Research Article

Detection and Analysis of Resistance Genes in *Escherichia coli* Bacteria Isolated from Children in Baghdad

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Urinary tract infections are common diseases and *Escherichia coli* is the most common cause of this disease, especially in infants and children. The 200 samples were collected for the period from July to December 2020 from the Central Children’s Hospital in Baghdad from infants and children and cultured on MacConkey and blood agar for primary isolation of *E. coli* bacteria. The VITEK 2 system was used for biochemical tests and final identification. Antibiotic susceptibility for 16 antibiotics was determined using the VITEK 2 system, as well as the ability for biofilm formation using the tissue culture plate method, while DNA was isolated for molecular antibiotic resistance genes *tet*A, *tet*B, CITM, *sul*1, *sul*2, and *sul*3 using the standard polymerase chain reaction. Results showed that 40/62 isolates (64%) belonged to *E. coli* bacteria (32 females and 8 males), and isolates were of varying resistance to antibiotics, with the highest resistance to ampicillin and sulfamethoxazole and the lowest resistance to tigecycline and most MDR. The percentage of biofilm formation ability was 62.5%. The ratio of resistance genes CITM, tetA, tetB, sul1, and sul2 for *E. coli* bacteria was 5%, 45%, 12.5%, 35%, and 40%, respectively, while the sul3 gene did not appear in isolation from any clinical local isolation. So, *E. coli* isolates isolated from children showed a high extent of antibiotic resistance as the genetic sequencing of resistance genes showed that there were many mutations with a type of transmission or silence or transition. In conclusion, *E. coli* isolated from children was highly resistant to antibiotics and had the ability to form biofilms as well as the presence of a resistance gene that could be transmitted among bacteria and spread antibiotic resistance.

1. Introduction

*Escherichia coli* is the most common cause of urinary tract inflammation; approximately 90% of urinary injuries can reach the bloodstream, cause blood septicemia, and develop injury to include the bladder and kidneys, as they possess virulence factors that facilitate adhesion and cell invasion [1]. In recent times, resistance to antibiotics has increased in *E. coli* bacteria by using various mechanisms, including limiting the absorption of antibodies, changing the location of the target of the antibodies, and disrupting the action of the antibodies or flow pumps. These mechanisms may be present on the bacterial chromosome and occur naturally in all strains or be acquired by plasmid [2]. The current study is aimed at isolating and diagnosing *E. coli* bacteria from hospitalized children under 12 years of age and identifying resistance to antibiotics in addition to genetic detection of resistance genes.

2. Materials and Methods

2.1. Sampling Collection. In the current study, 200 urine samples of Iraqi children under the age of 12 were collected under appropriate medical supervision for the period from July to December 2020 at the Central Children’s Hospital in Baghdad. Inclusion criteria: all children less than 12 years of age that suffered from urinary tract infections in hospitals. Exclusion criteria: patients treated with antibiotics for UTIs for less than 1
month. All samples are collected in the morning, depending on midstream specimens, and sent immediately to the laboratory for culture.

Primary cultured MacConkey and blood agar for initial diagnosis and confirmatory diagnosis of *E. coli* isolates were done using the VITEK 2 system. A sterile swab was used to transport bacterial colonies and transfer them to a sterile tube containing 3 milliliters of normal saline. The turbidity was adjusted to 0.5 McFarland turbidity range and measured using a spectrophotometer, DensiChek™ Plus. The bacterial suspension was used to inoculate the VITEK 2 system (bio-Mérieux, France). Interpretation of results was performed according to VITEK 2 compact system special software to identify bacterial species and strains.

2.2. Antibiotic Susceptibility Test. Susceptibility to the following antimicrobial agents was identified after the diagnosis of *E. coli* bacteria using the VITEK® 2 AST-GN test cassette, which included ampicillin, piperacillin/tazobactam, cefazolin, ceftazidime, cefoxitin, ceftriaxone, ceftepime, amikacin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, nitrofurantoin, and trimethoprim/sulfamethoxazole. The break point has been identified for each antimicrobial used according to [3]. These antibiotics were used to screen the ideal ones for children.

2.3. Biofilm Assay for Bacteria. Overnight bacterial growth was carried out in LB broth for 24 hours at 37°C. McFarland solution was used to reduce the culture to 0.01. In order to stimulate bacterial growth, 50 μl of LB broth was added to 150 μl of tissue culture plate wells and incubated at 37°C for 24 hours. The culture was carefully removed, and the wells were cleaned twice with 250 μl distilled water. The mixture was then incubated for 10 minutes at 25°C with 250 μl of (0.2%) crystal violet. The wells were washed three times with distilled water and dried at room temperature. Finally, 200 μl of 95% ethanol was injected into the wells. At 630 nm, the optical density (OD) was measured, producing an interpretation, according to [4].

