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Nosocomial E. Coli Isolated from UTI Identified Using DNA Markers Based on PCR and Pathogenic Markers

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most common bacterial infections, with Escherichia coli (E. coli) being the leading causative agent. The increasing resistance of E. coli to commonly used antibiotics, including β-lactam antibiotics, poses a significant clinical challenge. Understanding antimicrobial resistance patterns and genetic diversity among E. coli isolates is essential for effective treatment and infection control. This study aimed to determine the prevalence of E. coli in UTI cases, assess its resistance to multiple antibiotics, evaluate β-lactamase and extended-spectrum beta-lactamase (ESBL) production, and analyze genetic diversity using molecular techniques.

Materials and Methods: A total of 105 urine samples were collected from UTI patients at Rezali Hospital and General Hospital in Erbil (January–June 2024). Disk diffusion was used for antibiotic susceptibility testing, while MIC values were determined for penicillin and cephalosporin. β-lactamase production was assessed using the iodine titration method, ESBLs were confirmed using a disk proximity test, and RAPD markers were used for genetic diversity.

Results: E. coli accounted for 48.51% of UTIs, showing high resistance to tetracycline (75.71%) and ceftriaxone (95.14%), and complete resistance to ampicillin and amoxicillin. β-lactamase production was detected in 84.62% of isolates, while 5.77% were ESBL producers. Genetic analysis indicated strain diversity, suggesting multiple nosocomial sources.

Conclusion: The study highlights multidrug-resistant E. coli in UTIs, emphasizing the need for continuous surveillance and antibiotic stewardship programs to prevent treatment failures.

Keyword: *E. coli*, UTI infection, DNA polymerase chain reaction

INTRODUCTION

The multiple drug-resistant (MDR) bacteria cause nosocomial infections in the majority of hospitalized patients.[1] Furthermore, individuals infected during hospital stay are the main source of mortality and morbidity, and nosocomial urinary tract infections (UTIs) still account for a large percentage of the overall frequency or incidence of nosocomial infections.[2] Escherichia coli, most commonly associated with human infectious diseases, has developed antibiotic resistance, which poses a major challenge to treatment.[3] *E. coli* causes the majority of UTIs in outpatient and inpatient settings.[4] The first detection of β-lactamase in E. coli occurred prior to penicillin's medical approval.[5] Gram positive and Gram negative bacteria produce the enzyme of β-lactamases,

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which are the primary strategy of resistance to β-lactam medication.[6] The genes for β-lactamases are found on bacterial chromosomes, plasmids, and transposons.[7,8] β-lactam antibiotics. These inhibitors acylate β-lactamases indefinitely.[9] Various methods have been used to characterize *E. coli* isolates and help identify pathogenic strains. However, current bacteriological and serological methods for typing *E. coli* are often not sensitive enough to further differentiate between different bacterial isolates, including *E. coli*. Methods that recognize changes in the genetic composition of microbial communities have allowed these isolates to be divided into genetically distinct isolates.[10] The development of molecular markers based on DNA polymorphisms has brought tremendous benefits to multidisciplinary research in many areas of biology.[11] In particular, PCR-based techniques have revolutionized the identification of genetic diversity and infectious diseases.[12] E. coli isolates that were previously assumed to be homogeneous were studied using the random amplification of polymorphic DNA (RAPD) approach, which is based on the technique of PCR.[13] It is widely used due to its ease of use, high sensitivity and adaptability, low requirement for prior knowledge of the genome being studied, small amount of genomic DNA required, universal primers that can be used for any genome, non-radioactive detection, and reasonable cost. In recent years, RAPD has attracted great interest as a molecular typing technique.[14]

The RAPD approach detects DNA polymorphisms using a single primer of any nucleotide sequence. In this approach, a single kind of primer links to genomic DNA at two distinct places on the complementary strand of the DNA template; if these priming sites are amplified separately, thermal cycling amplification results in the formation of separate DNA products. Since each primer typically controls the amplification of many different loci in the genome, the method can be used to effectively screen for nucleotide sequence variation between people.[15] The location and frequency of primer annealing on the chromosome are ultimately determined by the genetic composition of each bacterial isolate, and the different patterns of DNA produced by RAPD technique for each bacterial isolates can be used as an indicator of genetic diversity within the species. Short sequences are more likely to repeatedly slow down the genome, so small 10-base oligonucleotides are used as primers.[16]

MATERIALS AND METHODS

Samples collection: All samples were collected from January to June of 2024, 105 urine samples of UTI patients were collected at the Rzgary and General Hospitals in Erbil City. A clean urine sample collected midstream was placed in a sterile sealed container, which was promptly forwarded to the laboratory for general urine examination and culture.

General analysis of the urine: A volume of 5 ml of urine is separated by centrifugation for a period of 5 minutes at 3000 RPM, the supernatant is then discarded. To determine if the infection is actually occurring or if it's simply a contamination, a sample's drop is positioned on a sterile slide and examined under a high-power microscope for bacteria, pus, red blood cells, and epithelial cells.

