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Genotyping of Esherichia coli isolated from urinary tract infections using ERIC method

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ABSTRACT— Twenty isolates of *Echerichia coli* were obtained out of (55) isolates collected from urinary tract infections patients in Baghdad governorate and from different hospitals. The results detected *E. coli* in females at (80%) and in males at (20%); also the percentage of *E. coli* in terms of age showed that the rate of infection was highest in the ages of 30-39 years with a percentage of (35%) and the lowest percentage of infection wasin the range 20-29 years with a percentage of (18%), while the ages of 40-49 and 50-60 years recorded (25%) and (22%) respectively. The resistance of *E. coli* to (15) antibiotics was studied, and the results revealed it is resistance to Ceftazidime (86%), Ciprofloxcin (50%), Tobramicin (40%), Gentamicin (41%), Nitrofurantion (5%), Augmentin (86%), Tetracycline (70%), Imipenem (20%), meropenem (94%), Rifampcin (99%), carbencillin (98%), Cefriaxone (50%), Levofloxacin (30%), Ampicillin (95%), and Amikacin (14%). Genotyping of bacterial isolates was performed to find out the genetic relationships between the collected isolates by using ERICPCR technique. The results of this study found that there are 14 genotypes containing seven groups, each group containing genetically close bacterial isolates, while the other isolates contain different genotypes.

KEYWORDS: *Escherichia coli*, antibiotic resistance. Genotyping ERIC- PCR

1. INTRODUCTION

Esherichia coli is one of the important bacteria belons to Enrerobacteriacea family; it is part of the normal fora in the colon of humans and other animals. *E.coli* was first diagnosed by the German scientist Escherich Theodore in 1885 through his study on the normal bacteria in the intestines in the stool of an infant, and found is a commensal bacteria that colonizes the intestine immediately after the birth [15], [30].

The characteristics of *E.coli* is Gram negative, facultative anaerobic, widespread in nature, positive for indole and methyl red, negative for oxidase test, positive for catalase test, negative for Vogas proskauer, H_2S and gelatin. Although some of this bacteria live naturally in the human and animal body, and some of their types come from feces, and at the same time they are opportunistic bacteria that cause many diseases, including meningitis, diarrhea, burn inflammation, wound infection, bacteremia, and urinary tract infection (UTI), which is the most common diseases caused by *E.coli*, especially in children between the ages of (5-10), as well as in the age groups between (30-39) and (60-69), and it is one of the main problems in hospital infections [12].

Interestingly, there are two types of urinary tract infections, the first is called lower urinary tract infections; this type of infection includes cyctitis and urethritis. Moreover, this type occurs as a result of recurring acute cystitis due to resistance of *E.coli* to antibiotics, and it causes obstruction of the urethra resulting in urine accumulation in the bladder. This type is more common in males than in females due to shorter urethra in males [11].

While the second type of urinary tract infections called upper urinary tract infections; this type includes infection of the renal pelvis (kidney) and a few cases causes infection of glomerulonephritis. This is a common type in females [9], the Japanse 2017.

The cause of *E. coli* pathogenicity is due to its possession of many virulence factors, which are a measure of the ability of the microorganism to invade the host tissues, and causing infection. *E.coli* possess many virulence factors, including cytotoxic necrotizing factor, cilia that help attach to the surface of the host, flagella, capsula, polysaccharides, hemolysin, and also has the ability to produce flagellar antigen H, somatic antigen O and capsular antigen K [23].

Furthermore, *E.coli* possess one of the most important virulence factors, bacterosin, which contributes to the protection of bacteria via killing other bacterial species. It also secretes many toxins, including stable entero toxin, heat-label entero toxin, plasmid-encoded toxins, and also secrete toxins similar to Shigella enterotoxinal.; it has also the ability to produce biofilm and possesses iron siderophoers that help bacteria to grow in a low-iron environment, as well as *E.coli*, after their implantation in the intestine lining, causes a disease called dysentery [18].

