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Genotyping and Beta–Lactamase Production of *Escherichia coli* Isolated from Different Sources

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Abstract

It was collected 145 samples from a variety of clinical sources, including urinary tract infections, wounds, blood, respiratory tract infections, stool, sputum, and high vaginal swabs, from various hospitals in Baghdad. Following the collection, we conducted microscopic examinations, biochemical examinations, and diagnosis. The Vitek-2 Compact System conducted the final analysis. It was gotten 50 isolates of *Escherichia coli*, and 34% of those were from urinary tract infections, wounds, respiratory system infections, sputum, and vaginal swabs. 72% of the isolates were from urinary tract infections, 14% were from wounds, and 2% were from respiratory system infections. Next, we conducted genotyping and tested for beta-lactamase resistance. It was genotyped bacterial isolates using the ERICPCR technique to understand their genetic relationships. The results showed three different patterns. We obtained (E19, E42, E50, E75) from each type, which consists of a group of isolates genetically related to each other on the genetic tree. We found genetic relatedness in isolates (E13, E140, E54, E30, E120, E60) that showed no genetic relatedness (E36, E38). Phenotypical detection revealed the presence of Extended-Spectrum B-Lactamase (ESBLS) beta-lactamase. The Double Disk Synergy Test (DDST) results revealed that E36, E13, E38, E120, and E60, with a percentage of 41.7%, possessed the ability to produce beta-lactamase, while E42, E50, E19, E30, E75, E140, and E54, with a percentage of 58.3%, lacked this ability.

Keywords: *Escherichia coli*, Beta-lactamase, Genotyping, Double disk synergy test.

1. Introduction

Esherichia coli is a facultative anaerobic, Gram-negative bacterium of naturally occurring intestinal bacteria that colonizes the mucous layer (1, 2). Theodore Escherichie initially named it "Bacterium coli commune" and isolated it from infant feces in 1885 (3). It does not contain spores in the form of rods (4), and it is a common cause of health concern (5). Hospitals regard it

as a contaminant (6). Humans and animals have digestive systems, and certain species, like *Escherichia coli* O157:H7, are known to be pathogenic. Therefore, *E.coli* bacteria cause many serious clinical symptoms, such as fever, bloody diarrhea, and hemolytic uremic syndrome, which can lead to death in both children and the elderly (7, 8). It thrives in temperatures ranging from 7 to 50°C, with an optimum temperature of 37°C. It possesses a single circular chromosome, some of which contain circular plasmids. *E. coli* bacteria include other species within the genus *E.hermanni*, *E.albertii*, *E.vulneris*, *E.blattae* and *E.fergusonii* (9). The *E. coli* bait has virulence factors that break down hemolysin and stick to surfaces (10), as well as biofilm (11, 12), colicin, aerobactin, cytotoxic necrosis factor, cell surface hydrophobicity, and some specific serotypes of the O and K antigens that are more resistant to phagocytosis and kill bacteria than normal serum (13). *E.coli* is the main pathogen that causes urinary tract infections (UTI), meningitis in children, middle ear infections, and wounds (14, 15). It has six species that cause diarrhea (Diarrheagenic *E.coli* (DEC)) according to (16): Enterotoxic *E.coli* (ETEC), Entero Aggregative *E.coli* (EAEC), Enteropathogenic *E.coli* (EPEC), EnteroHemorragic *E.coli* (EHEC), EnteroInvasive *E.coli* (EIEC) and Diffusely Adherent *E. coli* (DAEC). *E.coli* is the primary cause of biofilm-associated diseases; the biofilm enables bacteria to resist antibiotics up to 1000 times and suppresses the host's immune system, making biofilm-associated infections challenging to treat (17). Antibiotics play an important role in the treatment of bacterial infections, but the wrong use of antibiotics leads to the emergence of high multidrug resistance in different species of bacteria, especially negative bacteria, because they produce broad-spectrum β-lactamase enzyme (ESBLS) extended-spectrum beta-lactamase (18, 19). In genomics, not only is there amazing diversity among bacteria, but there is also great diversity even among *E. coli* strains, of which *E.coli* was first observed through phenotypic characterization (20, 21, 22).

