Microbes and Infectious Diseases 2025; 6(3): 4047-4057

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Enterobacterial repetitive intergenic consensus typing of pathogenic *Escherichia coli*

***Israa Radwan Ali*[1]*.**

Department of Science, College of Basic Education, Mustansiriyah University, Baghdad, Iraq

ARTICLE INFO

Article history:
Received 3 February 2025
Received in revised form 10 February 2025
Accepted 15 February 2025

Keywords:
Bacterial diversity
Biofilm formation
DNA typing
E. coli

ABSTRACT

Background: *Escherichia coli* (*E. coli*) is considered as an opportunistic pathogen. It is rarely detected in healthy people but occasionally discovered in medical and domestic sources. DNA typing techniques have regularly been employed to evaluate the diversity of *E. coli* specimens. The aim of this study was to evaluate the prevalence of various isolates responsible for the infection among specific population at a specific time. **Methods:** In this cross-sectional study, 60 *E. coli* isolates were collected from different clinical samples including urine, wound, burn, sputum, and blood from hospitals in Baghdad, Iraq from October 2023 to December 2023. Biofilm composition was tested using Congo red agar. The genetic diversity and antibiotic resistance of the isolates were examined. API 20NE and PCR tests targeting *rpsL* genes were performed, followed by ERIC-PCR for DNA sequencing amplification. **Results:** Biofilm formation on Congo red agar showed 39 positive isolates, indicating a high potential for persistent infections, while 21 isolates showed no biofilm activity. Antibiotic susceptibility testing revealed complete resistance to tetracycline, ceftazidime, and cefepime, with moderate resistance to amoxicillin-clavulanate. Significant sensitivity was observed for norfloxacin and azithromycin. The ERIC-PCR analysis identified 10 DNA fragments (200–2500 bp), forming two main clusters: Cluster A (26.6%) and Cluster B (73.3%), with sub-clusters B1 and B2, suggesting clonal spread within hospitals. **Conclusion:** The isolates from the same hospital were often genetically linked, indicating clonal spread, while those from the same patient were usually unrelated, highlighting diverse strain types. The ERIC-PCR effectively distinguishes *E. coli* strains, supporting the hospital surveillance and prevention of multidrug-resistant spread.

Introduction

The organism *Escherichia coli* (*E. coli*) is an opportunistic pathogen and frequently found in soil, water, plants, animals, and people. Normally, only a small portion of healthy people have *E. coli* in their digestive system [1,3]. It is occasionally discovered in saliva and on moist parts of human skin. With little nourishment it can grow in any damp area. Furthermore, it can withstand a broad range of environmental circumstances [2]. As a result, this microbe is commonly detected in both the medical setting and domestic sources such as floors, sinks, bathtubs, soap dishes, and dishcloths as it can cause hospital-acquired infections in immunocompromised hosts, burned patients, and people with malfunctioning homeostasis mechanisms, or those with metabolic abnormalities. Using conventional techniques like biotyping,

DOI: 10.21608/MID.2025.357686.2506
* *Corresponding author:* Israa Radwan Ali
E-mail address: *israaradwanali@uomustansiriyah.edu.iq*

ribotyping, serotyping, pyocin typing, and phage typing, *E. coli* can be internally divided into sub-clusters. Compared to the molecular typing techniques, the discriminatory power of these conventional techniques is significantly low. DNA typing techniques have frequently been employed to evaluate the diversity of *E. coli* specimens [1, 3]. Biofilms give the bacteria a means of life by arranging to efficiently utilize the nutrients at their disposal and blocking their access to the antimicrobial agents, antibodies, and white blood cells. The biofilm-forming bacterial populations benefit the matrix, which is made up of proteins, extracellular DNA, and polysaccharides. These benefits include protection from immune cells and help to adhesion aided by bacterial adhesins. One such method is pulsed-field gel electrophoresis (PFGE) [4]. Ribotyping and polymorphic DNA analysis methods encompass restriction fragment length polymorphism (RFLP) [5], random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), arbitrarily primed PCR (AP-PCR) [6], and PCR based on repeating elements (rep-PCR). The rep-PCR is a technique for identifying the bacterial genomes by examining the strain-specific patterns generated by amplifying repetitive DNA sequences present in the bacterial genome using PCR [7]. The three main types of repetitive elements used for typing are the repeating extragenic palindromic (REP) sequences, Enterobacterial repetitive intergenic consensus (ERIC) sequences, and BOX elements [8]. The REP-PCR and ERIC-PCR have been demonstrated to be effective methods for typing *E. coli* isolates. In this investigation, we typed clinical *E. coli* isolates using the ERIC-PCR technique. The rise of drug-resistant isolates has further impeded antibiotic effectiveness, decreasing the number of therapeutic alternatives available to the health services, which has led to an increase in medical costs, rates of morbidity, and mortality [9]. Therefore, this study aims to evaluate the prevalence, genetic diversity, and antibiotic resistance of *E. coli* isolates obtained from clinical samples using ERIC-PCR and biofilm formation analysis. This investigation provides insights into the clonal relationships of isolates, aiding in infection control and the prevention of multidrug-resistant *E. coli* dissemination in hospital settings.

Materials and Methods

Bacterial isolation and identification

This prospective cross-sectional study was conducted between October 2023 and December 2023 in various wards of the Municipal Hospital, the Main Hospital, and the Outpatient Departments in Iraq. The inclusion criterion was any clinical specimen including burn, wound, sputum, blood, and urinary tract infections from which *E. coli* was isolated using the standard laboratory methods. A total of 60 *E. coli* isolates were collected; 12 from burns, 21 from wounds, 2 from sputum, 2 from blood, and 23 from urine. The bacteria were identified as *E. coli* using biochemical patterns in the API 20NE system (bioMérieux). The NCTC 6749 control strain was also evaluated. The stock cultures were stored at -80°C in trypticase soy broth (TSB, Difco) with 20% glycerol [10].