2.4. Extraction of Bacterial DNA Concentration. Extract DNA from *E. coli* bacteria using the purification Wizard genomic DNA kit. Overnight bacterial growth was grown on LB broth at 37°C for 24 h and transferred to the Eppendorf tubes and then centrifuged at 1300 rpm for 2 minutes, and the extraction was completed based on the manufacturer’s instructions.

2.5. Polymerase Chain Reaction (PCR). PCR was performed using a specific primer set for the detection of resistance genes in bacterial extracted DNA. PCR amplification and antimicrobial resistance genes were performed according as in Table 1. PCR products were electrophoresed in 1.5% agarose gel.

2.6. Gene Analysis. The product of the PCR for the Sanger sequence was sent using an ABI3730XL, automated DNA sequencer, by Macrogen Corporation-Korea. The results were received via email and analyzed using Geneious software.

3. Results

3.1. Sampling Collection and Diagnosis. Samples were collected from children of different ages under 12 years of age who were likely to develop urinary tract infection (107 females and 93 males), of which 62 (31%) were bacterial growth-positive samples on MacConkey agar. Due to the low positive rate of microbial growth, a reculture was recommended.

According to the findings of this study, *Escherichia coli* was the most common gram-negative bacteria in infected children, with a percentage of 64% (40/62) from both sexes, 80% (32/40) in female samples, and 20% (8/40) for males, followed by Proteus sp. and Klebsiella sp. as 24% (15/62) and 11% (7/62), respectively, from the Central Children’s. The proportions varied according to the child’s age. Infant infections ranged from 30% to 12/40, with older children up to 12 years old infected at a rate of 70% (28/40). The initial diagnosis was based on the appearance, and chemical tests revealed grey, nonhemolysis colonies on blood agar and lactose fermentation on MacConkey agar. Indole, catalase, and red methylation were also found in the isolates, but oxidase and citrate utilization were not. The final diagnosis was made with the VITEK 2 compact system, which is one of the most recent and accurate techniques for diagnosing bacteria in as little as 6 hours and with 99% certainty.

3.2. Antibiotic Susceptibility Test. Antibiotics were tested on 40 *E. coli* bacteria isolates for 16 antibiotics, and the results were compared (CLSI, 2019). Isolates exhibited a wide range of antibiotic resistance. Because of the greater resistance to ampicillin antibiotics (97.5%), antibiotics that target the cell wall were the most resistant by bacterial isolates under study; the ratio of *E. coli* isolates with multidrug resistance was 31/40 (77.5%) shown in Table 2.

3.3. Biofilm Assay for Bacteria. The detection of biofilm formation in 40 isolates using TCP methods revealed that the total biofilm formation was 25/40 (62.5%), as follows: 10/40 (25%) isolates were strong biofilm producers, 15/40 (37.5%) isolates were moderate biofilm producers, and 15/40 (37.5%) isolates were nonbiofilm producers.

3.4. Molecular Detection of Resistance Genes

3.4.1. Molecular Detection of the CITM Gene in *E. coli* Bacteria. The presence of the CITM gene was investigated, and the results revealed that the ratio of isolates carrying the gene was 2/40 (5%), which is the gene responsible for ampicillin resistance, shown in Figure 1.

3.4.2. Molecular Detection of tetA and tetB Genes in *E. coli* Bacteria. Percentage of isolates harboring tetA and tetB genes is 45% and 12.5%, respectively, shown in Figure 2.

3.4.3. Molecular Detection of sul Genes in *E. coli* Bacteria. The percentage of isolates containing sul1 and sul2 gene is (35%) and (40%), respectively, while none of the isolates under study carry sul3 gene, shown in Figure 3.
3.5. DNA Analysis of Resistant Genes

3.5.1. Molecular Analysis for CITM Gene. The DNA sequence of resistant genes was analyzed, and the results for the CITM gene revealed that isolate no. 39 had five mutations of transmission type in situ 9345, the nitrogen base guanine was replaced by thymine, and the amino acid isoleucine was converted into the amino acid arginine. The second replacement type mutation occurred at site 9349, where guanine was replaced by adenine as the amino acid arginine was converted into the amino acid glycine, and the third mutation occurred at site 9478, where guanine was replaced by adenine as the amino acid isoleucine was converted into the amino acid valine. In the fourth silent type mutation at site 9603, replacement type guanine has been replaced by adenine, which results in the same amino acid trypsin. The fifth mutation occurred at the replacement type's site 9604, guanine was replaced by adenine as the amino acid valine was converted into the amino acid phenylalanine. The first two mutations of a silent type occurred at site 9603 of a replacement type, guanine was replaced by adenine, giving the same amino acid trypsin, and the second mutation occurred at site 9604 of the replacement type, guanine was replaced by adenine as valine was converted into phenylalanine.