The resistance of *E.coli* to antibiotics is one of biggest health problem in the world, that prompted many researchers to generate a new antibiotics to defeat the resistant strains; *E.coli* has several mechanisms of resistance to antibiotics, especially beta-lactams through their effect on the inhibition of transpeptidase and carboxypeptide enzymes, which affects the side connections in the cell wall that surrounds the bacteria, leading to its weakness and thus cell decomposition [5]. Additional, other mechanisms that *E.coli* possess for resistance to beta-lactams include production of beta-lactamase enzymes and a change in cell membrane permeability, efflux pum and target site modification [7], [28].

The genetic sequencing methods are critical in detecting the genetic relationships between bacterial isolates and distinguishing the most virulent strain. There are several methods for genotyping, and ERIC-PCR method is one of the easy and fast methods that do not need time to complete it in comoparison to other methods [26].

2. Materials and Methods

2.1 Isolation of E. coli

Fifty five samples were collected from urinary tract infections patients from different ages and from different hospitals in Baghdad governorate (Baghdad Teaching Hospital, Alalwia Hospital, Yarmouk Teaching Hospital, Imam Ali Hospital, Central Child Hospital) for the period from 12/18/2020 to 25 /2/2021.

2.2 Diagnosis the Isolates

E.coli samples were diagnosed by using MacConkey agar, blood agar, Hekton enteric agar, Chromium Orientation agar, biochemical assays (Catalase, Oxidase) and the final diagnosis of the isolates was done using API20E system and Vitek2 system.

2.3 Antibiotic sensitivity test

The sensitivity test for (15) antibiotics was performed according to the method of Kirby Bauer on Mueller Hinton agar using discs of the following antibiotics: Ceftazidime, Cefriaxone, Ciprofloxacin, Tobramycin, Gentamycin, Nitrofurantoin, Imipenem, Tetracycline, Ampicillin, Rifampin, Levofloxacin, Augmentin,

Amikacin, Meropenem, Carbenicillin. It was relied by measuring the diameter of inhibition zon (mm) around the discs of antibiotic and then compared with the global measurements according to [8].

2.4 DNA Extractiuon

The genomic DNA extraction kit (Genomic DNA Purification Kit) was used for DNAextraction from bacterial isolates according to the instructions of the manufacturer protocol (Promega, USA).

2.5 The Genotyping of E. coli using ERIC-PCR method

The genotyping of *E. coli* was performed using ERIC-PCR methodusing specific primers for the target gene. ERIC (F): 5 ′- ATG TAA GCT CCT GGG GAT TCA C-3, ERIC (R): 5 ′- AAG TAA GTG ACT GGG GTG AGC G-3. The master mix (25 µL) was prepared from GO Taq Green Master Mix which included (12.5 µL), 1 µL of DNA template, 2 µL of Primers and 9.5 µL of deionized distilled water); this was prepared according to the kit protocol (Promga USA). The reaction conditions were determined according to the following program with the some modifications [24]:- (The initial denaturation stage, only one cycle for 7 minutes at a temperature of 95°C. Second stage DNA amplification stage; only one cycle at a temperature of 95°C in order for the attaching of primers to to the DNAtemplate; it included 30 cycles according to the following steps: (Annealing stage: one cycle at 49 °C for one minute, Extension stage at 65°C for 8 min. Final extension: one cycle at 65°C for 16 min).

2.6 Products of reaction

Reaction products were electrolysed by using Bio Basic INC (Canada) agarose gel (2%) that containing 5 µL of Eithidium bromide (Promega USA) dye and using (100-1500) base pairs of DNA ladder at a voltage of 100 V for 80 min., and imaged using device of UV light Optima (Japan).