DNA extraction

Each isolate was cultured for 24 hr at 37°C in nutrient broth, and its purity was assessed on nutrient agar plates. The biomass was scraped from the agar plates, suspended in 100 µl of sterile distilled water, heated for 10 min, and centrifuged at 12,000 × g at 4°C. The remaining liquids were collected and 5 µl was used as PCR template [11].

The application of PCR

To verify that the isolates were *E. coli*, 25µL reaction volume containing GoTaq® Green Master Mix, DNA template, primers, and deionized water (nuclease – free) were prepared targeting the housekeeping gene (*rpsL* gene) using primers [12]. The PCR negative control containing no DNA template was also tested. The PCR reaction was carried out with the following parameters: an initial denaturation at 95°C for 5 min, then 30 cycles of 94°C for 30 sec, 57°C for 30 sec, 72°C for 1 min, and the final extension at 72°C for 7 min.

DNA amplification by ERIC-PCR

The chromosomal DNA of *E. coli* isolates was amplified using a forward primer) 5'-ATGTAAGCTCCTGGGGATTCAC-3') and a reverse primer (5'-AAGTAAGTGACTGGGGTGAGCG-3') to identify the repetitive sequences. The ERIC-PCR reaction was prepared with a total volume of 25 µL including Master Mix, primers (10 pmol/each), and 100 ng of E. *coli* DNA template. Filtered nuclease-free water was added to adjust the mixture to the final volume of 25 µL. The cycling protocol began with an initial denaturation at 95°C for 2 min,

followed by 35 cycles consisting of denaturation at 95°C for 40 sec, annealing at 51°C for 1 min, and extension at 72°C for 8 min. A final extension step was performed at 72°C for 16 min. Each PCR run included a positive control using *E. coli* DNA and a negative control containing only distilled water [13].

Gel Electrophoresis

The PCR products were mixed with loading buffer. Then, the mixtures were run on 1.5% agarose gel containing ethidium bromide at concentration of 0.5 µg/µL TBE (40 mM Tris-borate, one mM EDTA, pH 8). DNA size marker was also utilized. The bands were viewed and recorded under ultra-violet (UV) light. Repeated ERIC-PCR runs on similar strains were done to check for consistency [12,13].

Antibiotic Susceptibility Test and Detection of Biofilm Formation in *E. coli* Isolates

E. coli was isolated from various clinical isolates and a diverse range of antibiotics were selected for testing against the isolates. The disk diffusion method (Kirby-Bauer) was used by inoculating plates with *E. coli* isolates, placing disks saturated with antibiotics on the plates, incubating them, and then measuring the inhibition zones [14]. The Congo red agar (CRA) method was used to assess the biofilm formation in *E. coli* isolates, following the protocol by Freeman *et al*. (1989). The Congo red stain was prepared separately in an autoclaved aqueous solution and then added to the autoclaved brain heart infusion agar and sucrose. The test isolates were inoculated onto CRA plates and incubated aerobically at 37°C for 24 hr. Biofilm-producing isolates formed black colonies with a dry, crystalline appearance, while non-biofilm-producing isolates formed red or pink colonies [15].

Constructing Dendrograms and Assessing Genetic Relatedness

All isolates dendrograms were assessed for the cluster analyses. The banding pattern generated by ERIC-PCR served as the foundation for the construction of the dendrogram. Gel or antibiogram studies provided presence (1) or absence (0) data for each strain.

Statistical analysis

The genetic relatedness of *E. coli* isolates was determined using the Dice coefficient to analyze ERIC-PCR banding patterns. The banding patterns observed in gel electrophoresis were analyzed to construct a binary table, indicating the presence (1) or absence (0) of specific bands. A dendrogram was then created using the UPGMA method, visually representing the genetic similarities between different *E. coli* isolates. These analyses were performed using STATISTICA software, enabling the assessment of genetic relationships and the clustering of strains based on their ERIC-PCR profiles.

Results

The bacterial isolates were classified according to the infected part as follows: The lowest amounts of samples (5%) were obtained from blood cultures, and it was followed by the sputum (23.3%). The urine sample had the highest numbers of isolates (53.3%), indicating a high frequency of urinary tract infections. The numbers of isolates in wound and burn samples were significantly high but less than urine (46.7 and 36.7%, respectively), indicating a high frequency of infection in wound and burn cases. As shown in Figure 1 this distribution highlights the differences in spreading the bacteria in relation to the site of infection, with urinary tract and wound being the most predominant sites.

Among the isolates, 39 tested positive for biofilm formation, while 21 were negative. Antibiotic resistance patterns are displayed in the matrix (Figure 2), revealing robust co-resistance associations among certain antibiotics. Similar to ceftazidime (CAZ), which exhibited a strong positive correlation with cefepime (FEP) and Augmentin (Aug), norfloxacin (NO1) demonstrated a significant association with both ciprofloxacin (CIP) and chloramphenicol (C). Additionally, Ceftazidime/clavulanic acid (CLA) exhibited a strong correlation with tetracycline (TE) and chloramphenicol (C), suggesting a potential co-resistance mechanism. A substantial association was also observed between cefotaxime (CTX) and ceftazidime/clavulanic acid (CLA), as well as cefepime (FEP), indicating a shared resistance pathway.