3.5.2. Molecular Analysis for tetA and tetB Genes. In isolation 7, the first two mutations replaced adenine with guanine at the 808540th location; the mutation type transition was transformed into asparagine, and the second mutation replaced

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| Genes | Sequence | PCR product (bp) | Tm | References |
|-------|----------|-----------------|----|------------|
| tetA  | F-GGTTCACTCGAAGCGATCA R-CTGTCCGACAGTTGCAATGA | 577 | 30 sec at 57°C | [5] |
| tetB  | F-CCTCAGCTTCTCAAGCGTG R-GCAACCTTGCTGATGACIT | 634 | 30 sec at 56°C |
| CITM  | F-TGTCGAGAAACTGAGGCAAA R-TTTCTCCTGACAGTGCTGCG | 462 | 30 sec at 56°C |
| sul1  | F-TGGTGACCGGTGCAGCTTC R-GCGAGGTTCGCAGGAGAAGTG | 789 | 30 sec at 63°C |
| sul2  | F-CGGCATCGTCAACATAACC R-CTCTTGCGATGAAGTCAG | 722 | 30 sec at 50°C | [6] |
| sul3  | F-CATTCTAGAAAAACAGTGATGTTCCG R-CATCTGCAGCTAACCTAGGGCTTTGA | 763 | 30 sec at 51°C |

PCR: polymerase chain reaction; Tm: annealing temperature.

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| Antibiotic class according to mode of action | Antibiotic | Percentage | S | I | R |
|---------------------------------------------|------------|------------|---|---|---|
| Inhibit cell wall synthesis                 | Ampicillin | 2.5%       | — | 97.5% |
|                                            | Piperaclid/tazobactam | 80% | 5% | 15% |
|                                            | Cefazolin | 12.5%      | 5% | 82.5% |
|                                            | Ceftazidine | 17.5% | — | 82.5% |
|                                            | Cefoxitin | 77.5%      | 7.5% | 15% |
|                                            | Ceftriaxone | 20% | — | 80% |
|                                            | Cefepime | 20%        | — | 80% |
|                                            | Ertapenem | 92.5%      | — | 7.5% |
|                                            | Imipenem | 92.5%      | — | 7.5% |
| Inhibit protein synthesis                   | Amikacin | 90%        | 2.5% | 7.5% |
|                                            | Gentamycin | 75% | 2.5% | 22.5% |
|                                            | Tetracycline | 42.5% | — | 57.5% |
| Inhibit DNA synthesis                       | Ciprofloxacine | 40% | — | 60% |
|                                            | Levofloxacine | 42.5% | — | 57.5% |
|                                            | Nitrofurantoin | 85% | — | 15% |
| Inhibit folic acid synthesis                | Trimethoprim/sulfamethoxazole | 35% | — | 65% |

S: sensitive; I: intermediate; R: resistance.
thymine with adenine at 808599. The type of replacement mutation was also a transition mutation. Isolation 32 had three mutations; the first of which was a transition mutation at site 808540 that replaced adenine with guanine. At site 808744, guanine was replaced by adenine as histidine was converted into arginine in the second substitution type mutation.

Figure 1: Gel electrophoresis for CTIM gene 462 bp in *E. coli* samples by 1.5% agarose gel containing 1 μl of ethidium bromide dye using DNA ladder 100-1500 bp at 100 V for 1 hour.

Figure 2: Gel electrophoresis of *tetA* (577 bp) and *tetB* (634 bp) genes in *E. coli* samples using 1.5% agarose gel containing 1 μl of ethidium bromide dye using DNA ladder 100-1500 bp at 100 V for 1 hour.

Figure 3: Gel electrophoresis of *sul1* (789 bp) and *sul2* (722 bp) genes in *E. coli* samples using 1.5% agarose gel containing 1 μl of ethidium bromide dye using DNA ladder 100-1500 bp at 100 V for 1 hour.
obtained at site 808744. The third substitution type mutation is also a substitution mutation at 808762, where guanine was replaced by adenine as asparagine was converted into glycine. Isolation 38 had one transition mutation at the site 808540, where adenine was replaced by guanine.

