sites and cause various infections outside the gastrointestinal tract, with the urinary tract being most commonly affected<sup>3</sup>. Knowledge of the epidemiology of microbial populations is important in the field of medical microbiology. It is possible to identify infection reservoirs, examine the prevalence of hospitalization infections, and identify the type of microbial pathogenic agents via molecular typing<sup>1</sup>. Molecular typing can be used to identify nosocomial infections and infection

reservoirs and also to isolate specific genotypes in conjugation with a specific bacterium. Besides, it gives us more knowledge about the principles of epidemiology, evolution, and spread of many bacterial diseases<sup>4</sup>. With the development of molecular biology techniques, We believe that Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) methods could resolve the genotypes of the large number of O antigen serotypes, thereby enabling identification of an appropriate immunogenic strain. These researchers determined the sequence distribution and copy numbers on chromosomes for ERICs and showed interspecies specificity. This technique can be used in strain typing of ETECs based on their unique numbers and sizes of pattern<sup>5</sup>.

### Patients and Methods

A total of (150) clinical samples (Urine) were collected from the patients at admission to the hospital in a sterile container. A loopful of uncentrifuged urine samples was cultured onto blood agar, MacConkey agar, HiCrome UTI Agar and EMB media, the diagnosis of *E.coli* bacteria was confirmed by using VITEK 2 system (Biomérieux, France). Disc Diffusion Method (Kirby-Bauer Method) used to determine AB sensitivity test<sup>6</sup>. Biofilm assay using (microtiter plate method)<sup>7</sup>. The oligonucleotide primers supplied by Integrated DNA Technologies Company (IDT) / USA PCR using ERIC primers ERIC-IR CATTAGGGGTCCTCGAATGTA ERIC-2 AGTAAGTGACTGGGGTGAGCG was used to identify strains<sup>8</sup>. The PCR tubes were transferred to the

thermal cycler to start the amplification reaction according to specific program for the gene. PCR amplification programs was Initial Denaturation 94°C 3 min, Denaturation 94°C 1 min 40, Annealing 55°C 1 min, Extension 72°C 5 min, Final Extension 72°C 10 min.

### Finding

#### Distribution of *E. coli* Isolates According to Gender:

According to the gender, the incidence of *E. coli* isolates among patients with UTI showed a remarkable difference in percentage of *E. coli* isolation. It was noticed that the percentage of *E. coli* isolation was high in females (90%) in comparison with males (10%), as shown in table (1). This result is agree with many studies as well as with local study by Basima<sup>9</sup> in Baghdad City, who recorded that *E. coli* was isolated with a percentage of (81.8%) from female with UTI and (18.2%) from male. This higher frequency of UTI caused by *E. coli* in females than males is due to several causes, such as the shortness of the female urethra and close proximity of the perianal region of the urethral meatus to the vagina and anus and alternations in vaginal microflora that play a critical role in encouraging vaginal colonization with coliforms which may lead to UTI<sup>10</sup>. On the other side, this study disagree with Tawfiq<sup>11</sup>, that showed the bacterial cultivation had revealed positive results in (54.5%) of males and (45.5%) in females<sup>12</sup>. The UTI in female is influenced by several risk factors such as; marital status, number of child, operation history, delivery methods and other factors<sup>13</sup>.

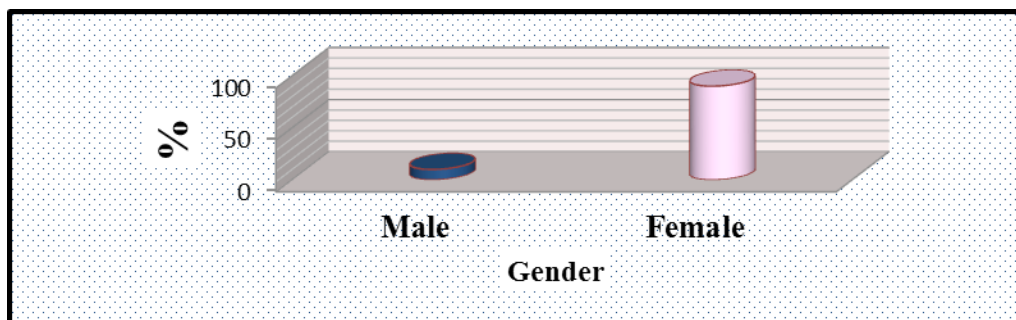


Figure (1): The isolation of *Escherichia coli* according to the gender.