Antibiotic susceptibility testing revealed high resistance levels among E. coli isolates, particularly against cephalosporins and tetracycline. All isolates (100%) exhibited resistance to tetracycline, ceftazidime, and cefepime (P = 1.7). Ceftazidime/clavulanic acid (CLA) showed resistance in 49 isolates, while 11 isolates were sensitive (P = 7.5). Chloramphenicol (C) resistance was observed in 46 isolates, whereas 14 isolates were sensitive (P = 4.2). Norfloxacin (NO1) and

azithromycin (AZM) demonstrated 41 sensitive and 19 resistant isolates each (P = 0.0062 and P = 0.006, respectively). Cefoperazone (CPO) exhibited a higher sensitivity rate, with 43 sensitive and 17 resistant isolates (P = 0.0011). Augmentin (AMC) displayed relatively a balanced sensitivity/resistance profile, with 40 sensitive and 20 resistant isolates (P = 0.01). Piperacillin (PIP) showed an almost equal distribution of resistance (31 isolates) and sensitivity (29 isolates) (P = 0.8). Cefepime (FEP) and ceftriaxone (CRO) had high resistance rates, with 50 and 52 resistant isolates, while 10 and 8 isolates were sensitive (P = 1.6 and P = 5.2, respectively). These findings indicate a significant level of antibiotic resistance among E. coli isolates, particularly against cephalosporins and tetracycline, while some antibiotics, such as cefoperazone, retained notable sensitivity levels. The dendrogram of *E. coli* isolates is displayed in Figure 3, arranged according to how similar their patterns of antibiotic resistance are. Very high similarity (over 0.90) is shown by Cluster A1 isolates (e.g., 45, 47, 49), suggesting similar resistance profiles. Cluster A3 isolates (e.g., 8, 14, 35) exhibited moderate similarity, whereas Cluster A2 isolates (e.g., 13, 12, 15) exhibited high similarity (0.80–0.90). Diverse resistance patterns are indicated by the low to moderate similarity (0.60–0.70) of Cluster B1 isolates (e.g., 3, 6, 9) and the low similarity (below 0.60) of Cluster B2 isolates (e.g., 32, 35, 39).

Figure 4 illustrates high bacterial resistance to several antibiotics, with complete resistance observed in cefotaxime (CTX), ceftazidime (CAZ), and tetracycline (TE) (100%), rendering them ineffective. Moderate resistance was noted in the second-line antibiotics like ceftriaxone (CRO) (86.6%), cefepime (FEP) (83.3%), and ceftazidime/clavulanic acid (CLA) (81.6%), indicating their partial efficacy. Chloramphenicol (C) exhibited a resistance rate of 76.6%. Lower resistance rates were found for piperacillin (PIP) (51.6%), augmentin (AMC) (33.3%), azithromycin (AZM) (31.6%), norfloxacin (NO1) (31.6%), and cefoperazone (CPO) (28.3%), suggesting some effectiveness for these antibiotics. Red dots represent statistical significance (p-value), where higher positions on the graph indicate higher P-values**.**

In the current investigation, variations in the amount and arrangement of bacterial recurring elements in the genomes of clinical *E. coli* isolates were identified using PCR with the ERIC primers sequences. The PCR identification of ERIC sequences obtained 10 DNA fragments spanning from 200 bp to 2500 bp. These fragments were found in 30.66%, 65%, 35%, 32%, for 1200 bp, 1500 bp, 2000 bp, and 2500 bp, respectively, with four isolates remaining untypeable. This data somewhat supports the results of the study by Nasif *et al.*, [13]. Two major clusters were identified by the dendrogram of similarity produced by the ERIC-PCR (A-B).The majority, cluster B, included 44 isolates (73.3%) that shared the same band pattern. Group B1 contained 32 isolates that shared 100% similarities, while 12 isolates were present in group B2. Cluster A contained 16 isolates (26.6%) that shared 3–6 bands. Thirteen isolates exhibited 100% similarity, resulting in 4 authentic clones with 3 distinct patterns at 100% similarity

Molecular weights and corresponding percentage values of ERIC-PCR bands are shown in Table 1. The most frequently occurring band has a size of 400 base pairs (bp), with an incidence of 65%, while the least frequent band, sized 1000 bp, is found in only 1.50% of samples. These percentages express distribution patterns as well as dominance of particular gene markers within analyses carried out on these samples.

The genetic similarity was represented by a dendrogram made using ERIC-PCR data (Figure 5). The horizontal axis shows the samples, and the vertical axis indicates the similarity level. The branches that are closely clustered each have higher genetic similarities, which provide insights into the genetic relationships and diversities of the samples. The ERIC-PCR and biofilm formation methods were used to classify *E. coli* isolates, producing different groupings. Based on the genetic similarity, the ERIC-PCR approach separated the isolates into clusters A and B, which were then further subdivided into smaller clusters (A1, A2, A3, B1, and B2). For example, the isolates from Cluster A1 (Ec3, Ec4, and Ec26) and Cluster B1 (Ec7, Ec8, and Ec18) exhibited a significant level of genetic similarity, whereas isolates from Cluster B2 (Ec14 and Ec15) had a more pronounced genetic profile. Furthermore, an examination of the biofilm formation showed that 21 isolates were biofilm-negative, and 39 isolates were biofilm-positive. Combining these techniques yields a thorough profile of the isolates' genetic similarities and capacity to produce biofilms.

Table 1. The percentage of ERIC-PCR bands and their sizes in base pairs

Band No.	Base pair	Percentage value
ERIC1	200	30.66%
ERIC2	400	65%
ERIC3	500	35%
ERIC4	600	32%
ERIC5	800	18.30%
ERIC6	1000	1.50%
ERIC7	1200	11.66%
ERIC8	1500	6.55%
ERIC9	2000	16.70%
ERIC10	2500	42.66%

Figure 1. Distribution of the isolates by the sample type. The lowest and the highest amounts of isolates were detected in blood (5%) and urine (53.3%) samples.