Separation: All samples were coated with blood and cultivated on MacConkey agar using a sterile needle. The colonies were originally composed of pink lactose processors that were cultivated on nutrient agar to identify them. Additionally, the colonies were grown on eosin-methylene blue, which produces a shiny green appearance that is metallic.

Microscopic examination: A minute amount of bacterial suspension was made by combining a colony of the tested bacteria with a drop of D.W. The mixture was then put on a glass slide, heated to get the bacteria to stick to the surface, and examined at a magnification of x100. *E. Coli* cells are scarlet, gram-negative rods.

Biological processes: Biochemical tests were employed to verify the recognition of *E. coli*. Catalase production by E. coli was detected by catalase test through the formation of oxygen bubbles, as well as an oxidase test that detects color development within 10 seconds of the creation of an oxygen bubble. The Vogels-Proskauer test produces a red brown color that is indicate of acetone, while the indole test produces an indole ring in the event of a positive result. The consumption of citrates is assessed; if the result is positive, the test is considered successful. Otherwise, it is considered negative. The beneficial color is royal blue. For the production of urease, positive test produces pink color while negative results is yellow color. Kriegler Iron Assay (KIA) After the incubation period, the color of the slash and rump will change, a positive finding is showed by rise in the production of gas and H2S will increase. According to the motility test [17, 18] (see Table 2), a turbid appearance in the culture medium indicates the presence of motile organisms.

The Kirby-Bauer test employs the disk diffusion method to assess the susceptibility of antibiotics.

This method is considered one of the most effective standardized tests on the NCCLS (National Committee for Clinical Laboratory Standards), and its consensus is regularly updated by the project. Here's the format of the test. The preparation of the inoculum, the placement of the disc in the test plate and the inoculation of the test bacteria are all described in detail. Müller-Hinton agar plate was used for antibiotic sensitivity test (AST) and its surface was spread with a cotton swab of the inoculum. Insert the antibiotic disc into the agar as close to the inoculation point as possible in 15 minutes. The antibiotics is slightly attached to the plate surface with forceps. The plate is then reversed and incubated overnight at 37°C. To create an inoculum for exponential growth, take a single colony and transfer it to a new container with 5 ml of nutrient broth and incubated 18-24 hrs at 37°C. After incubation, the zone of inhibition was determined with a caliper in millimeters (mm) and

compared to standard to determine if the isolate is sensitive, intermediate, or resistant to a certain medication.[17,18]

Minimum inhibitor concentration test [18]

With the use of this method, it was discovered that the following antibiotic concentrations inhibit the organism's apparent growth the lowest: -0.8 milliliters of nourishing broth were aseptically placed into a sterile screw-cap container, which was then autoclaved at 121 degrees Celsius for 15 minutes.

After observing the optical density of the broth's culture and comparing it to the Macfrland standard solution, 0.1 ml of the test overnight cultured bacteria were added to a separate 9.9 ml sterile sample that contained 9.9 ml of sterile D.W. This sample was then transferred to prepare a 10-2 dilution of the selected bacteria. The broth's culture is then incorporated into this. The antibacterial agents were diluted two times each time until concentrations between 0.25 and 1024 mg/ml were reached. The cultures were then treated sequentially with 0.1 ml of the dilution, while a control tube remained agent-free.

All cultures were incubated at 37 Co for a duration of 24 to 48 hours.

The cultures' turbidity development was examined. The bacteria were vulnerable to the antibiotic concentration if there was no growth, but resistant if there was.

Check for the presence of β-lactamase

Quick iodometric technique: After being infused with microorganisms, MacConky agar was cultured for twenty-four hours at 37°C. After selecting several colonies using a loop, they were placed in an Epindorf tube with 100 µl of penicillin G and cultured for 30 minutes at 37°C After adding 50 µl of starch solution, thoroughly mix it with the other ingredients. After adding 20 µl of the iodide solution to the tube, it was stirred for one minute, causing a dark blue hue to appear. When there was a quick shift in color from blue to white the outcome was deemed favorable. When a false positive result was achieved (after five minutes), the test was repeated.[19]

Extended spectrum β-lactamase (ESBLs) detection test

Disc approximation test: A test tube filled with nutrient broth was used for cultivation (18-24 hrs at 37°C) of a single colony of the selected bacteria. The plate was then augmented with salt using a sterile cotton swab to produce a 10-2 ratio of the broth's culture to the salt's, which was then uniformly spread on Müller-Hinton agar plate surface. After a few minutes, a sensitive disk with Augmentin (amoxicillin-clavulanic acid) was positioned in the middle of the plate. Three disks (cefotaxime, ceftriaxone, and ceftazidime) were then positioned 3 mm away from the center disk. The medium was then incubated overnight at 37°C. The synergistic effect of clavulanic acid in the amoxicillin-clavulanic acid disk increased the effectiveness of each drug (cefotaxime, ceftazidime, and ceftazidime). This was a significant outcome.[20]