3. Results and Discussion

After diagnosis we obtained, (20) isolates were detected as *Escherichia coli* collected from patients infected with UTIs out of (55) patient. The results found the percentage of *E. coli* in females was (80%) while it was (20%) in males (Figure 1). Moreover, the percentage of *E. coli* at the age of 20-29 was (18%), while for the ages 40-49 and 50-60, the percentage were (25%) and (22%) respectively (Figure 2).

This study almost is in consitent with the results of study which observed the most bacteria isolated from UTI from multiple hospitals in Baghdad recorded the incidence of UTIs in females at (90%) and (10 %) in males. While the study of [16] in Kirkuk city and [4] study in Tikrit city revealed that the incidence of UTIs in females was (58%) and the incidence in males was (42%). Moreover, The findings of the current study closely consitent with those [21] study, where they found that the incidence of urinary tract infections in females was (73.9%), and in males was (26.1%) in the samples isolated from several hospitals in the Kurdistan city/Dohuk. In addition, This study is consistent with many local and international studies that showed that *E.coli* was the main cause of urinary tract infections. In contrast, The study of [3] in the city of Qadisiyah showed that the percentage of *E.coli* isolation was (56%) from urine samples. The reason for the difference in infection rate in males and females may be due to the shortness of the urethra in females and its proximity to the anus, which causes the entry of bacteria into the urethra and bladder, in addition to that women are more susceptible to UTIs due to hormonal changes and anatomical differences. While the reason for the decrease in the incidence of UTIs in males is due to the presence of zinc in the prostate fluid which acts as a killer of bacteria, and thus the incidence of infection decreases in males compared to women [10], [22]. A study by two researchers showed [25]. [25] observed that the high rate of *E.coli* in UTIs is due to the transmission of bacteria from the anus which is a natural habitat for it, to the urinary orifice.

The results of the current study were consistent with the observation of [6] for the antibiotic Carbencillin, in which the percentage of bacterial isolates isolated from UTIs was (100%). Furthermore, This study was consistent with the local results by [13], where the resistance of bacterial isolates to the antibiotic Carbencillin was (100%). It was also found from the present study that the resistance of *E.coli* to Ampicillin was (95%), and the results of the study closely agree with the findings of [21] study. In contrast, the resistance of *E.coli* to Ampicillin was (88%) and this was consistent to the observations of [17] where *E. coli* was resistant to Ampicillin at 87%). The reason for the resistance of *E. coli* to the group of penicillins is due to their possession of beta-lactamase enzymes that break down the beta-lactam ring and prevent its binding with transpeptidases enzymes.

The results of the current study also revealed that (86%) of *E.coli* isolates were resistant to Augmentin, and this was in agreement with [14] study. This resistance is due to the production of chromosomally encoded beta-lactamase enzymes by this bacteria.

The results of the current study for third-generation of cephalosporin antibiotics which include Ceftazidime and Cefriaxone; the result of this study in relation to Cefraixone is consistent with the results of study, where the rate of resistance to Cefriaxone was (46%). On the other hand, this study of Cefriaxone is not agree with the results of [21], as the bacterial resistance to studied antibiotic was (63.9%). The findings of this study with respect to the antibiotic Ceftazidime were closely consistent with the observations of [6] study where the rate of bacterial resistance to Ceftazidime was (88%). The main reason for resistance of *E.coli* to third generation of cephalosporins is that they possess efflux pumps that eject the antibiotic out of a cell and then removing its effect [29].

The results of the current stud with regard to Levofloxacin are in agreement with the results [21] as the resistance of *E.coli* to Levofloxacin was (23.4%). Additionally, the results of a study with Ciprofloxcin were consistent with the results of [20] study, where the percentage of resistance to Ciprofloxcin was (57.1%). Moreover, the findings of this study is in agreement with those of [1] in Nigeria, and the percentage of resistance to Ciprofloxcin was (46%), and Levofloxacin and Ciprofloxacin of the quinolones group were the first choice for the treatment of UTIs, and this causing the excessive use and thus *E.coli* become resistant to it.