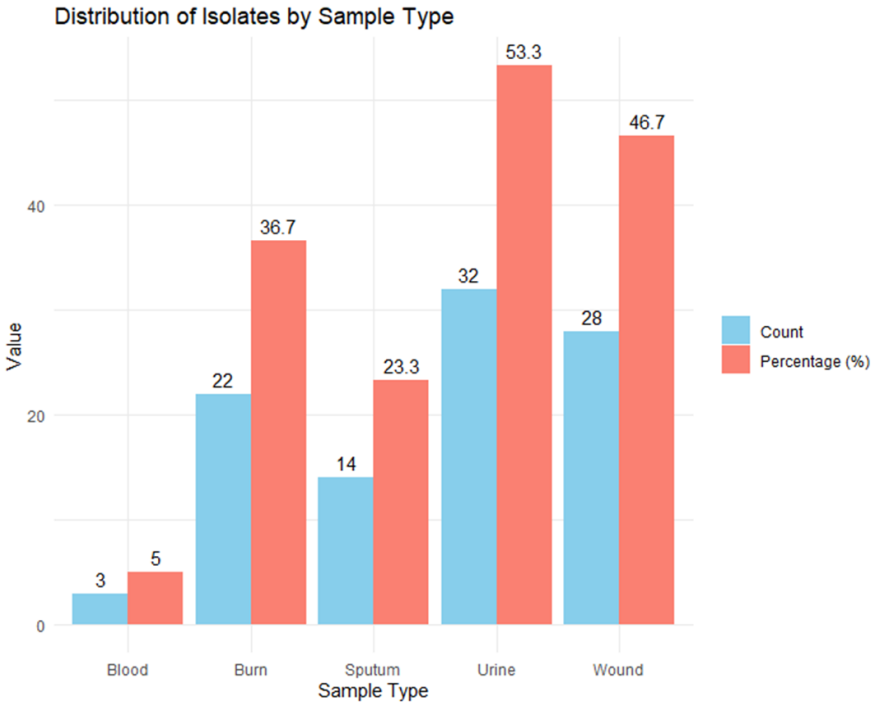


Figure 2. Patterns of co-resistance and inverse relationships among antibiotics.

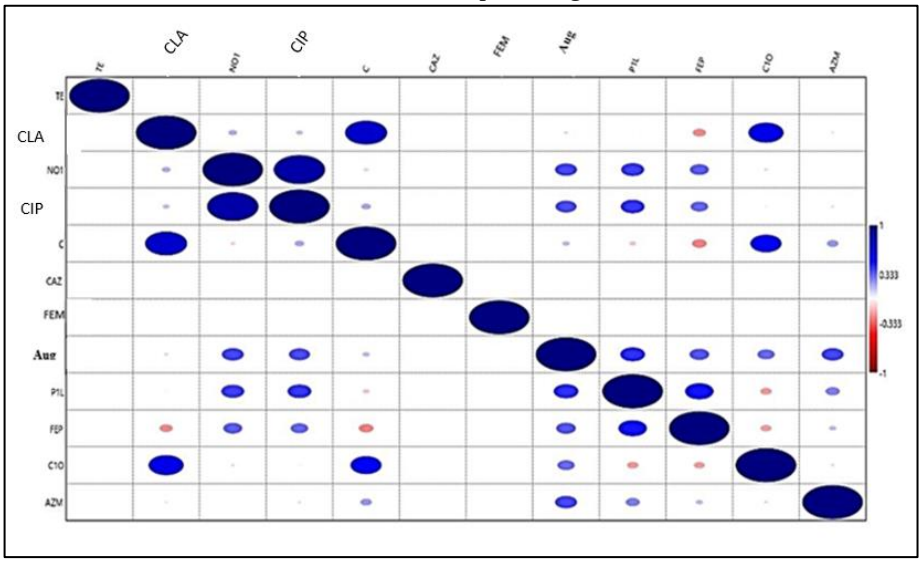


Figure 3. A dendrogram with genetic dice similarity coefficients analysis based on the antibiotic susceptibility test results.