#### Distribution of clinical states with *Escherichia coli* according to age groups:

The clinical samples of present study were recovered from patients their age ranged from 17 to 64 years.

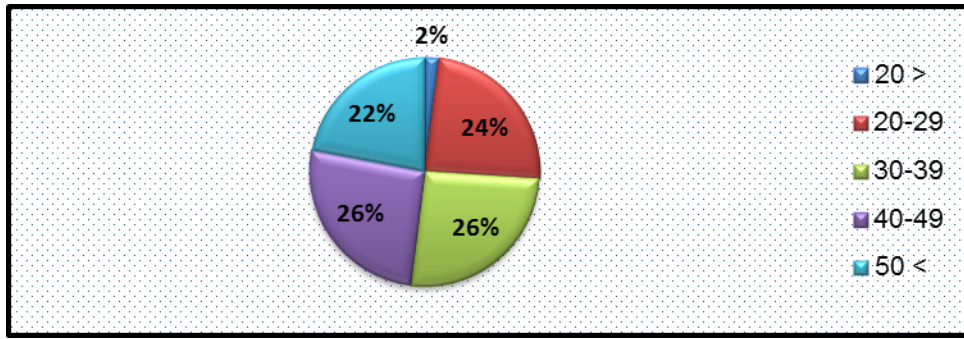


Figure (2): The isolation of *Escherichia coli* according to the age group.

The study recorded more than half of the isolation rate in UTI state was among the age (30-39) years and (40-49) years with (26%), while other local study have reported (30.51%) with age (>35 years) with UTI caused by *E. coli*<sup>13</sup>. And other study in Nigeria have reported (22.4%) with age (>45) years with UTI caused by *E. coli*<sup>14</sup>. Other study in Ethiopia have reported (20.6%) with age (36-45) years with UTI caused by *E. coli*<sup>15</sup>.

**Disc Diffusion Test (DDT):**

Antimicrobial susceptibility tests were done for all *E. coli* isolates (50 isolates) by using the disk diffusion method (Kirby-Bauer Method) against 4 different clinically important antimicrobials (Ceftriaxone, Ciprofloxacin, Gentamicin, Rifampine ) (Appendix III). These isolates showed different susceptibility toward antimicrobials used in this study. The susceptibility to different antimicrobials was determined depending on<sup>16</sup>.

The result illustrated in Figure (3) show a high resistance level of *E. coli* isolates to most of the antimicrobials. The present study showed a highest resistance to Rifampine (100%) and Garamycine (86%). Other findings revealed that *E. coli* isolates had resistance to Ciproflaxcine (66%) and The lower percentage of resistance was recorded to Ceftraxone (46%). All isolated *E. coli* show resistance to one or more antimicrobial agents.