As for the tetB gene, there were four mutations of the first 7 transmission type isolation in situ 1937900 of the transmission type. Cytosine was replaced by adenine, and lysine was converted into glycine; the second mutation occurred at site 1937981, guanine was replaced by adenine as asparagine was converted into the aspartic acid; the third mutation occurred at site 1938053, adenine was replaced by cytosine as valine was converted into isoleucine; the fourth mutation occurred at site 1938060, guanine was replaced by adenine and the amino acid glutamine was converted into arginine; isolate no. 31 had a single mutation at site 1938176 replacing adenine with guanine as glutamic acid was converted into lysine.

3.5.3. Molecular Analysis for sul Genes. As for the sul1 gene, there have been three mutations of isolate no. 38. The first mutation occurred at site 131937. The cytosine nitrogen base was replaced by guanine and the amino acid glycine was converted into arginine. The second mutation occurred at site 131991. Cytosine was replaced by guanine as asparagine was converted into histidine. The third mutation occurred at site 132058. The nitrogen base of guanine was replaced by the nitrogen base of cytosine, and the amino acid threonine was converted into the amino acid arginine.

For the sul2 gene, four mutations occurred in isolate no. 40, at site 804483, silent type, where adenine was replaced by thymine and gave the same amino acid valine. The second mutation of transmission type occurred at site 804669, where the code changed entirely from GTT to CCC and thus replaced the amino acid proline with the amino acid valine. The third mutation occurred at site 804673 of a replacement type where adenine was replaced by cytosine as leucine was converted into isoleucine. The fourth mutation was a silent type, obtained at site 804746. Guanine was replaced by thymine. It gave the same amino acid threonine. Isolate no.7 had one mutation of silent type obtained at site 804746. Guanine was replaced by thymine. It gave the same amino acid threonine, shown in Table 3.

The seven isolates used in gene analysis (7, 23, 31, 32, 38, 39, 40) were multidrug resistant and possessed at least three of the resistance genes studied in the current study.

4. Discussion

Acute urinary tract infection is the most common infection in children, and an estimated 30% of infants suffer from repeated infection after 6 to 12 months of initial infection. Symptoms are different between infants and older children [7]. Children may have urinary tract infections under two years of age due to birth defects in the kidneys or in the urinary system [8]. In addition, there are causes that increase the risk of recurrence of injury, such as alkaline bladder reflux and bladder-intestinal dysfunction [9]. There are no specific symptoms at the time of injury, but the most important feature is high temperatures at less than two years of age or after two years of age, including abdominal pain and the urgent need for intermittent deterioration and abnormal excretion and odor [10, 11].

Based on the results of the VITEK 2 compact system, the cause of antibiotic resistance to *E. coli* isolated from children under the age of 12 is its production of large-spectrum beta-lactamase enzymes with a percentage of 70% (28/40). These enzymes have the ability to analyze penicillin and cephalosporins by breaking the beta-lactam ring and becoming ineffective. Resistance genes are either portable on chromosomes or on plasmids in many types of germs that lead to multiple resistance to different antibiotics [12]. The reason it is resistant to aminoglycosides is because it contains the resistance genes of this group, including *tob*, *gen*, *net*, and *ami*, inhibiting the synthesis of protein by preventing the binding of tRNA from attaching to the 30sRNA small ribosome unit.

The results of the current study were an approach to the researcher's findings [13]; in Diyala in Iraq in a study including 100 isolation from *E. coli* bacteria to detect their susceptibility to antibiotic resistance, the results show that 22% of