Molecular Methods

Genomic DNA Isolation: The DNA of various E. coli was isolated using the method described in [21] with minor alterations: bacterial colonies were added to 30 ml of LB broth and the mixture was stirred continuously at 37 °C for 24 h. After centrifugation which carried out at 4000 round per minute (rpm)for 25 min, the cells were resuspended in 3.5 ml of TE25S buffer and mixed thoroughly. Next, 100 µl of the lysozyme solution was added to the mixture and incubated at 37 °C for one hour. After adding 50 µl of the proteinase K and 200 µl of the 10% SDS to the mixture, it was shaken and incubated at 55 °C for one hour. After adding 650 µL of NaCl and 0.65 mL of the CTAB/NaCl, it was incubated in a water bath for 10 minutes. The tubes were stirred and placed in a warm room for five minutes, followed by an equal amount of chloroform and isopropyl alcohol. They were then repeatedly stirred for 30 minutes and then centrifuged at 4000 RPM for 30 minutes. This procedure was repeated twice. After transferring the supernatant to a sterile container, add 0.6 liters of cold isopropanol. Cover the bottle and repeatedly invert it to incorporate the mixture. Then place the tube in a 4°C refrigerator for 30 minutes and rotate at 4000 RPM for 20 minutes. After collecting the supernatant and leaving it for half an hour on filter paper, add 1 ml of 70% ethanol and briefly rotate the mixture at 4000 RPM. After disintegrating the DNA pellet in 300 µL of TE buffer, store at -20°C until it's needed.

Gel Electrophoresis

Agarose Gel Electrophoresis: After heating 100 ml of 1X TBE buffer to its boil, 0.7 g of agarose was added to create an agarose gel (0.7%). After cooling to 55 degrees Celsius, the agarose was transferred to a plastic sheet to solidify. A comb was positioned on one of the boundaries of the gel and it allowed to freeze. After augmenting the gel tank with 1X TBE, and placing the gel plate in the electrophoresis tank horizontally, the samples were then placed in different wells. 2 ml of the DNA's composition was combined with 3 ml of a buffer that loads DNA. The current was intended to be 45 volts for 15 minutes and 85 v for 4-7 hours to complete the DNA. To imbue the agarose with ethidium bromide, it was placed in D.W. for 30-45 minutes. The dye was coated with 0.5 µg/ml of final concentration. DNA bands could be observed using an ultraviolet transilluminator that had a wavelength of 365nm. The gel was rinsed after 30-60 seconds with D.W.'s Destaining Solution to eliminate any remaining color that could be identified as background. Then take pictures with a digital camera.[22]

Agarose Gel Electrophoresis Agarose; A mixture of 100 ml of the hot 1X TBE buffer and 1.2 g of agarose was used for preparation of 1.2% agarose gel. After cooling to 55 degrees Celsius, place the agarose on a plastic sheet to solidify. Face the gel's edge and allow it to solidify. After augmenting the gel tank with 1X TBE, and placing the gel plate in the electrophoresis tank horizon-

tally, add 2 milliliters of DNA sample and 3 microiliters of buffer to each well.[23]

Primers: These primers were provided by Cinnagen (Iran). Five different primers were employed in this experiment; the primer sequences and their respective lengths are listed in Table 1.

Table 1: Primer and their sequence employed in the study

Primers	Sequences
A	5-CGGAGAGCGA-3
B	5-GGTCTCCTAG -3
C	5-CCGGCATAGA-3
D	5-TGGGCTCGCT-3
E	5-ACTTGTGCGG-3

Preparation the RAPD-PCR master mix: The chemicals in Table (1) represent the components necessary for the RAPD-PCR method. Five different samples were used to create the master mix in Eppendorff tubes (1.5 µL) that contained 25 µL 10XPCR, 25 µL dNTPs, 20 µL primers, and 2 µL Taq polymerase. Later, deionized D.W. was incorporated into the mix to bring the total volume to 230 µL (all procedures were conducted on ice). Every 0.5 ml Eppendorff bottle had 23 µl of the primary mixture. Next, 2 micro-liters of DNA from each sample were incorporated and the mixture was thoroughly stirred. As a result, the final volume of each tubule was 25 µL. 25 µL of mineral oil was incorporated into the mixture in each tube to prevent it from evaporating.

Amplification of genomic DNA: They were all put in a heat cycler to start the amplification process. The following settings were used for the amplification program: 94C for two minutes, 40 cycles, 92C for one minute, 36C for one minute, and 72C for one minute, followed by a ten-minute cycle at 172C.