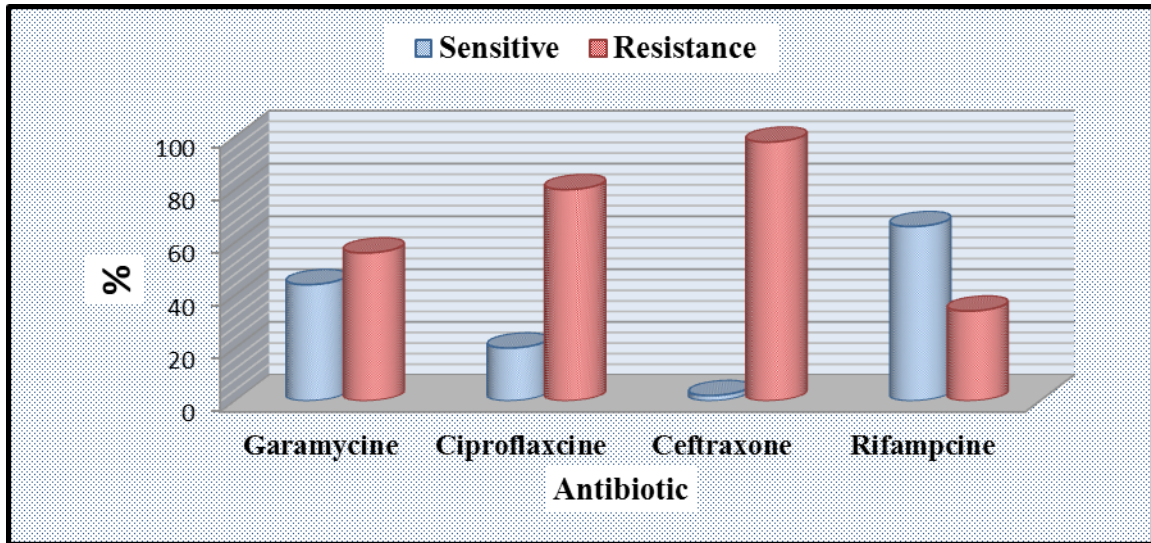


Figure (3): Percentage of resistant *E. coli* isolates against some antibiotics.

Rifampine is the first “broad spectrum” Rifampine are components in the first-line treatment of bacterial infections, As with many antibiotics, the clinical utility of these therapeutics has declined due to the increased incidence of antibiotic resistant bacterial pathogens, Resistance to the rifamycin family of antibiotics commonly occurs in clinical isolates as a result of point mutations in the antibiotic’s target<sup>17</sup>. The study findings showed that all *E. coli* isolates were resistant to Rifampine with percentage (100%).The resistance level of *E. coli* against gentamicin was obtained in this study (86%). while the result of present study is lower than the result obtained by<sup>12</sup>.

in Ethiopia have reported that resistance was (67.7%). And other study in Mexico have reported (50%) done by<sup>17</sup>. Another study obtained by <sup>18</sup>. in Mongolia, who found that resistance was (44%).The study showed resistance to Ciproflaxcine with percentage (66%) other study in Ethiopia have reported (57.7%) done by <sup>10</sup>. while the result of present study is lower than the result obtained by <sup>19</sup>. in Mongolia, who found that resistance was (42%).The ciprofloxacin are an important class of antibiotics prized for their large spectrum of activity and ease of use in oral versus parental forms, but emerging resistance to these antibiotics is limiting their usefulness <sup>20</sup>. Ceftriaxone is a third-generation cephalosporin class used to treat infections caused by Gram negative bacilli especially *E. coli* <sup>21</sup>. The low resistance level of *E. coli* against Ceftraxone was obtained in this study (46%) other local study in Zakho have reported (52%) done by <sup>22</sup>.

