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Isolation and Molecular Identification of Multidrug Resistance *Escherichia coli* Isolated from Patients with Urinary Tract Infections

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Abstract

Background Urinary tract infection (UTI) is the leading cause of bacteremia in hospitalized patients and the most prevalent nosocomial infection, making up as much as 35% of all infections in healthcare facilities. Clinical microbiology labs deal with many cases of UTI, and the most common cause of UTI is still enteric bacteria, namely *Escherichia coli* (*E. coli*).

Objective To isolate and identify *E. coli* in urine and determine their connection to antibiotic resistance.

Methods A consultant doctor from many hospitals oversaw the patients and a nurse was collected of 100 urine samples from patients suffering from UTI. Due to the large number of *E. coli* isolates (50 in total) and the use of polymerase chain reaction (PCR) technology for molecular detection of the diagnostic element 16SrRNA, the conventional techniques of bacterial identification were verified before PCR.

Results A total of 50 (50%) of the bacterial isolates were determined to be *E. coli* after culturing, biochemical, and molecular testing. *E. coli* was shown to be multidrug resistant (MDR) after an antibiotic susceptibility test. Assessment of antibiotic resistance was done on 25 isolates, and revealed that one (4%) is resistant to Nitrofurantoin, seven (28%) to Gentamicin, fifteen (60%) to Ciprofloxacin, and sixteen (64%) to Tetracycline. A total of 24 (96%) bacterial isolates are Carbenicillin-resistant; 18 (72%) of these isolates are resistant to Novobiocin, 20 (80%) to Cefotaxime, 22 (88%) to Ceftazidime, 23 (92%) to Rifampin, and 24 (96%) to Carbenicillin. Based on PCR results, all *E. coli* bacteria had the diagnostic gene 16SrRNA.

Conclusion This study found that nitrofurantoin is the antibiotic of choice for treating *E. coli* UTI, and the 16SrRNA gene is a confirmative diagnostic gene for *E. coli* infection.

Keywords *E. coli*, antibiotics, multidrug resistance, 16SrRNA

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List of abbreviations: *E. coli* = Escherichia coli, MDR = Multidrug resistance, PCR = Polymerase chain reaction, UTI = Urinary tract infection,

Introduction

The rod-shaped, motile or non-motile, aerobic or facultative anaerobic, *Escherichia coli* (*E. coli*) belongs to the Gram-negative Enterobacteriaceae family, ferments lactose, principally rhamnose and sorbitol, produces the enzyme β-glucuronidase, and its optimal growth temperature is (36-37)˚C [1,2]. Produces indole but does not consume citrate; tests positive for catalase but negative for oxidase; tests positive for methyl red but negative for the Vogase-Proskauer. Bacteremia may be caused by this kind of bacterium, which is found naturally in the

intestine of both animals and humans [3]. Diarrhea, meningitis, sepsis, and bacteremia are only a few of the numerous illnesses caused by this opportunistic bacterium. Children are particularly vulnerable to urinary tract infections (UTIs), which account for 90% of all cases globally. [4,5].

Siderophores, cytotoxic necrotizing factor, colicin, and surface structures such as flagella, capsules, and lipopolysaccharides (LPS) give these bacteria their pathogenicity and antigenic properties, causing them to produce flagellar antigen H, somatic antigen O, and capsular antigen K. Additional features that aid in adhesion to host tissues and biofilm production include fimbriae or pili [6,7].

There is a capacity of *E. coli* germs to acquire resistance to multiple drugs (MDR) [8]. A high degree of antibiotic resistance is seen in these bacteria because they contain resistance enzymes such as β-lactamases, which provide resistance to β-lactams, as well as enzymes that give resistance to aminoglycosides and quinolones. These bacteria are resistant to antibiotics like rifamycin, novobiocin, and the macrolide group because they have additional mechanisms that confer resistance, such as changing the permeability of target cells, inhibiting protein synthesis, and possessing efflux pumps [9]. Also, the use of 16S ribosomal RNA genes was one of sequence-based microbiome studies which used as molecular marker for classification the bacteria [10].