Agarose gel electrophoresis of amplified product, staining and photograph: The amplified DNA was then subjected to a 360 degree of UV light, and 1.2% agarose gel was used for electrophoresis of DNA, the DNA fragments was then tainted with ethidium bromide, and the images were recorded with a digital camera.[23]

Data Analysis: To examine the results of the RAPD, a table that summarizes all of the information in the figure is provided. These data include the occurrence or absence of DNA bands, the overall number of bands that are amplified in all E. coli instances, and the number of instances in which DNA is horizontally transferred.[24]

RESULTS

Identification of infection with *E. coli:* Out of 105 urine samples examined for the General Urine Examination (G.U.E.), 51 (48.51%) isolates yielded positive results. Since a general urine examination may provide prompt findings that confirm the initial diagnosis of urinary tract infection, it is considered an essential test. In the present study for the diagnosis of UTI, only samples with bacteria (number of bacteria cells in urine ≥10 cells / HPF) and pyuria (number of W.B.C. ≥ 10 cells / HPF) are considered positive findings [25]. This test is essential for differentiating between UTI and contamination. Additionally, as shown in Table 2, 52 (49.52%) isolates were determined to be E. coli based on standard biochemical assays as well as physical and cultural traits on selective medium such MacConky and Eosin Methylene Blue agar. Numerous variables, including the fact that *E. Col*. is a component of the faecal flora and uropathogenic *E. Coli* virulence factors, are responsible for this high value of *E. Coli* distribution.[26] The results of this investigation supported those of [27], who found that 53.7% of UTI cases were caused mostly by *E. coli*. *E. coli* was identified by several studies as the most frequent etiological cause of UTIs [28].

Table 2: E. Coli isolates are identified using biochemical assays

Biochemical test	The results
Indole (I)test	+ ve
Methyl red (MR) test	+ ve
Voges Proskauer (VP) test	- ve
Citrate utilization (C) test	- ve
Urease production test	- ve
Oxidase test	- ve
Catalase test	+ ve
Klegller Iron Agar (KIA)	
Gas production	+ ve
H2S production	- ve
Slope	Acid
Bottom	Acid
Motility test	+ ve

Antibiotic Sensitivity Test of Isolated Strains: The NCCLS guidelines advocate testing 51 different E. coli strains for resistance to 9 different antibiotics using the agar disk diffusion method. All of the bacterial isolates were susceptible to the antibiotic nitrofurans, this is an antibiotic that is toxic to bacteria in the urine at a therapeutic dose and has multiple strategies of action that have contributed to its longstanding effectiveness against *E. coli* over the course of more than 50 years of use. The increased sensitivity of *E. coli* to nitrofurans may be attributed to a limited spectrum of action, limited indications, limited tissue distribution (undetectable or low amounts in serum), and limited interactions with other organisms in addition to the urinary system. Nalidixic acid was the first quinolone to be employed to address urinary tract infections; it was encountered in 17 (32.69%) of the E. coli samples collected. This outcome is in line with another study that stated that 27% of the E. coli found in Spain were resistant to nalidixic acid [31]. Ciprofloxacin is considered a second-generation fluoroquinolone. Ten (19.23%) strains of the E. coli in this research were resistant to this chemical, although they possessed a large zone of inhibition in the susceptible strains, this demonstrated their effectiveness against these. Other investigations have documented

that 20% of E. coli found in hospital patients are resistant to the antibiotic ciprofloxacin, the percentage of resistant bacteria in the urine can be as high as 20.6% [32]. Additionally, a study in the hospitals for determination the prevalence and bacterial susceptibility of urinary tract infections revealed that a large percentage (22%) of patients were resistant to ciprofloxacin [33]. The revelation and widespread release of fluoroquinolones (such as ciprofloxacin) in the late 80's manifest the beginning of a innovative era in the history of antibiotic therapy. These substances have a unique mechanism of action, which is characterized by a favorable pharmacokinetic profile, a safe side effect, and a wide variety of effectiveness against G +ve and G -ve bacteria. Fluoroquinolones have continued to be highly effective against common uropathogens, but these organisms have been noted to develop resistance to these chemicals as a result of their association with trimethoprim-sulfamethoxazole and β-lactam resistance [34].

Table 3: The results of the antibiotic susceptibility test conducted on each E. Coli isolate

Antibiotic	Susceptible	S %	Resistant	R%
Ampicillin	0	0	52	100
Amoxicillin	0	0	52	100
Cephalothin	2	3.85	50	96.15
Cefotaxime	29	23	55.77	44.23
Tetracycline	12	23.08	40	76.72
Gentamicin	31	59.62	21	40.38
Ciprofloxacin	42	80.77	10	19.23
Nalidixic acid	35	76.3	17	32.69
Nitrofuronation	52	100	0	0