2. Materials and Methods

2.1. Bacterial sample collection

One hundred forty five samples were collected from different clinical sources, of different ages and both sexes, from several hospitals in the city of Baghdad, including (Baghdad Teaching Hospital, Educational Laboratories, Shaheed Ghazi Al-Hariri Hospital for Specialized Surgeries in the Medical City, Al-Imamin Al-Kazemin (PBUH) Teaching Hospital, Central Children's Teaching Hospital, and Al-Yarmouk Hospital). The study was conducted for the period from (6/11/2022) to (22/12/2022) at different clinical sources.

2.2. Isolation and identification of bacteria

The collected samples were cultured on blood agars, MacConkey agars, and Eosin agars.

2.3. Biochemical tests

The biochemical testes were also conducted (citrate Utilization, indole Prodution) to identify *E.coli* from other Enterbacteriacae bacteria (23), as well as Methyl red-Vogues Prosquer, Catalase, Glucose, Fermentation, Hemolysin, and oxidase test. The final diagnosis of the isolates was made by the Vitek-2 system compact (24) and (25). After that, it was obtained (50) isolates of *E.coli* from different clinical sources.

2.4. DNA extraction

Genomic DNA was extracted from the preserved bacterial isolates (E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, and E54) for *E.coli* according to the company's protocol and using a DNA kit (ZYMO RESEARCH).

2.5. The primers used in the interaction

The primers were lyophilized, dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution, and kept at -20 to prepare a 10 pmol/µl concentration as work primer suspended. 10 µl of the stock solution in 90 µl of the free ddH_2O water to reach a final volume of 100 µl was investigated by IDT (Integrated DNA Technologies Company, Canada).

2.6. The primers used in the interaction

By using the specific primer ERIC (45), as shown in **Table 1**,

Table 1. The specific primer ERIC.

Primer	Sequence	Tm (°C)	GC (%)
Forward	5′- ATGTAAGCTCCTGGGGATTAAC -3′	54.4	45.5 %
Reverse	5′- AAGTAAGTGACTGGGGTGAGCG -3′	59.0	54.4 %

The contents of the PCR tubes were mixed well using the Vortex, then placed in a PCR thermal cycler, as shown in **Table 2**.

Table 2. The optimum condition of detection gene.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	5 min.	1 cycle
2-	Denaturation -2	94°C	30 sec	40 cycle
3-	Annealing	36°C	45 sec	
4-	Extension-1	72°C	45 sec	
5-	Extension -2	72°C	7 min.	1 cycle

2.7. Agarose gel electrophoresis

It was prepared according to the method (26) as follows: dissolve (1.5%) grams of agarose gel in 100 ml of TBE Buffer solution at a concentration of 1x and microwave at a temperature of 60 °C for a period of 15 minutes, then let the gel cool to a temperature of 45 °C, and then add 5 microliters of red dye and mix well with the gel. Pour the agarose gel into the tray after fixing the combs, then leave to solidify at room temperature for 30 minutes. After that, the combs were carefully removed from the gel to obtain the holes.

2.8. Detection of Extended Spectrum β–Lactamases (ESβLs)

Twelve cuts of 50 isolates were taken from *E.coli* bacteria to perform the investigation of βeta-lactamase. The ability of *E.coli* isolates to produce beta-lactamase was revealed by double-disk synergy, where the bacterial suspension of *E.coli* bacteria was present at the age of 18-24 hours, where its density was compared with the solution of the standard constant turbidity McFarland, whose cell number is approximately equal to 1.5×10^8. Mueller-Hinton medium was inoculated by using a sterile cotton swab in the method of brushes (mat method) and left to dry for 10 minutes. After that, antibiotic tablets were installed, and the anti-tablets (Amoxicillin, Clavulanic acid) were placed in the medium of the dish and offset by the antibiotic (Cefotaxime) on one side and the other side, in which the antibiotic (Ceftazidime) provided that the distance between each antibiotic is not less than 20-25 mm.

Then, after the end of the incubation period (18-24hours) and a temperature of (37 °C), where the result is positive and the isolate produces enzymes (β–Lactamases) broad-spectrum if the inhibition zone of Cefotaxime, Ceftazidime is towards the antibiotic tablet (Amoxicillin, Clavulanic acid) (27).

3. Results

3.1. Isolation identification

Fifty (34%) isolates of *E.coli* were obtained from different clinical cases (urinary tract infections (72%), wounds (14%), respiratory tract infections (2%), sputum (10%), and vaginal swabs (2%)), and then E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, and E54 were taken for genotyping and β-lactamase antibodies (**Table 3**).