This study aimed to isolate and identify *E. coli* from the urine of patients with UTIs using culture, biochemical, and molecular methods using the diagnostic gene 16SrRNA, and to test the sensitivity for various groups of antibiotics. This study is important because *E. coli* is a medically significant bacteria that is associated with UTIs and resistant to antibiotics.

Methods

This study involved 100 urine samples collected from patients with different ages that suffered from UTIs referred from urology consultant clinic in Al Imamein Al kadhimein Medical city and excluded those who were on treatment with antibiotics for UTIs for less than 1 month. The study was approved by the Al-Imamein Al kadhimein Medical City authority.

The bacterial isolates were first diagnosed using traditional techniques, and then confirmed using molecular detection of the diagnostic gene 16S rRNA by polymerase chain reaction (PCR).

Each patient's mid-stream urine sample was collected in the morning using a specific container. As a further step, 10 ml of urine was collected and grown on several differential media (MacConkey and EMB from Himedia, india). The bacteria were diagnosed by using catalase, oxidase, O/F, IMVIC and TSI). After that, the mixture was incubated at 37°C for 24 hr. Following that, the samples were spun in a centrifuge at 2000 rpm for 5 minutes. After that, the homogenizer was used to mix the precipitate for 30 seconds. then, a drop of the precipitate was put on a glass slide and analyzed under a light microscope with a 40X magnification. If there are at least 10 white blood cells in the microscope field and 50-200 pure colonies in the dish, it is deemed a good result. the Kirby-Bauer technique was used to determine the antibiotic sensitivity of E. coli on Muller Hinton agar [11].

Molecular detection of the diagnostic gene 16S rRNA was done by PCR using 16srRNA gene specific primer sequences as shown at table (1).

After preparing 2% agarose, it was allowed to cool at 60°C. Then, 0.5 µg/ml of ethidium bromide was added, and the mixture was placed onto the taped plate. Near one edge of the gel, a comb was inserted. After the gel had hardened to an opaque consistency, the comb and tape were delicately removed. Before placing the slab horizontally in the electrophoresis tank, 0.5X Tris-Borate-Ethylenediaminetetraacetic acid buffer (TBE) buffer was produced and put into the gel tank.

Each 10 µl of DNA sample was mixed with about 5 µl of loading buffer that had been produced. The mixture was transferred to the

wells using a micropipette, and 5 µl of 100 bp DNA ladder was added to one well. Genomic DNA and PCR products were electrophoresed using a power supply set at 5 V/cm (70) for 1 hour.

Amplification was performed by initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C for 1.5 min, and 72°C for 45 s, and a final extension at 72°C for 5 min. the volumes of components of PCR are mentioned in table (2).

Table 1. The primer sequences of the gene used in this study

Gene		Primer sequence (3-5)	Result (base pair)	Reference
16SrRNA	F	CGAGTGGCGGACGGGTGAGT	727	(10)
	R	TCGACATCGTTTACGGCGTGGA		

Table 2. Volume of component of PCR

PCR component	Volume (µl)
Master Mix 2X	12.5
Forward primer	1
Reverse primer	1
Template DNA	3
Nuclease free water	7.5
Total	25

Muller Hinton agar with 5% sheep blood was recommended for antimicrobial disc diffusion susceptibility testing of *E. coli* i.e., Nitrofurantoin, Gentamicin, Ciprofloxacin, Tetracycline, Novobiocin, Cefotaxime, Ceftazidime, Rifampin and Carbenicillin, as shown in table (3).

Table 3. Antibiotics used in this study (Bioanalysis, Turkey)

Antibiotic	Concentration (µg)
Nitrofurantoin	5
Gentamicin	25
Ciprofloxacin	10
Tetracycline	10
Novobiocin	10
Cefotaxime	20
Ceftazidime	10
Rifampin	10
Carbenicillin	10

Statistical analysis

In this research, the Statistical Analysis System (SAS) version 23 (12) was used to calculate chi-square values for comparing various percentages.