Gentamicin is an aminoglycoside antibiotic used to treat urinary tract infections. According to the data collected, gentamicin resistance was detected in 21 isolates (40.38%). Most ESBL-producing bacteria have been shown to be resistant to medicaiton other than β-lactams, such as trimethoprim, gentamicin, and ciprofloxacin [35]. Tetracycline resistance: 40 of the 52 isolates (76.92%) were not susceptible to tetracycline. A resistance protein called TetA expressed on the plasmid promotes the efflux of active drug, thereby reducing drug accumulation. Changes in cell permeability are mainly associated with resistance to this antibiotic. Two other pathways by which bacteria develop resistance to tetracyclines are the formation of bacterial proteins that prevent the drug from attaching to the ribosome and enzymatic inactivation of the drug. It is well known that any organism resistant to one tetracycline is also resistant to other tetracyclines. [36] The results of this study are consistent with another study conducted in Baghdad [37], which found that tetracycline resistance was more common in their isolates. Cephalosporins and penicillins: Cephalosporins, which are first-generation cephalosporins, were resistant to 50 isolates (96.15%), while all isolates showed 100% resistance to the two aminopenicillins (ampicillin and amoxicillin). This result means that ampicillin, amoxicillin, or ceftriaxone cannot successfully treat E. coli-associated urinary tract infections. Another study [38] reported E. coli strains which isolated from patient during hospital stay (nosocomial UTIs) shown significant resistance to ampicillin and ceftriaxone. In addition, data from other researchers in the United States [34] showed that 97.8% of isolates were ampicillin resistant and 86.6% were ceftriaxone resistant. The resistance of bacterial isolates to β-lactam medications such as penicillins and cephalosporins is usually caused by bacterial formation of β-lactamases. These β-lactamases cause the breakdown of β-lactam medication. TEM-1 is a common β-lactamase that hydrolyzes early cephalosporins such as ceftriaxone and cefuroxime as well as penicillin [39]. Cefotaxime is a third-generation cephalosporin that is more effective than first- and second-generation cephalosporins, although 23 (44.23%) isolates were resistant to it. It is a β-lactam antibiotic with a broad spectrum of action. When broad-spectrum cephalosporins were widely used in hospitals, strains of Enterobacteriaceae developed resistance to these drugs [20].

Lowest inhibitory concentration (LIC) determination: The LIC that prevents infection's spread and growth is called the minimal inhibitory concentration (MIC). One of the most significant objectives of antibiotic therapy is to have a sufficient amount of drug at the infection site in relation to the MIC in order to achieve optimal effectiveness. The MIC of various β-lactam medication, including ceftriaxone, cefotaxime, amoxicillin, 1and ampicillin, was assessed according to the guidelines of NCCLS (1987). This was performed with the aim of investigating the degree of resistance of overall *E. coli* strains. The Isolates can then be considered resistant or susceptible. The concentration of the antibiotic that shows highest activity against the majority of the bacteria is called the cutoff value. If the MIC is less than the threshold value, the microorganism in question is considered to be susceptible. The results showed that the MIC for ampicillin was between 64 and 1024 µg/ml, and the minimal inhibitory concentration for amoxicillin was between 32 and 1024 µg/ml. This outcome is similar to that documented in [42]. The migration of the global population, the selection pressure due to antibiotic usage, and the genetic flexibility of microorganisms have all contributed to the worldwide development and spread of bacterial isolates that are resistant to medications [43]. Among the 50 resistant isolates, the MIC was between 16 and 512 µg/ml, and only two strains with an inhibitory concentration of 8 mg/ml were susceptible to ceftriaxone. However, 24 of the isolated strains had ceftriaxone concentrations greater than the breakpoint, this indicates that they are resistant to the antibiotic; 28 of the isolated strains had concentrations less than the breakpoint, which indicates that they are susceptible to the antibiotic. The primary cause of cephalosporin resistance in both the general population and hospital isolates is the emergence of CTX-M ESBLs. Typically, the most frequented-resistant bacteria are those that produce CTX-M. Other important routes are non-CTX-M ESBLs and ampC β-lactamases [44]. EARSS which stand for European Antimicrobial Resistance Surveillance System documented a rise in the

percentage of resistance of *E. coli* strains to cephalosporins (third-generation) in 2004. This may be caused by the increasing number of ESBL producers [45].

Extended Spectrum-β-lactamase (ESBLs) enzyme detection: The probability of each strain to harbor ESBLs was evaluated using the Jarlier method of disk evaluation [20]. This is derived from the combined effect of clavulanic acid on the expansion of the inhibitory zone of oxo-β-lactam in amoxicillin-clavulanic acid tablets, this indicates a positive result and the potential presence of ESBLs. Of the 52 samples, three of them (5.77%) showed an increase in the disk's enhancement. This outcome is consonant with previous studies that reported that 9.3% of E. coli strains possessed ESBLs, the majority of which were non-TEM and non-SHV. In [13], 7.5% of the isolates had ESBLs. These enzymes are probably derived from the increasing prevalence of newer broad spectrum cephalosporins in the medical community. The widespread distribution of organisms that create ESBLs and demonstrate these enzymes via plasmids will have a significant impact on the available therapeutic options for doctors regarding these organisms, as they pose a significant threat to the treatment of common diseases in the future. The increase in antibiotic resistance is attributed to multiple causes, including increased global travel, animal consumption, and the transmission of resistant organisms from humans to animals.[40,47,48]