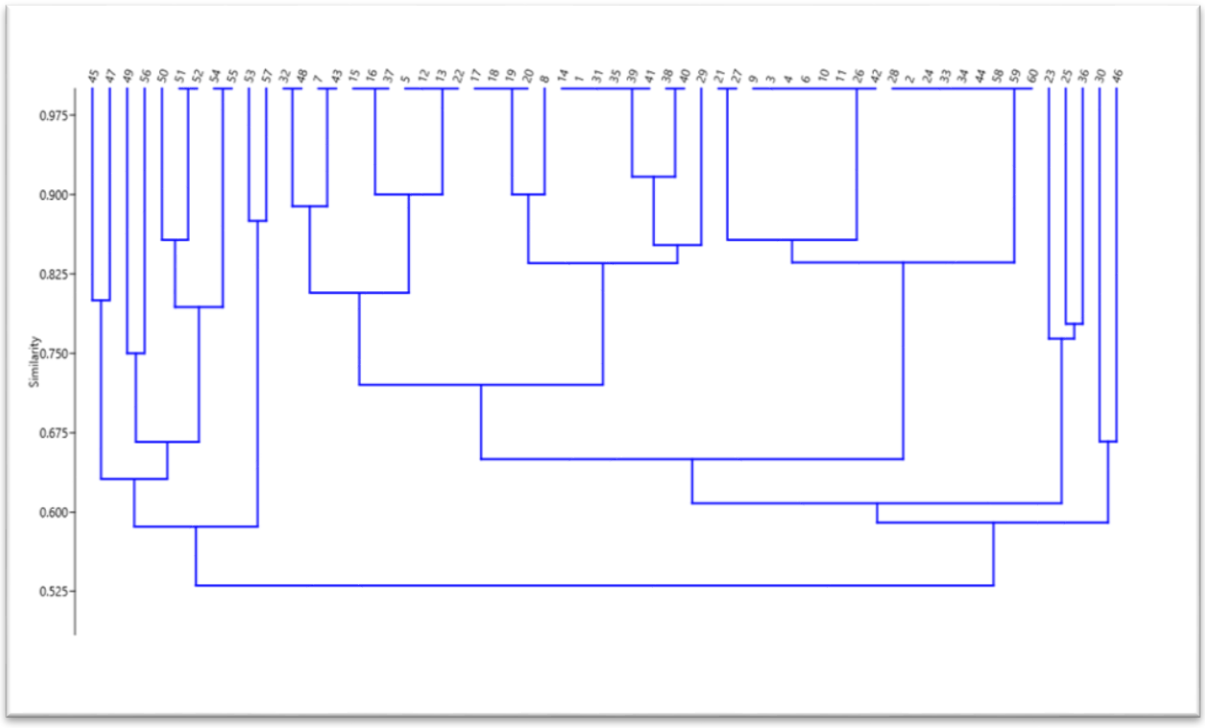


Figure 4. Assessment of bacterial resistance rates and statistical significance across antibiotics

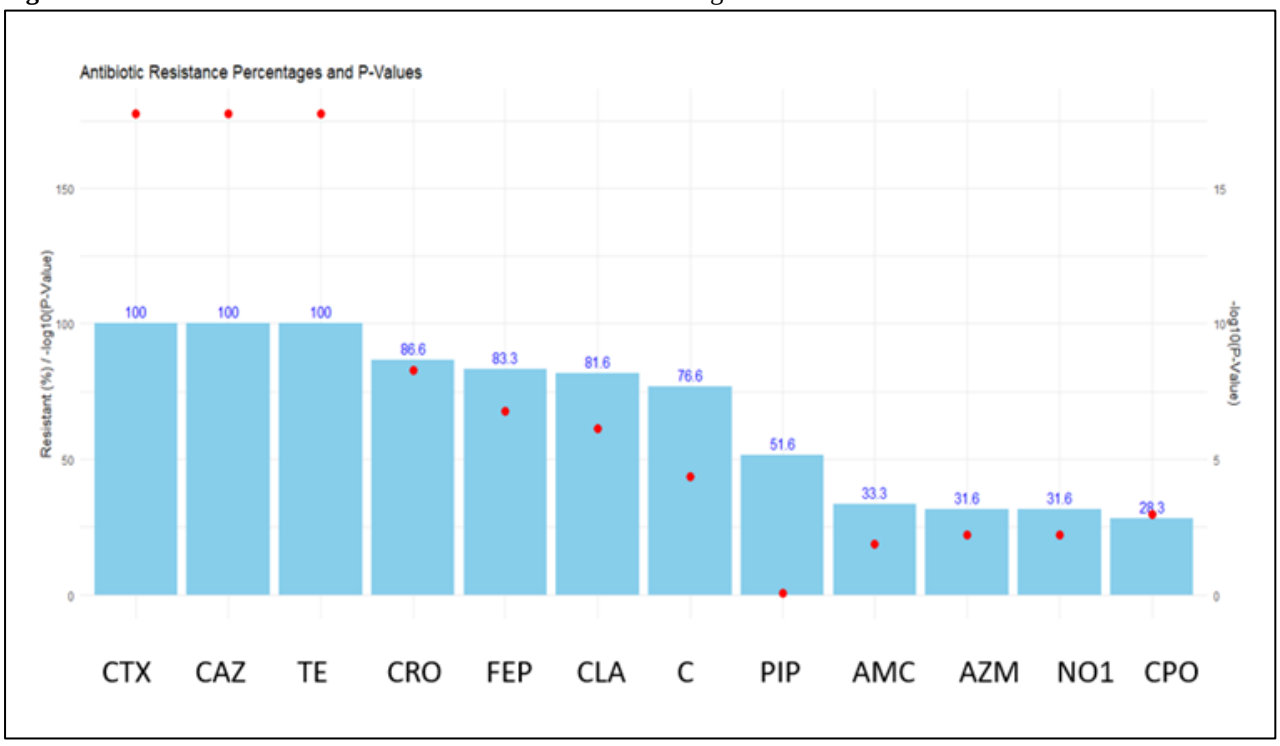
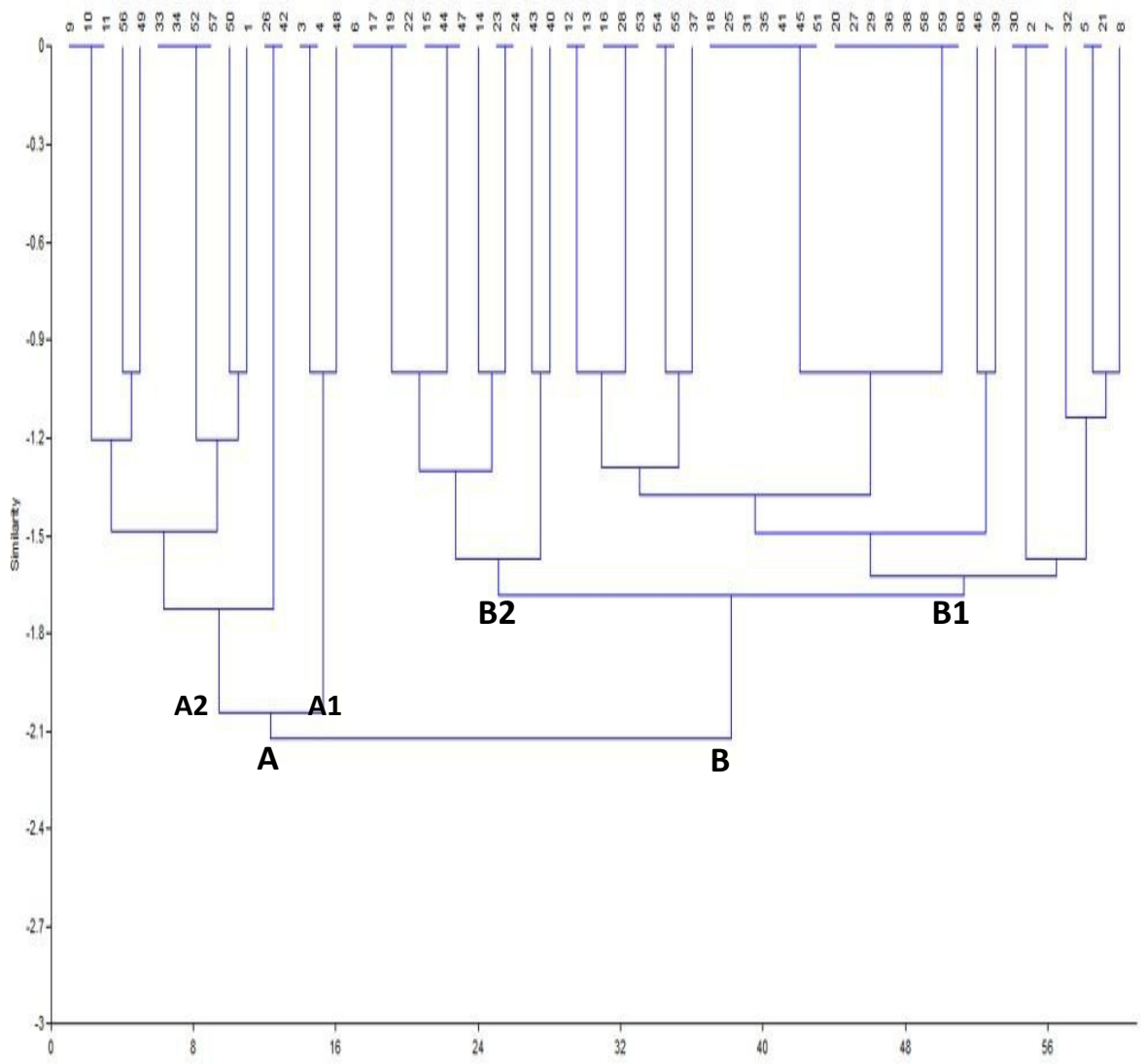