Results

Prevalence of *E. coli* infection

Fifty percent of the bacterial isolates were determined to be *E. coli* after culturing (Figure 1). Biochemical test includes the catalase test demonstrated that the isolated catalase enzyme decomposed hydrogen peroxide, leading to the creation of bubbles.

The oxidase test comes out negative because it does not contain cytochrome c oxidase and cannot create the crucial β-glocuronidase enzyme.

The indole reacts with the aldehyde in the Kovacs reagent, turning the pale-yellow hue to red, indicating a positive result for isolates in the indole test. It should be mentioned that when bacteria are able to ferment glucose and create acid, the red methyl indicator changes color from yellow to red, indicating a good result for the methyl red test.

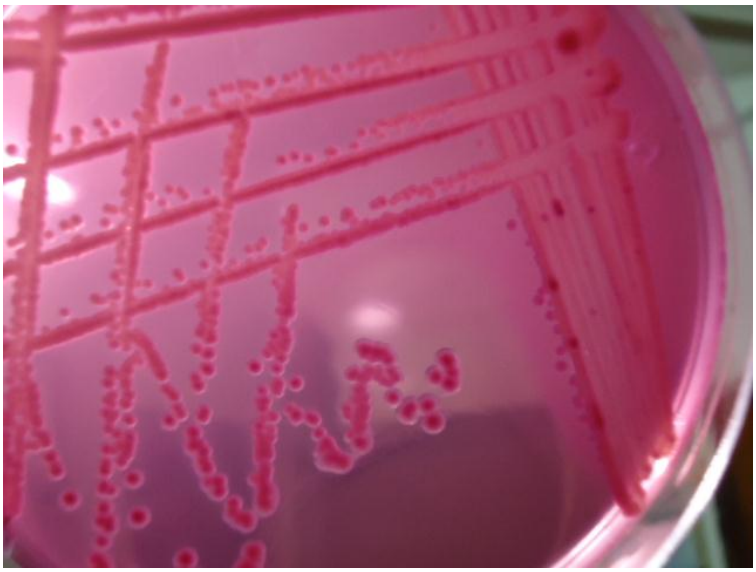

Figure 1. *E. coli* on MacConkey agar

The persistent green hue of the medium indicated that the bacteria were unable to utilize citrate as their sole carbon source. Furthermore, their growth was inhibited in the presence of potassium cyanide, and they exhibited an inability to ferment cellobiose, as illustrated in figure (2).

Molecular identification

The DNA ladder and multiplexed bundles were compared to the resultant bundles, which had a molecular weight of 727 base pairs, as shown in figure (3). The results showed that all bacterial isolates contained the *E. coli* diagnostic gene sequence, and the results were based on molecular diagnostics using PCR. The diagnostic gene 16SrRNA is known to be stable and rarely changes in bacteria over long periods of time.