The findings of the current study of the group of aminoglycoside antibiotics, which include Gentamicin and Tobramicin, showed results that consisyent to [29] study which found that the percentage of resistant to Gentamicin was (37%). While the results were inconsistent with the results of [21], [27] studies, where the percentage of their resistance to Gentamicin were (48.1% and 28.2%) respectively. Furthermore, this result was inconsistent with the results of [21] as *E. coli* resitant to Tobramycin wa (37.3%). The reasons for the resistance of *E. coli* to the group of aminoglycosides is that the bacteria possess Aminoglycosides modifying enzymes, which leads to their resistance to the aminoglycosides. The result of the current study was inconsistent with the results of [6] study whose reported that the resistance to Tetracycline was (66%). The reason for *E. coli* to this antibiotic is that they have efflux pumps that eject the antibiotic outside the bacterial cell (Kapoor, 2017). The results of this study also found that the resistance to Amikacin was consistent with the results of [17] study which was (12%). In addition, the results of this study of carpenems antibiotics that include Imipenem and Meropenem, the optimal and effective treatment for urinary tract infections, were inconsistent with the observations of [17] study, where the percentage of resistance to Imipenem was (5%) and it was not in agreement with the findings of [21] study among the percentage of resistance to Imipenem was (3.9%).

Nitrofurantoin is one of the oldest and best antibiotics to be used in the treatment of UTIs caused by *E.coli*. This study was consistent with many previous studies, including the results of the [6] study that reported the resistance rate to this antibiotic was (4%), and also consistent with Tajbakhsh. (2016) study conducted in Iran as the resistance of *E. coli* to Nitrofurantoin was (6%).

The ERIC-PCR genotyping method was performed to detect the genetic relationship between the bacterial isolates. The results of this study detected there is genetic relationship between *E. coli* isolates, and also detecting 14 patterns, while the molecular weight of detected bundls was between 100-3000 base pairs (Table 1). Moreover, as shown in (Figure 4), the results revealed the presence of 7 groups containing similar and genetically close genotypes, while the other isolates showed different genotypes with no genetic relationship. The first group contained two isolates (1,5) isolated from patients with urinary tract infections; these isolates also belonged to females at the age of 30 years and showed high resistance to carbencillin and ampicillin.

The second group also contained two isolates (3,4), and these isolates were with genetic relationship, and isolated from 25-year-old females with urinary tract infections and showed high resistance to Tetracycline and Ciprofloxacin). Furthermore, the third group included two genetically related isolates (2,8) isolated from patients admitted to Yarmouk Teaching Hospital with urinary tract infections belonging to males with 40 years with high resistance to antibiotics. While the fourth group contained two isolates (9,6) isolated from people with urinary tract infections with low resistance to antibiotics, but were sensitive to Imipenem and Tobramycin. The fifth group contained two isolates (1,6) belonging to 25-year-old females with urinary tract infections that showed high resistance to cephalosporins (ceftazidime, cefraixone).

The sixth group detected two isolates (1,3) isolated from people hospitalized in a Baghdad Teaching Hospital/Medical City, belonging to males at the age of 20 years, and the isoaltes showed high resistance to Rifampcin and Gentamacin. In contrast, the seventh group contained two isolates (2,8) that showed sensitivity to Nitrofurantion.

The findings of the current study were consistent with those of a that there were 14 genotypes isolated from people with UTIs, while it is inconsistent with the observations of who detected 20 genotypes isolated from patients with UTIs, and the results were compatible with [24] which found 9 genotypes.

Thus, genotyping methods are important in detecting the genetic relationship between the bacterial strains, and also are important in the bacteria classification, identifying the infection sources and identifying and eliminating the most virulent strain [2].