Extraction of the genome: DNA extraction kits are sold by several companies, including Sigma, Amersham, Cinnagene, and others. There are numerous methods for obtaining whole DNA these days. The use of CTAB, a cationic detergent that precipitates DNA and binds tightly to it to create the CTAB-DNA complex while other cell components are still in the solution, was part of the quick, simple, and efficient approach used in this investigation. Using a 100 bp DNA ladder sample as a reference, migration via 0.7% agarose gel electrophoresis demonstrated a satisfactory concentration of DNA in the results of several trials. The concentration of the DNA samples was assessed using the spectrophotometer. DNA with a purity of about 1.8 and an average yield of 50µg/ml was obtained using the spectrophotometric ratio A260/A280.

DISCUSSION

RAPD–PCR Based Technique: Investigating the differences between hospitalized individuals is crucial, as it derives from the clinical scenario in which the pathogen first appeared. Understanding the differences between strains is crucial to the follow-up of recently introduced strains, and it's important to recognize the different strains in order to treat patients and eradicate pathogens. PCR-based RAPD is particularly beneficial for typing because it employs arbitrary nucleotides at low temperatures to initiate DNA replication and expose genetic diversity in the genome. This is drastically different from the traditional method of polymerase chain reaction analysis, which require detailed information of the DNA sequence and the utilization of precise sequences from the target species. As a result, it's extremely powerful and versatile. In this research, RAPD was employed to identify nosocomial E. coli that are obtained from the urine sample of patients with UTIs in order to identify genetic variants. Of the seven selected E. coli strains, three produced ESBL enzymes, but the other four did not. The most difficult component of RAPD analysis for bacterial identification is the selection of the appropriate primers. In this research, 10 (10-mer) random primers from Cinnagen were employed to assess the diversity of E. coli in seven different isolates. All of these primers led to DNA diversity, except for primer C, which was replicated multiple times. In all of the isolates, they generated an overall of 55 bands, of which 46 were monomorphic, and 2340 bp was the largest band. Additionally, they were easily repeatable. The primary causes of polymorphism are variations in the primer's binding site's sequence that manifest as the existence or absenteeism of a specific RAPD band, or as variations in the primer's binding site's (e.g., point mutations) that lead to the expansion of the target DNA blocks (insertions, deletions, and inversions) [49]. Several experiments were conducted in this research, including experiments with primer concentrations,PCR buffer, Taq polymerase, and DNA concentrations in order to improve the conditions of amplification. DNA polymorphism is typically expressed in two ways: when the amplified DNA fragment is absent or present, or when the molecular size of the amplified DNA fragment is different. The outcomes of each primer investigated in this research are documented separately, and the amplified fragments are organized by decreasing molecular weight. Each primer generates a unique amplified product (RAPD profile).

Primer A: The 5-CGGAGAGCGA-3 sequence of the primer produced a total of 12 bands in all samples. Eleven different bands with multiple polymorphisms were identified. The estimated molecular weight of the bands was between 120 and 2340 bp. The aislates were recognized as follows: isolate a had 6 bands with a total molecular size of 120-2340 bp, isolate b had 8 bands (300-2000 bp), and isolate c had only 4 bands (600-2000 bp). Isolate D appeared to have 8 bands (200–2000 bp), but isolate E had 7 bands (200–2340 bp). The isolate that had the greatest number of bands was f, it had 10, while the isolate that had the fewest bands was g, it had 3. Figure (1) demonstrates that each isolate possessed a singular monomorphic band (700 bp of molecular size). The association of the primers with the conserved regions of the E. coli genome for each isolate is responsible for this discovery [51].

Primer B: This primer has the sequence 5-GGTCTCCTAG-3. amplified a total of two bands from every isolate. One or two bands made up the banding pattern of the isolates. One polymorphic band has been found. Roughly speaking, the isolates may be recognized as follows: isolates c and e, as shown in Figure (2), are distinctive because they have two bands (120-600 bp),

whereas the other isolates only have one band with a molecular size of 120 bp.