Table 3. Number of *E.coli* isolates for each source.

Isolate source	Number of isolate	Percentage
Urinary tract infections	36	72%
Wounds	7	14%
Sputum	5	10%
Respiratory tract swab	1	2%
Vagina swab	1	2%
Total	50	

3.2. Microscopic and Biochemical examination

It was used on all 50 isolates after staining them with a gram stain, so the result was negative rods. Other tests were also conducted on *E.coli* to confirm them on the isolates (E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, E54), so the results of the biochemical tests were negative on the (E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, E54) isolate in the test oxidase, urea, Vogues-proskauer, citrate, and gram stain. The biochemical tests were positive tests on E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, and E54. The isolate tests are catalase, indole, and methyl red. The positive and negative results are consistent with the results of (28).

3.3. The ability of *E.coli* to produce β-lactamase

The results showed that (E36, E13, E38, E120, E60) can make beta-lactamase (41.7%), but (E42, E50, E19, E30, E75, E140, E54) can't (58.3%). **Figures 1** and **2**. When the effects of the inhibition zone (Cefotaxime and Ceftazidime) extend towards the central disk (Amoxicilin and Clavulanic acid), the isolate is considered a producer of beta-lactamase, and this is considered an indicator of the presence of beta-lactamase. The results of the current study are similar to those of (29), with 56% of bacteria producing beta-lactamase and 64% not producing beta-lactamase. The results of (30) contradict the findings of the current study, indicating a higher production of beta-lactamases compared to those that were not produced. As well as the results of (31), it was found that 70% is a producer of beta-lactamases, which does not correspond to the results of the current study. The percentage of 26.8% in the previous study (32) closely aligns with the findings of the current study. Whereas the results of the current study are not consistent with the results of the (33) study, which showed that *E. coli* is producing beta-lactamase enzymes by 53.8% and non-producing beta-lactamase by 38.8%.

The results of the current study are consistent with the results of the (34) study, which recorded *E. coli* producing beta-lactamase (45.7%) and non-producing (54.2%). Beta-lactamases have been widely used to treat several different types of infections that affect humans; the cause of

them is *E.coli*. *E.coli*, which breaks down the beta-lactamase ring, is one of the important causes of resistance to beta-lactamase (35; 36; 37). The study's results indicate that doctors should consult before using antibiotics to prevent severe resistance in *E. coli* bacteria and to prevent the development of drug resistance in bacteria.