Figure 5. Dendrogram derived from the ERIC-PCR data referred to as the ERIC cluster.



Discussion

Different *E. coli* isolates were collected from Baghdad hospitals. Using the Congo red agar (CRA) method, the ability of isolates to form biofilm as a key factor in the persistence and resistance of infections was tested. Among the isolates, 39 were tested positive for biofilm formation, meaning these bacteria have a strong potential to attach to the surfaces and build protective layers. Meanwhile, 21 isolates were tested negative: showing no signs of biofilm production. These outcomes give us a clear picture of the diversity among the isolates: while some can create biofilms and potentially lead to more stubborn infections, others lack this characteristic. This insight highlights the role of biofilm-forming *E. coli* strains that might play in infection severity and treatment challenges, reinforcing the need to consider biofilm production in clinical diagnostics and infection control.[16]

The antibiotic resistance patterns are displayed in the matrix (Figure 2), with certain antibiotics exhibiting robust co-resistance associations. Similarly, ceftazidime showed a strong positive correlation with cefepime and amoxicillin/clavulanate, while norfloxacin was significantly associated with both cefaclor and chloramphenicol. Ceftazidime/clavulanic acid also demonstrated a strong correlation with both tetracycline and chloramphenicol. Additionally, a substantial link was observed between cefotaxime and the combination of Ceftazidime/clavulanic acid , as well as cefepime. Conversely, weak, or erratic correlations were noted between Ceftazidime/clavulanic acid and piperacillin/lactamase inhibitor with other antibiotics. Because of Cluster A1 high level of

similarity, it is likely that these isolates are from the same strain or have a similar origin, which would explain their similar antibiotic tolerance. In a similar way, similarities in Cluster A2 might result from similar treatment histories or environmental circumstances. The difference in Cluster A3 is probably caused by different genetic backgrounds or treatments, as indicated by the considerable similarity. Given Cluster B1 low to moderate similarity, distinct resistance patterns may have developed because of separate evolution or a variety of antibiotic exposures. Lastly, the minimal similarity in Cluster B2 points to independently evolved strains and indicates a high degree of genetic and environmental variability.

Closely related strains are found in certain clusters, whereas independently evolved strains are found in others, highlighting the variability in *E. coli* resistance. These findings highlight a significant challenge in treating bacterial infections due to high resistance levels in commonly used antibiotics, particularly cefotaxime, ceftazidime, and tetracycline, which are no longer viable options. The moderate resistance observed in ceftriaxone, cefepime, and Ceftazidime/clavulanic acid suggests partial efficacy, but frequent use could lead to further resistance. Lower resistance rates in piperacillin, augmentin, and cefoperazone indicate they may still be somewhat effective options. However, the elevated P-values observed for multiple antibiotics underline the urgent need to consider alternative therapeutic strategies and reduce dependence on highly resistant antibiotics.

For typing *E. coli* strains, numerous molecular approaches have been developed [17]. Unlike PFGE, the highly specific rep-PCR approach has low labor costs, is a simple operation, and yields results quickly [18]. Rep-PCR is a fingerprinting method that analyzes the electrophoretic patterns of the PCR products containing dispersed repeating sequences throughout the genome. It may represent the location variation of repeated sections in the bacterial genome, and it has the cost advantage of being accomplished using just one PCR per strain [18].