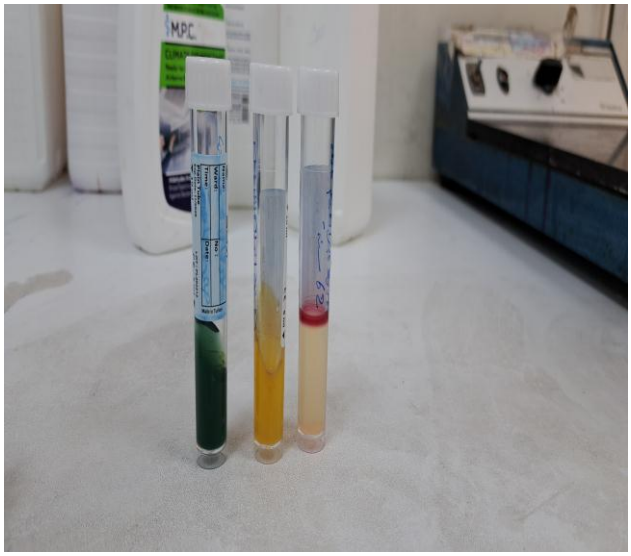

Figure 2. Results of a biochemical test

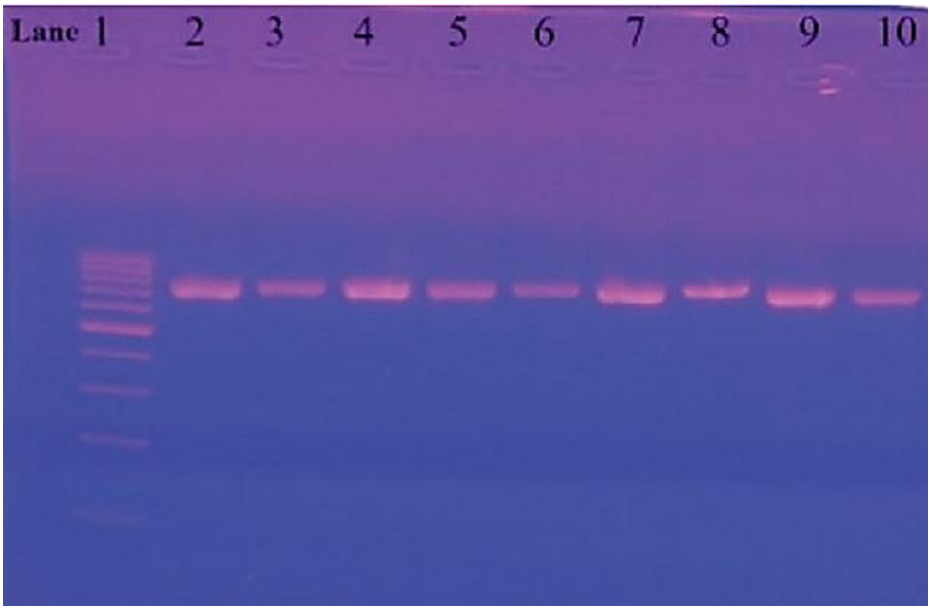

Figure 3. Electrophoresis of 16SrRNA gene (727 base pairs) of *E. coli* isolates at 1.5% agarose and a voltage of 100 V for 80 minutes, Lane 1 ladder, other lanes are positive for gene

Antimicrobial resistance

For this test, 25 isolates were chosen to assess the resistance. Although all of the bacterial isolates tested positive for MDR, as shown on Muller Hinton agar in (Figure 4), the specific antibiotics against which the bacterium showed resistance differed. A total of nine antibiotics were used to determine the sensitivity of the bacteria. As shown in table (4), one bacterial isolate (4%) is resistant to Nitrofurantoin, seven (28%) are resistant to Gentamicin, and fifteen (60%) are resistant to Ciprofloxacin. A total of twenty-four (96%) bacterial isolates are resistant to Carbenicillin, with sixteen (64%) being Tetracycline-resistant, eighteen (72%) Novobiocin resistance, 20

(80%) Cefotaxime-resistant, twenty-two (88%), Ceftazidime-resistant, twenty-three (92%) Rifampin-resistant.

Figure 4. Muller Hinton agar for sensitivity test

Table 4. Comparison of antibiotic sensitivity of *E. coli*

Antibiotic	Sensitivity %	Resistance %
Nitrofurantoin	96	4
Gentamicin	72	28
Ciprofloxacin	40	60
Tetracycline	36	64
Novobiocin	28	72
Cefotaxime	20	80
Ceftazidime	12	88
Rifampin	8	92
Carbenicillin	4	96
(P<0.01)		

Discussion

Nearly 90% of all UTI patients worldwide are infected with *E. coli*, making it the leading cause of UTIs [13]. This study's findings showing *E. coli* is a major culprit in UTIs are in line with those of several other research conducted around the world; the percentage of bacterial isolation was found to be 41.6% in a study by Özgür et al. [14] and 55.6% in a study by Ponnusamy et al. [15] in hospitals in Baghdad and the surrounding areas. The bacteria causing UTIs were *E. coli* of the general bacteria where these results corroborated those the percentage, 53.85% in Egypt by Hegazy et al. [16] and 54.7% in India by Contreras-Alvarado et al. [17]. Although the findings of this study differed from those of [18], who found a bacterial infection rate of 39.06% in their study, the discrepancy in infection rates can be explained by variations in health conditions,