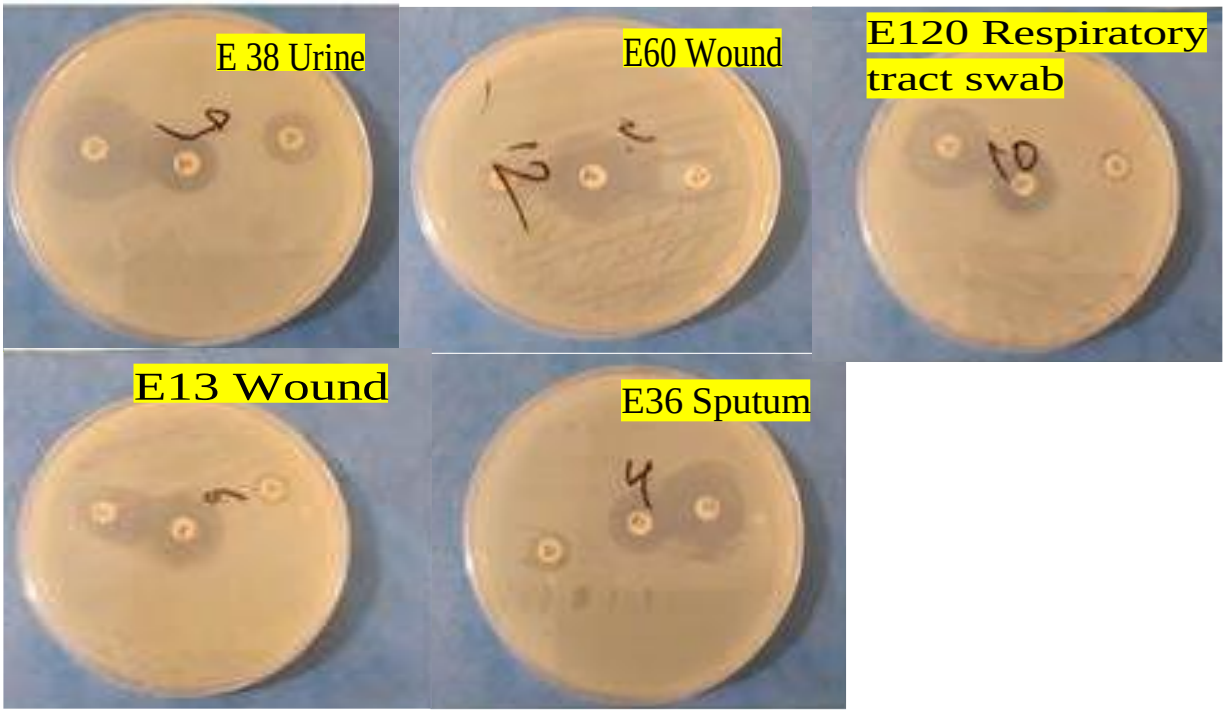

Figure 1. Production of extended-spectrum beta-lactamase (ESBLs) in *E.coli* isolates.

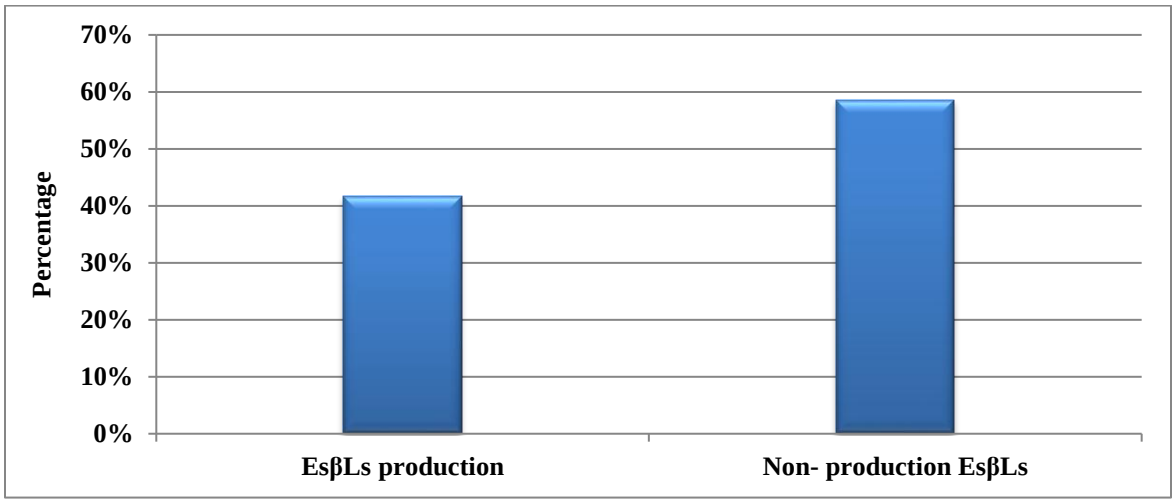

Figure 2. The percentage of ESBLS production by *E. coli* bacteria.

The number of isolates (E36, E13, E38, E120, E60, E42, E50, E19, E30, E75, E140, E54) was chosen due to their high resistance. Three types of antibiotics were used (Cefotaxime), (Ceftazidime), and (Amoxicillin- Clavulanic Acid) according to the method used for the source (27).

3.4. Genotyping of *E.coli* using the ERIC method

Genotyping is used to find a relationship between bacterial strains in terms of their genetic content. It is also important in finding genetic relations between strains and is also considered less expensive and faster than other techniques related to genetic similarity, as well as important