Observational data suggest that some of the patients after certain period were colonized with the same or different strains, which supports the theory of a single strain persisting in the colonization environment. This further calls for the dynamics of colonization and the risk of treatment failure that necessitates the need for surveillance to tell apart persistence from reinfection and replacement of one strain with another.

According to our investigation, the majority of *E. coli* isolates collected from the patients was unrelated. This revealed the existence of several sorts in a single individual [19]. This contrasts with studies showing comparable clonal types within the same patient and confirming that horizontal transmission of specific ERIC types likely occurs via the healthcare professionals' hands or environmental factors [19, 21]. Additionally, ERIC-PCR has successfully typed *E. coli* isolates, revealing 15 ERIC1 patterns in Saudi Arabia and 12 in Egypt. These patterns included from four to eleven bands in Saudi Arabia and three to nine bands in Egypt, with amplified DNA bands of the isolates range from 110 bp to 1535 bp [21].

The current finding indicates that isolates from the same hospital were likely related, suggesting patient-to-patient transmission of clonal *E. coli* strains across wards. Preventing this can be achieved through basic hygiene and proper disposal of the infected materials [13]. Since ERIC-PCR is faster and simpler than other molecular techniques [20] it can be used for the epidemiological typing of nosocomial bacteria while maintaining patient segregation and cleanliness [7].

The ERIC-PCR is an inexpensive, rapid, repeatable, and discriminating DNA technology that enables efficient monitoring of potentially transmitted *E. coli* strains among patients [22]. This result is consistent with the findings of the 'Comparative Study', which assessed the efficacy of different molecular techniques for the strain typing [23]. Although the causes of observed differences remain speculative, the ERIC-PCR offers a genome-wide view of diversity, capturing strain variations from single-point mutations to large-scale genomic rearrangements [22]. However, this study relies solely on the ERIC typing technique without incorporating other complementary typing methods, which may limit the resolution or comprehensiveness of the findings [8]. It was demonstrated that the best correlation was found between PFGE and ERIC-PCR, indicating that combining the two methods may be unnecessary [24]. The findings from ERIC-PCR and biofilm analysis provide a multi-dimensional view of the *E. coli* isolates characteristics.

The genetic clustering observed in ERIC-PCR suggests that isolates within the same sub-clusters, such as A1 and B1, may share genetic lineage or similar environmental exposures, potentially influencing their antibiotic resistance profiles. Biofilm formation further refines this analysis, as biofilm-positive isolates within clusters A and B, particularly in sub-clusters like B1 and B2, are more likely to exhibit resistance due to the enhanced protective layers. Notably, the presence of biofilm-producing isolates in genetically diverse clusters (e.g., B2) suggests that biofilm formation is a key factor independent of the genetic lineage, emphasizing the role of biofilm in the treatment challenges. These combined approaches underscore the need for the targeted therapeutic strategies that consider both genetic similarity and biofilm-forming capabilities among *E. coli* isolates.

Conclusion

Based on the study, ERIC-PCR is an efficient and low-cost technique for the genetic diversity detection in *E. coli* isolates appropriate for the application in hospital epidemiological monitoring. By ERIC-PCR, the strains can be distinguished easily, thus the application of other more sophisticated techniques like PFGE is spared. The correlation of biofilm formation with ERIC-PCR brings additional information on the severity of the infection and the level of resistance of the strain to antibiotics. The use of ERIC-PCR during the outbreak contributes to comprehension of the outbreaks as well as determining the planned treatment. Proper hygiene practices and appropriate handling of the infectious materials are essential in containing the spread of the multi-resistant *E. coli* and enhancing the patients' condition. The insights can enhance the health policy, clinical practice, research, and medical education, aiding healthcare providers in managing various *E. coli* infections effectively.

Acknowledgment

The author would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) in Baghdad - Iraq for its support on this accomplishment.

Ethics approval

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Mustansiriyah University (Ethical code#198/MOH/Approval/281). Prior to any intervention, all participants provided written informed consent. The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing–original draft, Writing–review & editing is done by Israa Radwan Ali.

Funding

This study was not funded by any institution.

References

1- Niranjan V, Malini A. Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. Indian J Med Res 2014 Jun;139(6):945-8. PMID: 25109731; PMCID: PMC4165009.

2- Nowicki S, deLaurent ZR, de Villiers EP, Githinji G, Charles KJ. The utility of Escherichia coli as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. PLoS One. 2021 Jan 22;16(1): e0245910. doi: 10.1371/journal.pone.0245910.

3- Contreras-Alvarado LM, Zavala-Vega S, Cruz-Córdova A, Reyes-Grajeda JP, Escalona-Venegas G, Flores V, Alcázar-López V, et al. Molecular Epidemiology of Multidrug-Resistant UropathogenicEscherichia coli O25b Strains Associated with Complicated Urinary Tract Infection in Children. Microorganisms 2021Nov5;9(11): 2299.doi: 10.3390/microorganisms9112299. PMID: 34835425; PMCID: PMC8623209.

4- Shayea RH, Ali MR. ERIC-PCR Genotyping and Clonal Genetic linkage Between Carbapenem-Resistant Acinetobacter baumannii Isolates. Jordan Journal of

Biological Sciences. 2022;15(4): 689 696 - .doi.org/10.54319/jjbs/150418

5- Jonas D, Spitzmüller B, Weist K, Rüden H, Daschner FD. Comparison of PCR-based methods for typing Escherichia coli. Clin Microbiol Infect 2003 Aug;9(8):823-31. doi: 10.1046/j.1469-0691.2003.00661.x. PMID: 14616703.