geographical factors, sample size, and the use of antibiotics prior to the sample collection [15].
Another possible explanation for the high concentration of *E. coli* bacteria could be their adaptation to the urinary tract and their ability to resist the hard conditions. These bacteria also have several virulence factors that make them more likely to cause infections, such as adhesion factors, which allow them to adhere to injured surfaces, and the ability to produce toxins and capsules that break down host tissues. Bacterial migration from the anus, the bacteria's native habitat, to the urinary opening is the primary mechanism by which this bacterium causes UTIs [18].
The results of current study support those of the previous study by Lin [19], in which all bacterial isolates had the 16SrRNA gene. They also agree with the conclusions drawn by Maleki et al. [10] in Iran, where the diagnostic gene 16SrRNA was used for molecular diagnosis of all bacterial isolates. The results of this investigation support those of the earlier work by [20], which demonstrated that every *E. coli* isolate had the diagnostic gene 16SrRNA.
When rapid and precise diagnosis of bacterial infections or other diseases is imperative, this procedure stands out as one of the most reliable options. The diagnostic gene 16SrRNA, ubiquitous across bacterial species, exhibits minimal heterogeneity—undergoing only minor random changes in its genetic sequence over time. This gene features conserved regions that intersect with the nine variable regions crucial for the identification and differentiation of specific bacteria [21,22].
This study's findings on Carbenicillin are in line with earlier research, Ibrahim et al. [23], which found that 100% of bacterial isolates were resistant to the antibiotic. The reason behind this is that Carbenicillin, Ampicillin, Cephalothin, and other antibiotics belonging to the same class are all degraded by the same β-lactamases enzyme, which is controlled by genes carried on plasmids in Gram-negative bacteria. Findings from this research are in line with those by Hinthong et al. [24], where 92.2% of bacterial isolates were Carbenicillin resistant.
Concerning the results of this investigation into the third generation of cephalosporin antibiotics, which comprise Ceftazidime and Cefotaxime, as reported in the study by McDougall et al. [25], *E. coli* bacteria exhibited a high level of resistance to β-lactamases group antibiotics, with a percentage of bacteria resistant to Ceftazidime at 78.6% and Cefotaxime at 76.6%. It is in agreement with the findings of an Indian study by Suresh et al. [26], which found that 98% and 100% of bacterial isolates were resistant to these two antibiotics, respectively. The researchers concluded that bacteria's efficient efflux pumps, which remove antibiotics from cells, are the main cause of this high resistance [26].
Hegazy et al. [16] found that 74.4% of *E. coli* bacterial isolates were resistant to cefotaxime; the current results were comparable to theirs. Because 64.3% of bacteria were resistant to the drug ceftazidime, this study's findings contradicted those of [16]. In Iran, the percentage of bacterial resistance to ceftazidime was 6.8%, while for cefotaxime it was 15.5%, 42.2%, and 30%. The researchers in those studies suggested that the presence of natural efflux pumps in the bacteria could be the cause of their resistance [10,26].
The present study's results for the aminoglycoside antibiotic Gentamicin are in line with those of Lee et al. [27], which found that 37% of the isolates tested were resistant to this antibiotic. Because *E. coli* isolates exhibit only a modest level of resistance to the antibiotic Nitrofurantoin, this long-standing medication remains a top choice for the treatment of UTIs in patients. The findings of this research were in agreement with those of other earlier investigations, such as the ones conducted in India by Mittal et al. [28], Egypt by Abujnah et al. [29], and Iran by Tajbakhsh et al. [30]. The findings demonstrated that the rate of bacterial resistance to this antibiotic was relatively modest, reaching 6.6%, 1.9%, and 6.25%, respectively, after isolating bacteria from individuals with urinary tract infections.
In conclusion, Nitrofurantoin is the antibiotic of choice for treating *E. coli* UTIs, and the 16SrRNA gene is a confirmative diagnostic gene for *E. coli* infection.

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Conflict of interest
There is no conflict of interest.

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