**Biofilm-forming ability of 50 *E. coli* isolate**

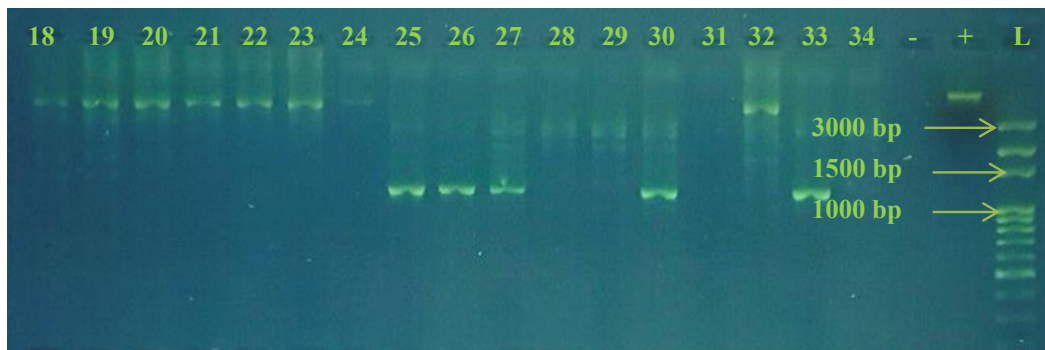
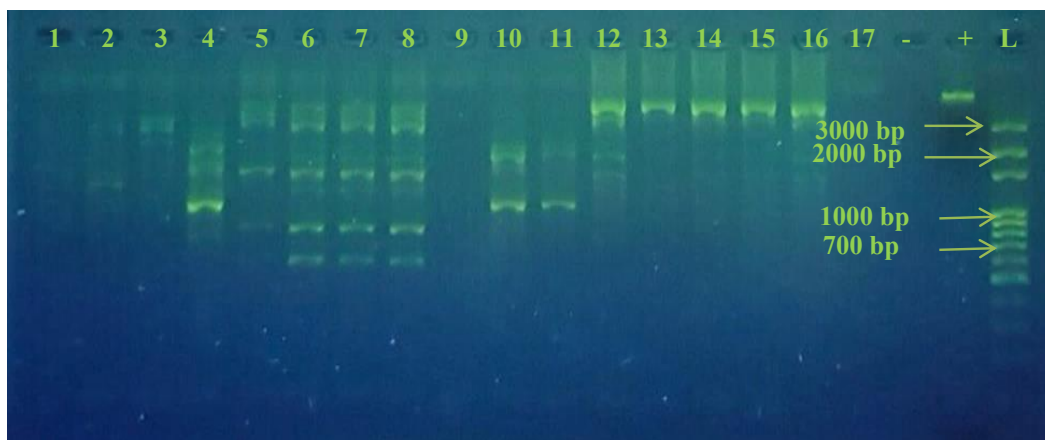
Biofilm formation has been described as an important virulence factor in various pathogenic bacteria causing human UTI.A total 50 *E. coli* isolated from UTI were divided into three groups : weak, moderate, strong biofilm formation(figure 4).The result showed out of 50 *E. coli* isolate (12%) were weak biofilm formation, (72%) were moderate biofilm formation and (16%) were strong biofilm formation, This study disagree with <sup>23</sup>. in Iran who found the biofilm formation was seen in 100% of isolates, of which (48.6%) were strong, (11.4%) were moderate .

**Table (1): Biofilm Formation results using plate method depending on OD Value.**

Biofilm -forming ability	Mean (%)	Percentage%
weak	0.0985	(12%)
Moderate	0.1623	(72%)
strong	0.3017	(16%)

<0.120 Weak biofilm producer ; 0.120-0.240 Moderate biofilm producer; >0.240 Strong biofilm producer

**Genotyping by ERIC-PCR of *Escherichia coli* isolate from UTI cases:**



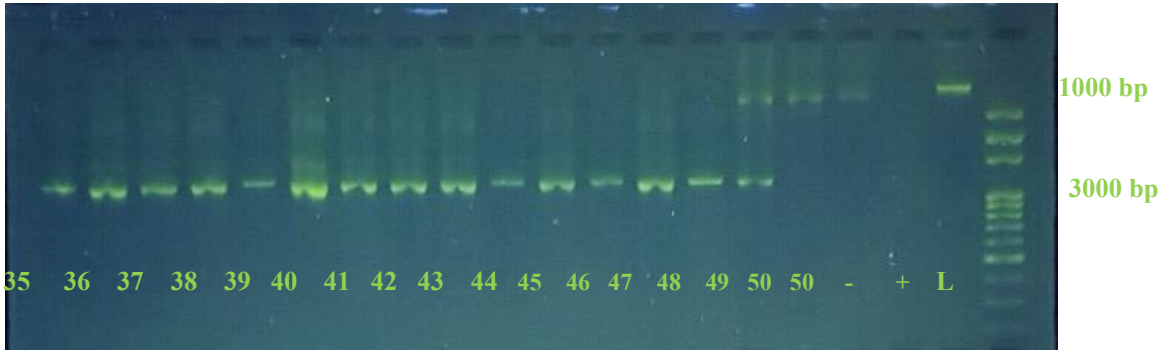


Figure (4): Analysis of ERIC genotyping of *E. coli* isolates on agarose gel (1%) at 5 V/cm for 1 hour. Lane(L): 3000bp DNA Marker. A - Lane(+,-):control isolates from the present study isolates. Lane (1-17) showing ERIC-PCR fingerprint of *E.coli* isolates; B- Lane (18-34) showing ERIC-PCR fingerprint of *E.coli* isolates; C- Lane(L): 3000bp DNA Marker. Lane(+,-):control isolates from the present study isolates. Lane (35-50) showing ERIC-PCR fingerprint of *E.coli* isolates.