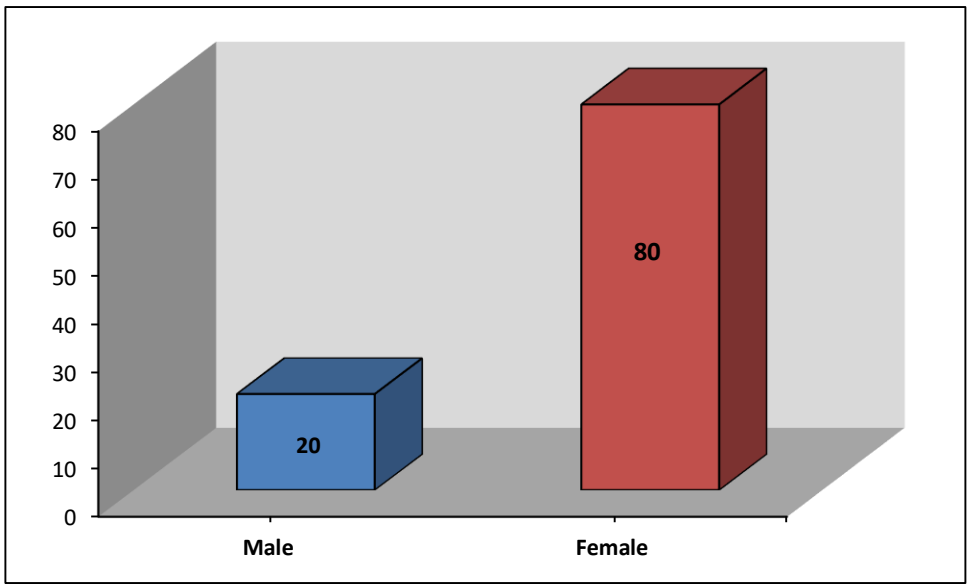

Figure 1: Distribution of *E. coli* according to the sex.

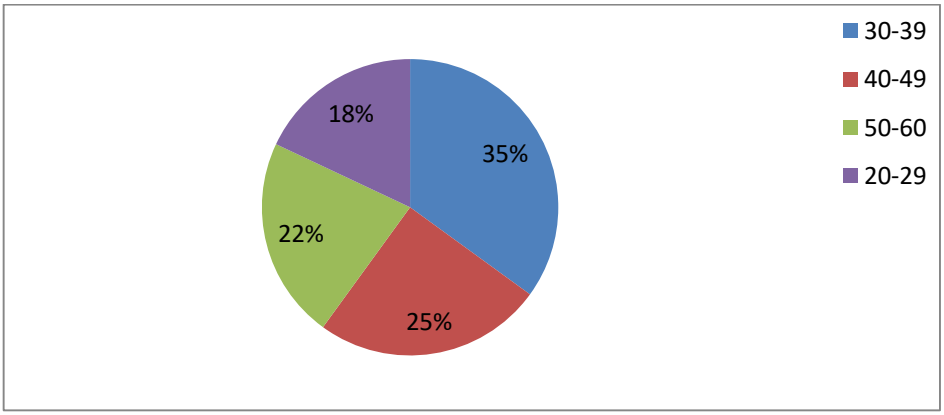

Figure 2: Distribution of *E. coli* according to the Age.