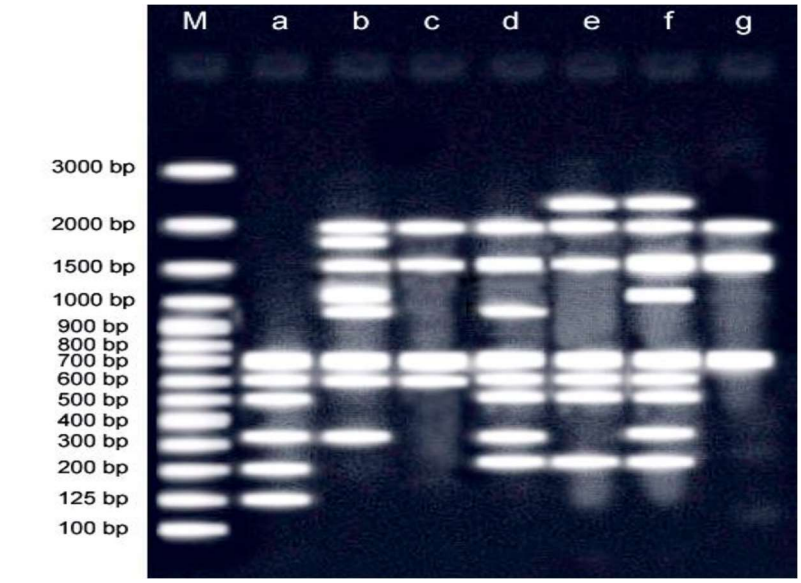

Figure 1 Lanes a, b, and c in the RAPD pattern of the bacteria experienced with primer A indicate Col isolates that produce the ESBLs enzyme; lanes d, e, f, and g represent isolates that do not. The molecular marker is Lane M, which is a 100 base pair DNA ladder.

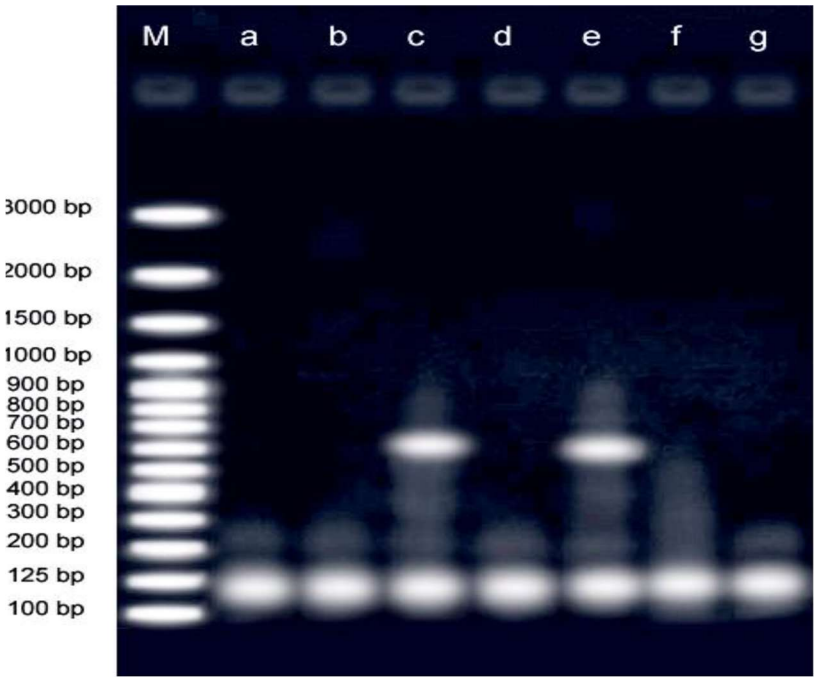

Figure 2 primer B was used to test the E. coli RAPD pattern. Lane M is the molecular marker (a 100 base pair DNA ladder), and lanes a, b, and c indicate isolates of the bacteria that generate the ESBLs enzyme, whereas lanes d, e, f, and g indicate isolates of the bacteria that do not produce the ESBLs enzyme.

Primer C: This primer has the sequence 5-CCGGCATAGA-3, and did not produce any amplification product despite repeating the experiment many times because the nucleotide sequence of this primer may not match the nucleotide sequence at any region of the template strand of DNA.

Primer D: 5-TGGGCTCGCT-3 is the sequence of this primer. six bands in total were amplified among all isolates. There were five polymorphic bands found. The isolates may be distinguished by their unique banding patterns, which are seen in two bands on isolates A and B (850–1000 bp and 700–1000 bp, respectively). The banding patterns of isolates c, d, and e are identical; they each have one band with a molecular size of 1000 bp, but isolates f and g have two and five bands, respectively, ranging from 500 to 1000 bp and 500 to 1600 bp, respectively, representing the maximum number of bands as shown in Figure (3).

Primer E: Primers with the sequence 5-AACTTGTGGCG-3 amplified two different bands from each isolate. Only one monomorphic band was identified in each sample. Isolates a, d, and e can be recognized using the following methods: Figure (4) demonstrates that isolates b, c, f, and g have the same banding pattern, but are distinguished by the existence of a single band of 1400 bp (molecular size) different from the pattern.