in the field of epidemiological studies and also in the classification of bacteria, methods of infection, and the distinction of bacterial strains that have high virulence in order to prevent their spread and eliminate them (38, 39). Genetic relation was identified and found by profiling *E.coli* under study by using the Enterobacterial Repetitive Intergenic Consensus (ERIC) method, which was found to be sequenced in multiple regions of the bacterial genome. The results of the current study showed the existence of genetic relations between bacterial isolates that were isolated from different bacterial sources, as well as the presence of (3) genotypes ranging in molecular weights for their bands between 2000 and 300 base pairs, as shown in **Figure 3** and **Table 4**. These results are consistent with the third group of the (39) study, which showed five patterns and one clone with genetic relation and are close to the results of the current study by using the ERIC method, while the results of the current study do not correspond to the results of the (40) study, where it showed 27 genotypes and 4 clones that have genetic relation. The reason for the difference in the source of isolation .The results showed in **Figure 4** the presence of one clone while (E19, E42, E50, E75, E13, E140, E54, E30, E120, E60) isolates contained different genotypes. The clone contained two isolates (E38, E36), while isolate No. (E36) was from a 36-year-old male isolated from sputum from Al Imamain Al-Kadhimain Medical City. As for isolate No. (E38), it was a 38-year-old female and was isolated from urine and also from Al Imamain Al-Kadhimain Medical City. The reason for the genetic relatedness is the location (Al Imamain Al-Kadhimain Medical City) and age (close), and the E38 and E36 also possessed genes (*CsgA*, *YajA*). Also, the results of the current study do not correspond to the results of the (41) study, which consists of 27 genotypes and 14 clones genetic relation, while the results of the current study do not correspond The reason for the difference in geographical location is its source to the (42) study that results were recorded for 8 genotypes and 10 clones. Also, the results of the current study do not correspond to the reason for the geographical location (India, Vietnam, China, Saudi Arabia) as well as the difference in source to the (43) results, which show 7 patterns and 12 clones. It also does not correspond to the results of the (44) study that showed 14 genotypes and 7 clones. Thus, genetic relationships are detected through profiling methods in the classification of bacteria, the identification of the most deadly strains, as well as the identification and elimination of sources of infection (38), as shown in **Table 4**.

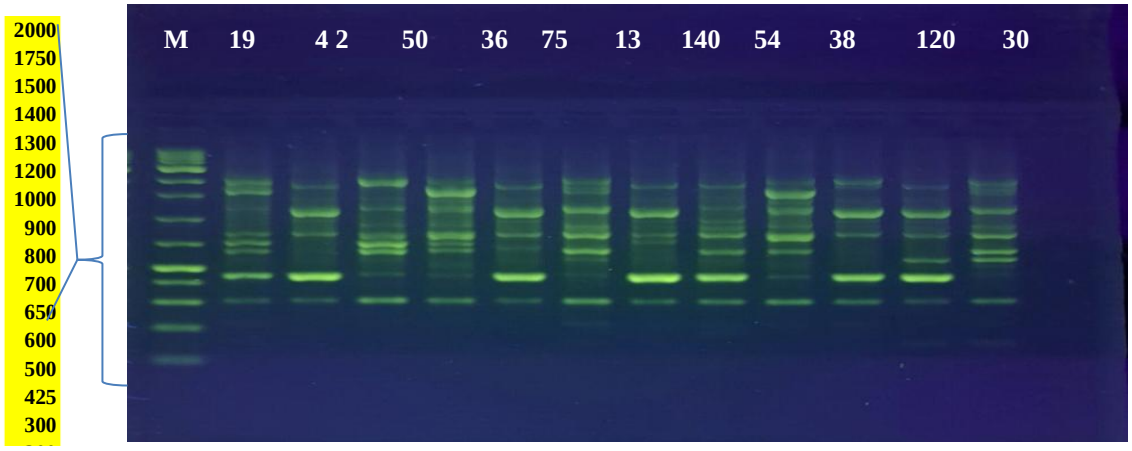

Figure 3. Electrophoresis of the polymerase chain reaction (PCR) product of *E.coli* bacteria isolates, using a primer to perform genotyping using the ERIC method (100-2000) base pairs on agarose gel at a concentration of (2%) and a voltage difference of 5 volts and 1:30 hours. Path (M) represents the size guide (100) base pairs. Paths (E 19, E 38, E 4 2, E 50, E 30, E120 (E140, E13, E75, E36, E 54, E 60) for positive isolates. The bands that appeared start from 300 to 2000.

Table 4. Molecular weights and percentages of bands produced by the ERIC method.