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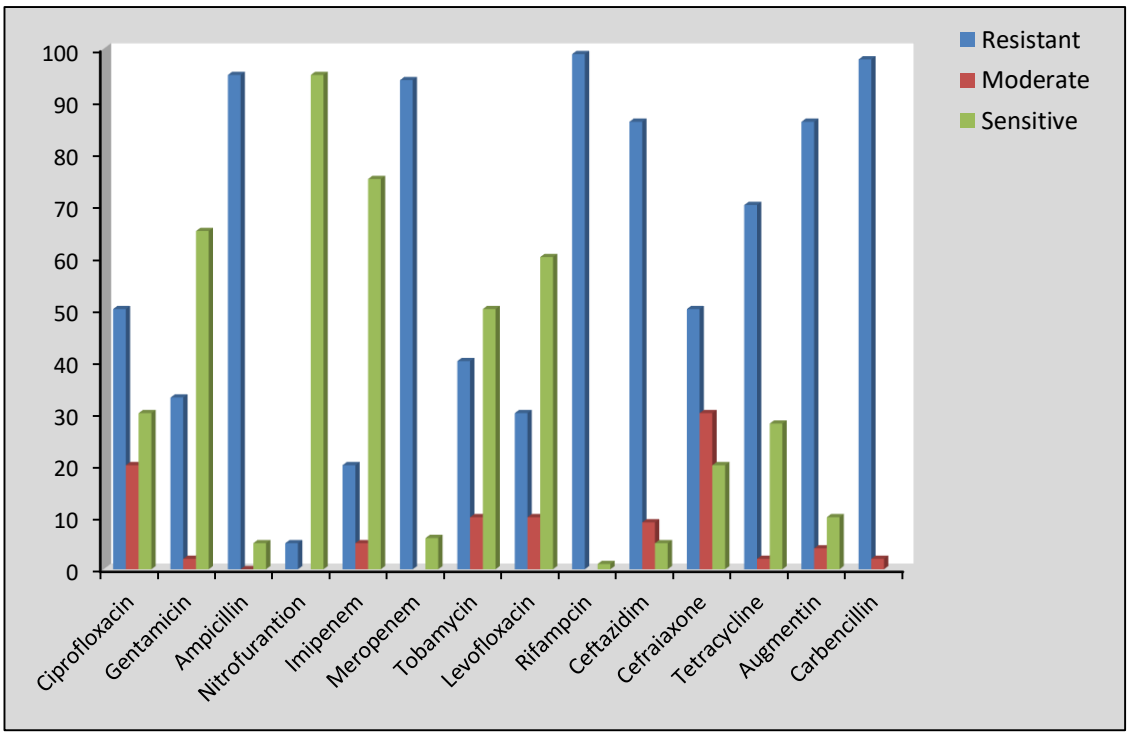

Figure 3: The percentage of resistance and sensitivity of *E. coli* using different types of antibiotics

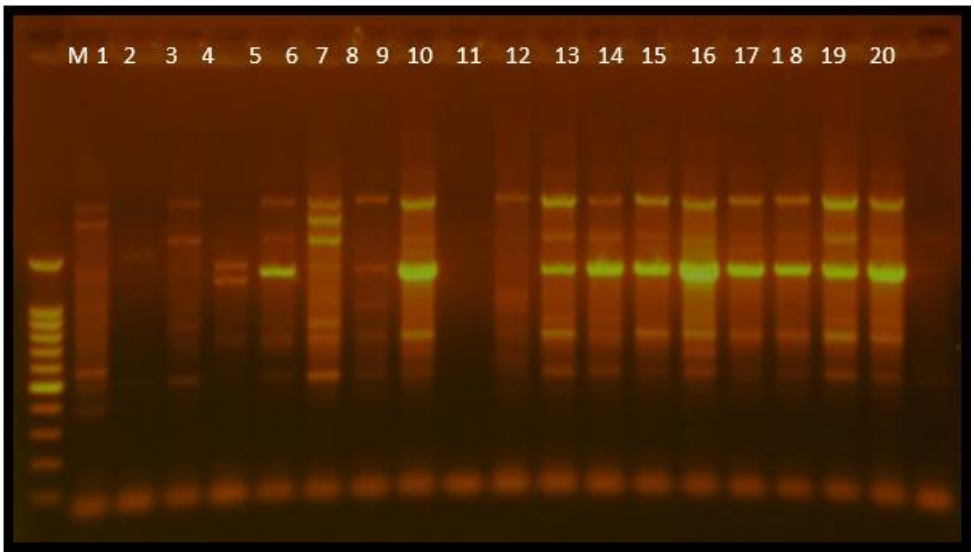

Figure 4: The gel electrophoresis of PCR product of E. coli isolates using specific primers using ERIC-PCR method (100-1500) base pairs on agarose gel under 100 volts of voltage for 80 minutes.