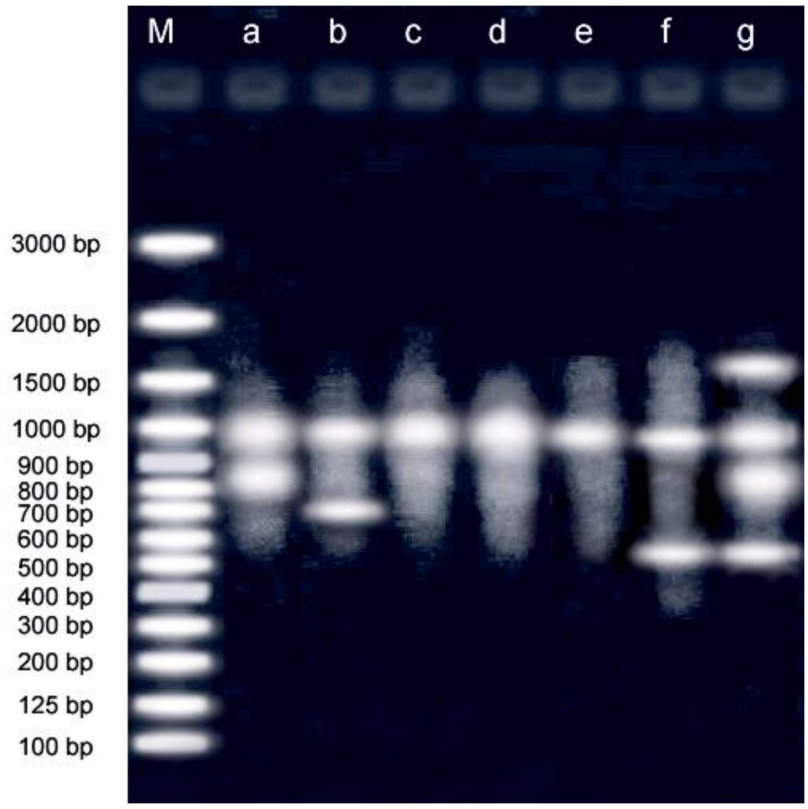

Figure 3 primer D was used to test the E. Coli RAPD pattern. Lane M is the molecular marker (a 100 base pair DNA ladder), and lanes a, b, and c indicate isolates of the bacteria that generate the ESBLs enzyme, whereas lanes d, e, f, and g indicate non producing bacterial isolates of ESBLs enzyme.

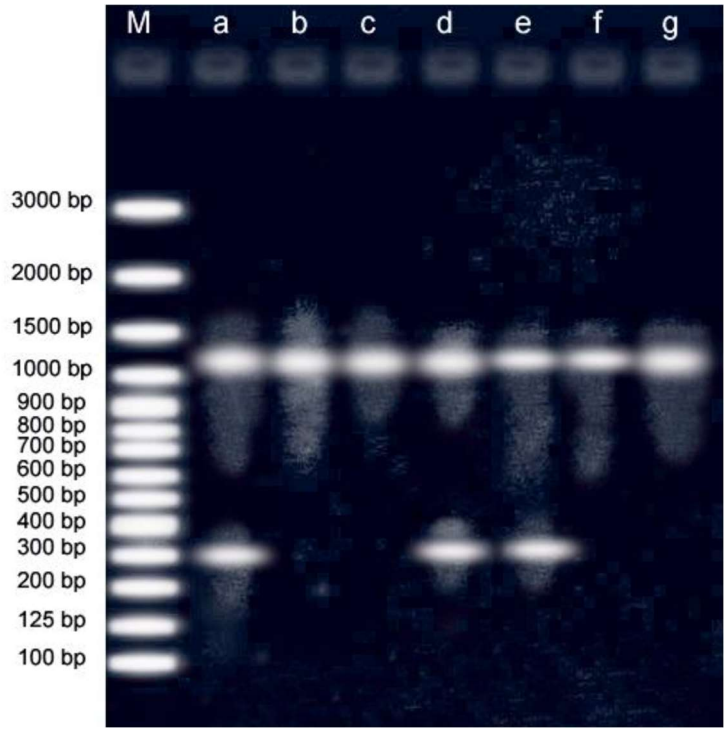

Figure 4 Lane M = molecular ruler (100 bp DNA ladder), lanes a, b and c denote strains of E. coli isolates that express ESBLs, and lanes d, e, f and g represent *E. coli* strains that do not have ESBLs. This is the pattern of E. coli's RAPD gene tested with primer E.

CONCLUSION

The investigation revealed that 48.51% of the UTIs were caused by Escherichia coli. Many of the isolated bacteria demonstrated multiple antibiotic resistance, a condition that is associated with the production of β-lactamase in 84.62% of the isolated bacteria. Additionally, 5.77% of the isolates possessed ESBLs due to overuse of β-lactam medication, this was especially prevalent in antibiotics with a broad spectrum of action. Furanone is the most effective medication for UTI treatment, followed by ciprofloxacin and nalidixic acid. β-lactam antibiotics like ampicillin, amoxicillin, and ceftriaxone are effective in the treatment of UTIs, and all isolates of the bacteria were resistant to amoxicillin and ampicillin (100%) as well as resistant to ceftriaxone (96.15%). Using a PCR-based RAPD test, DNA variants that are consistent with *E. coli* were detected in the isolate's DNA. The association between this variation at the DNA level and the frequency of UTIs and multiple strains in the hospital in Erbil is significant.

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