| Isolates  | Site   | Normal code | Change | Change in amino acid | Mutation |
|-----------|--------|-------------|--------|----------------------|----------|
| CITM (CP047012) |
| 39 | 9345 | AGA | ATA | Arg > Ile | Transition |
| 39 | 9349 | GGG | AGG | Gly > Arg | Substitution |
| 39 | 9478 | GTT | ATT | Val > Ile | Substitution |
| 23,39 | 9603 | ACG | ACA | Trp > Trp | Silent |
| 23,39 | 9604 | TTT | GTT | Phe > Val | Transition |
| tetA (CP073360) |
| 7,32,38 | 808540 | GAC | GGC | Asp > Gly | Transition |
| 7 | 808599 | TGG | AGG | Trp > Arg | Transition |
| 32 | 808744 | CGC | CAC | Arg > His | Substitution |
| 7,32,38 | 808762 | GGC | GAC | Gly > Asp | Substitution |
| tetB (CP069657) |
| 7 | 1937900 | CAA | AAA | Gln > Lys | Transition |
| 7 | 1937981 | GAT | AAT | Asp > Asn | Substitution |
| 7 | 1938053 | ATT | GTT | Ile > Val | Substitution |
| 7 | 1938060 | CGA | CAA | Arg > Gln | Substitution |
| 31 | 1938176 | AAA | GAA | Lys > Glu | Substitution |
| sul1 (CP074577) |
| 38 | 131937 | CGA | GGA | Arg > Gly | Transition |
| 38 | 131991 | CAC | GAC | His > Asp | Transition |
| 38 | 132058 | AGG | ACG | Arg > Thr | Transition |
| sul2 (CP073360) |
| 40 | 804483 | GTA | GTT | Val > Val | Silent |
| 40 | 804669 | GTT | CCC | Val > Pro | Transition |
| 40 | 804673 | ATC | CTC | Ile > Leu | Substitution |
| 7,40 | 804746 | ACG | ACT | Thr > Thr | Silent |
isolates were of multiple resistance while the resistance to ampicillin was 100%; the researcher is likely to cause resistance to the use of antibiotics for long-term treatment [14]. The reason for tetracycline resistance in _E. coli_ bacteria is the presence of flow pump efflux pumps of major facilitator super family (MFS) flow pumps that expel anti-tetracycline to the outside and do not expel minocycline, the protein that is encoded by the _tetB_ gene only for it. There are many genes responsible for the beta-lactam resistance to which ampicillin belongs since the presence of _blaTEM, blaSHV, blaOXA, blaPSE, and blaCTXM_ genes was detected in clinical isolations of _E. coli_ bacteria [15]. Based on the foregoing, our explanation of the difference in this study between the phenominal detection of the resistance of ampicillin when the results of this study show a high resistance ratio of the study. A low percentage of 5% for genetic detection may be due to the presence of another gene or a number of genes encoded to resist this antibiotic as well as other bacteria to which antibiotics are resistant.

The biofilm quantification test was found to be effective in detecting biofilm production by clinical isolates. Urinary tract infections are significantly associated with microbial biofilms that form on catheters and account for a high percentage of all nosocomial infections, as well as being the most common source of gram-negative bacteremia in hospitalized patients [16]. Biofilm formation is also regarded as a virulence determinant, as it is responsible for bacteria’s long-term persistence in the genitourinary tract [17]. In the current study, the ability for biofilm formation was 62.5%. In a 2020 Hungarian study, 250 isolates of _E. coli_ from UTI patients were screened for the ability to produce biofilm using the crystal violet tube-adherence method, and 119 (47.6%) were found to be positive for biofilm formation [18].

The total presence of both _tetA_ and _tetB_ genes together was 21/40 (57.5%), the same as the appearance of anti-tetracycline resistance in bacteria. In another study, researchers [19] investigated the spread of tetracycline resistance genes in 50 isolations of _E. coli_ bacteria from the urinary system, finding that the _tetA_ gene was 32% while the _tetB_ gene was 38%. Another study by researchers [20] involved 114 isolations of _E. coli_ bacteria isolated from going out to detect the proportion of resistance genes because the proportion of isolates carrying the _tetA_ gene (9.6%) also does not correspond to another study by researchers [21] including 65 isolations of _E. coli_ bacteria. The study is aimed at detecting the spread of resistance genes because of the proportion of isolators carrying the _tetA_ gene (95.4%). The reason for the different ratios is a difference in the number of isolates under study as well as a difference in the age of patients.

The combined presence of _sul_ genes in _E. coli_ bacteria totaled 30/40 (75%). This ratio is an approach to the appearance pattern of sulfonamide resistance at 85% resistance. Resistance of _E. coli_ resistance to antibiotics such as sulfonamide and aminoglycosides used to treat urinary system injuries that are often associated with the presence of certain plasmids, for example, sulfonamide resistance responsible for three genes ( _sul1, sul2, and sul3_ ). The _sul1_ gene is found on large conjugative plasmids unlike the _sul2_ gene usually portable on small nonconjugative plasmids while the _sul3_ gene is the lowest known to be portable on plasmids less commonly in _E. coli_ bacteria [22]. Therefore, the _sul2_ gene was more prevalent in the local isolates under study, indicating the similarities and genetic differences of local isolations than others diagnosed in other countries. A comprehensive study in Mexico by researcher [23] to detect different resistance gene limbs in 200 isolation of _E. coli_ bacteria showed _sul1_ (35.5%), respectively.

### 5. Conclusions

Female children are more likely to develop urinary tract infections than males, and isolated _E. coli_ isolates from babies have shown a high degree of antibiotic resistance. Genetic sequencing of resistance genes has also shown that there are many mutations with a type of transmission or stigma replacement.

### Data Availability

The datasets generated and/or analyzed during the current study are not publicly available.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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