Of 50 *E.coli* isolates subjected to ERIC-PCR, 47 isolates have amplications ranging from 700 to < 3000bp (figure 4). The generated banding profile showed highly polymorphic DNA fragments in these 47 isolates (4, 12, 13, 14, 15, 16,17).ERIC-PCR was formerly reported in clinical isolates of *E. coli* in India by <sup>24</sup>,and by have been recorded in <sup>1</sup>. ERIC was also recorded in other type of *E.coli* such as enterotoxigenic *Escherichia coli* (ETEC) <sup>5</sup>.

**Phylogenetic Analysis:**

In molecular typing phylogenetic tree, there were 100% matching in several strains as showing in figure

(5), such as (9&16) who both showed moderate biofilm production, while in antibiotic strain number 9 was moderate to Ciproflaxcine, while strain 16 was resistant to all antibiotics.Strain number (43&49) also where identical, strain 43 showed strong biofilm production while 49 showed weak biofilm production, strain 43 was resistant to all antibiotics and strain 49 was moderate to Ciproflaxcine and Ceftraxone.Strain number (40&47) both where moderate biofilm production, strain 40 was resistant to all antibiotics, while 47 was moderate to Ciproflaxcine and Ceftraxone.Strain number (41&44) both where moderate biofilm production, and both were resistant to all antibiotics.

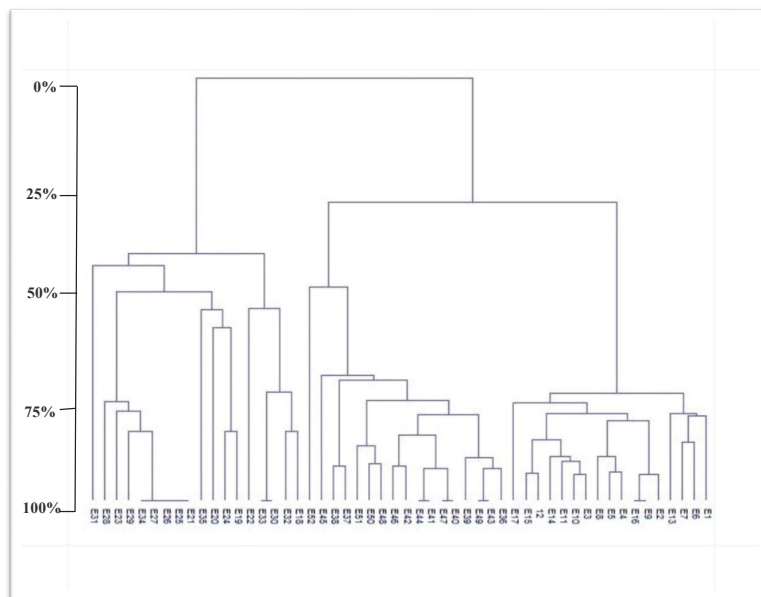


Figure (5): Neighbor-joining trees based on ERICgene molecular typing .

## Conclusion

1- *E. coli* isolation was high in females than with males and more than half of the isolation rate in UTI state was among the age (30-39) years and (40-49) years.

2- High resistance level of *E. coli* isolates to most of the antimicrobials and highest resistance to Rifampicine and Garamycine .

3- *E. coli* isolate (12%) were weak biofilm formation, and (72%) were moderate biofilm formation and Of 50 *E.coli* isolates subjected to ERIC-PCR, 47 isolates have amplications ranging from 700 to < 3000bp .

4- In molecular typing phylogenetic tree, there were 100% matching in several strains , such as (9&16) who both showed moderate biofilm production, while in antibiotic strain number 9 was moderate to Ciproflaxcine, while strain 16 was resistant to all antibiotics. Strain number (43&49) also where identical, strain 43 showed strong biofilm production while 49 showed weak biofilm production, strain 43 was resistant to all antibiotics and strain 49 was moderate to Ciproflaxcine and Ceftraxone..

**Conflict of Interest:** Non

**Source of Findings:** Self findings.

**Ethical Clearance:** Non

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