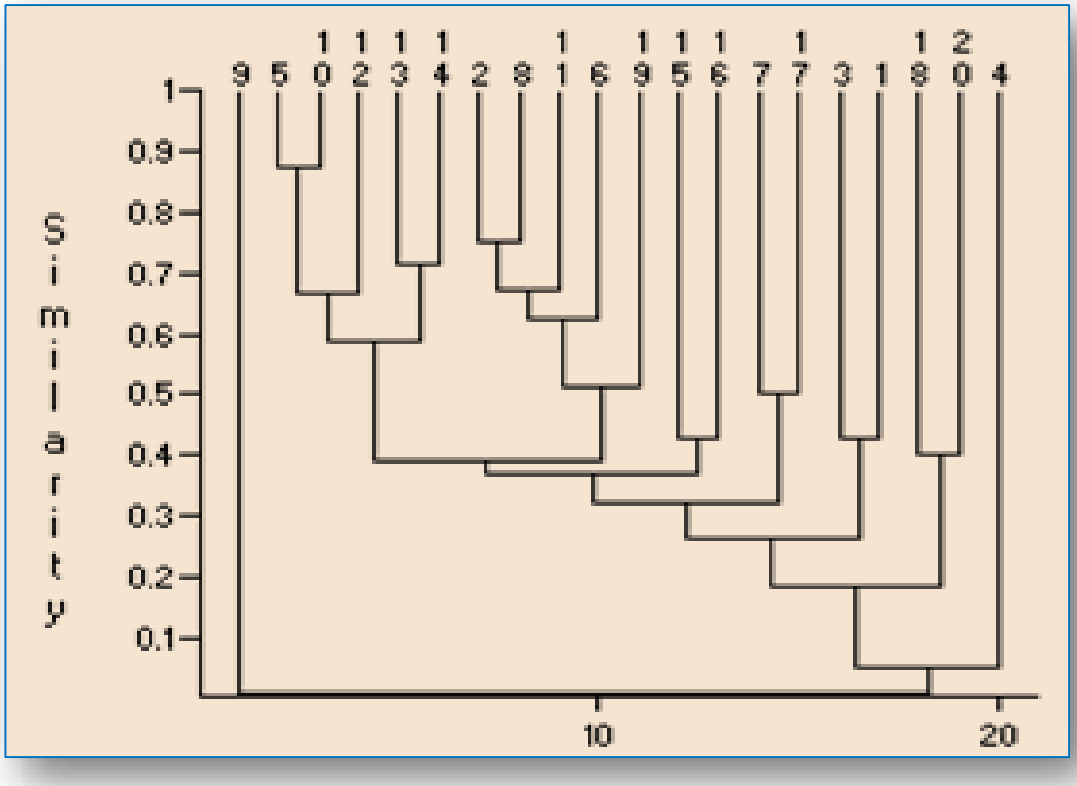

Figure 5: Dendogram of E. coli isolates using up/GMA Jaccard.

Table 1: The moleculr weights and percentage of the bundls produced using ERIC-PCR

Bundles	The molecular weight (bp)	No. of Isolates	The percentage (%)
ERIC1	100	2	4
ERIC2	200	4	22
ERIC3	250	2	4
ERIC4	300	10	18
ERIC5	350	4	22
ERIC6	400	9	16
ERIC7	500	8	15
ERIC8	600	14	25
ERIC9	700	4	22
ERIC10	800	3	6
ERIC11	900	3	6
ERIC12	1000	8	15
ERIC 13	2000	11	20
ERIC 14	3000	16	29

4. Conclusions

All isolates of *E.coli* that isolated from urinary tract infections showed MDR multidrug resistance, and the isolates showed the presence of 14 genotypes using ERIC-PCR method.

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