Bands	Molecular weight (bp)	Isolates number	Percentage %
ERIC1	300	12	100%
ERIC2	450	12	100%
ERIC3	500	3	25%
ERIC4	600	5	41%
ERIC5	650	7	58.3%
ERIC6	700	7	58.3%
ERIC7	800	12	100%
ERIC8	900	9	75%
ERIC9	1000	5	41%
ERIC10	1200	7	58.3%
ERIC11	1300	11	91.6%
ERIC12	1400	5	41%
ERIC13	1500	6	50%
ERIC14	1750	9	75%
ERIC15	2000	7	58.3%

Table 4 and **Figure 3**, show the bands with molecular weights starting from (300 to 2000) showing how many isolates appeared in it, as band (ERIC1) appeared in its 12 isolates with a molecular weight of 300 and its percentage is 100%, and band (ERIC2) also appeared. It contains 12 isolates with a molecular weight of 450, and their percentage is also 100%. As for band (ERIC3), there appeared 3 with a molecular weight of 500, and their percentage is 25%, etc. for the rest of the bands. This is the commonality between **Table 4** and **Figure 3**.

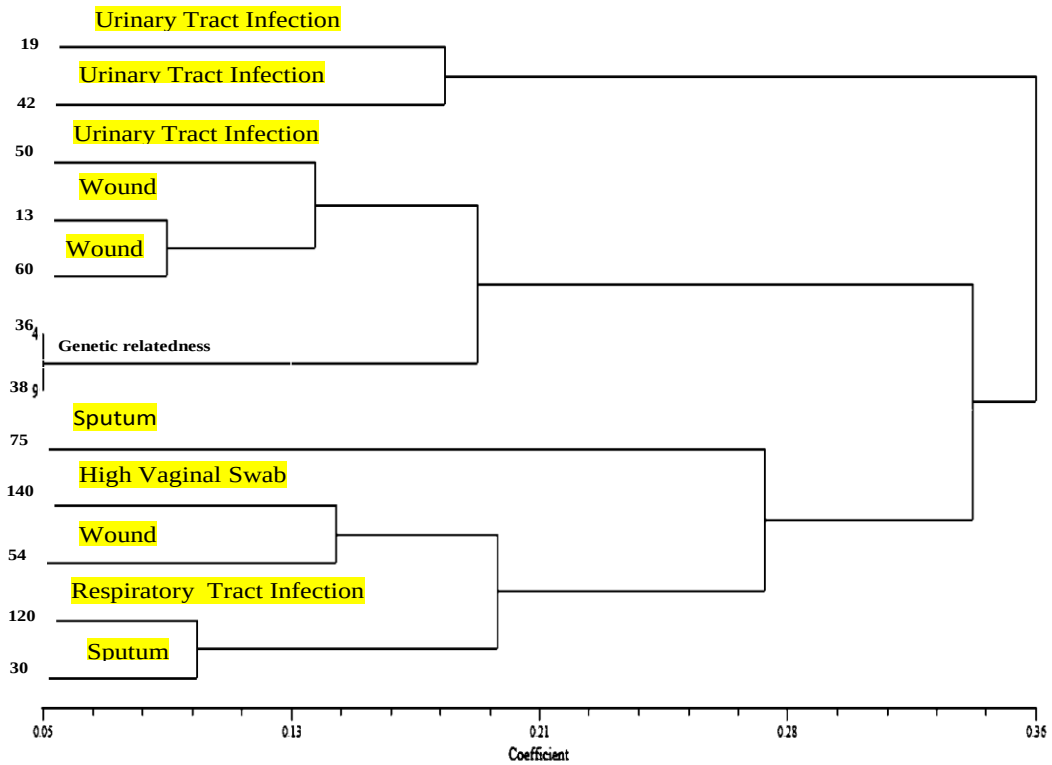

Figure 4. Cluster Analysis Diagram of *E.coli* isolates using ready-made program NTSYS-pc (Numerical Taxonomy System) to obtain the relation of the genetic tree.

4. Conclusion

The genotyping results revealed that (E38, E36) share a genetic relationship with (E38, E36, E19, E42, E50, E75, E13, E140, E54, E30, E120, E60) isolates, while (E19, E42, E50, E75, E13, E140, E54, E30, E120, E60) isolates do not share a genetic relationship. In terms of beta-lactamase production, it was observed that (E36, E13, E38, E120, E60) produced beta-lactamase at a rate of 41.7%, while (E42, E50, E19, E30, E75, E140, E54) did not produce beta-lactamase at a rate of 58.3%.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health No. 5824 in 2/12/2022.

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