6- Liu Y, Davin-Regli A, Bosi C, Charrel RN, Bollet C. Epidemiological investigation of Pseudomonas aeruginosa nosocomial bacteraemia isolates by PCR-based DNA fingerprinting analysis. J Med Microbiol 1996 Nov;45(5):359-65. doi: 10.1099/00222615-45-5-359. PMID: 8918952.

7- Syrmis MW, O'Carroll MR, Sloots TP, Coulter C, Wainwright CE, Bell SC, Nissen MD. Rapid genotyping of Pseudomonas aeruginosa isolates harboured by adult and paediatric patients with cystic fibrosis using repetitive-element-based PCR assays. J Med Microbiol 2004 Nov;53(Pt 11):1089-1096. doi: 10.1099/jmm.0.45611-0. PMID: 15496385.

8- Shin JI, Ha JH, Kim KM, Choi JG, Park SR, Park HE, et al. A novel repeat sequence-based PCR (rep-PCR) using specific repeat sequences of Mycobacterium intracellulare as a DNA fingerprinting. Front Microbiol 2023 Apr 6; 14:1161194. doi: 10.3389/fmicb.2023.1161194. PMID: 37089534.

9- Alsultan A, Elhadi N. Evaluation of ERIC-PCR method for determining genetic diversity among Escherichia coli isolated from human and retail imported frozen shrimp and beef. Food Contamination. (2022) Nov; 9(12). doi.org/10.1186/s40550-022-00098-1

10- Ali MR, Khudhair A. M.Detection of Colony Adhesion Factors and Genetic Background of Adhesion Genes among Multidrug-Resistant Uropathogenic Escherichia coli Isolated in Iraq. J Pure Appl Microbiol 2018; 12(4): 2017-2026. doi.org/10.22207/JPAM.12.4.38.

11- Ali IR, Abdulsatar DM, Salman RS, Khudhair AM. Exploring the Sequence analysis of the Two-Component System Response Regulator OmpR in Multi-Drug Resistant (MDR) Acinetobacter baumanni. Adv Life Sci 2024Aug; 11(3): 533-538. doi.org/10.62940/als.v11i3.3256

12- Nasif SH, Ali MR, Alsakini AH. Characterization of UropathogenicEscherichia coli by Phylogenetic Grouping, Integron, and Antibiotic Resistance Properties. Trop J Nat Prod Res 2024; 8(4): 7035-7041. doi:10.26538/tjnpr/v8i4.41.

13- Nasif SH, Ali MR, Alsakini AH. Phylogenetic Groups and Antibiotic Resistance Characteristics of UropathogenicEscherichia coli. J Commun Dis 2023; 55(1): 36-44.doi:org/10.24321/0019.5138.202306.

14- Mohsin AS, Alsakini AH, Ali MR. Outbreak of drug resistance Escherichia coli phylogenetic F group associated urinary tract infection. Iran J Microbiol 2022 Jun;14(3): 341-350. doi: 10.18502/ijm.v14i3.9771. PMID: 37124864; PMCID: PMC10132334.

15- Katongole P, Nalubega F, Florence NC, Asiimwe B, Andia I. Biofilm formation, antimicrobial susceptibility and virulence genes of Ur pathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infect Dis 2020 Jun 29; 20(1): 453. doi:10.1186/s12879-020-05186-1. PMID: 32600258; PMCID: PMC7325280.

16- Ballén V, Cepas V, Ratia C, Gabasa Y, Soto SM. Clinical Escherichia coli: From Biofilm Formation to New Antibiofilm Strategies. Microorganisms 2022 May 26; 10(6): 1103.

doi: 10.3390/microorganisms10061103. PMID: 35744621; PMCID: PMC9229135.

17- Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM, Feng P. Advances in Molecular Serotyping and Subtyping of Escherichia coli. Front Microbiol 2016 May 3; 7:644. doi: 10.3389/fmicb.2016.00644. PMID: 27199968; PMCID: PMC4853403.

18- Crnich CJ, Duster M, Warrack S, Maki D, Safdar N. Comparison of pulsed-gel electrophoresis and a commercial repetitive-element PCR method for assessment of methicillin-resistant Staphylococcus aureus clustering in different health care facilities. J Clin Microbiol. 2014 Jun;52(6):2027-32. doi: 10.1128/JCM.03466-13. Epub 2014 Mar 26. PMID: 24671801; PMCID: PMC4042735.

19- Shin JI, Shin SJ, Shin MK. Differential Genotyping of Mycobacterium avium Complex and Its Implications in Clinical and Environmental Epidemiology. Microorganisms 2020 Jan 10;8(1):98. doi: 10.3390/microorganisms8010098. PMID: 31936743; PMCID: PMC7022546.

20- Sukumaran D, Hatha M. Molecular Epidemiology of Multidrug-resistant Escherichia coli from Urinary Tract Infections. J Microbil Infect Dis June 2021;11(02):66-73. doi:10.5799/jmid.951495.

21- Mansour SA, Eldaly O, Jiman-Fatani A, Mohamed ML, Ibrahim EM. Epidemiological characterization of P. aeruginosa isolates of intensive care units in Egypt and Saudi Arabia. East Mediterr Health J. 2013 Jan;19(1):71-80. PMID: 23520909.

22- C. Elliott, A.l Justiz Vaillant, Antimicrobials and Enterobacterial Repetitive Intergenic Consensus (ERIC) Polymerase Chain Reaction (PCR) patterns of nosocomial Serratia marcescens isolates: A One Year Prospective Study (June 2013-May 2014) in a Rural Hospital in the Republic of Trinidad and Tobago, Prog. Chem. Biochem. Res. 2020, 3(2), 114-120.DOI: 10.33945/SAMI/PCBR.2020.2.4.

23- Kidd TJ, Grimwood K, Ramsay KA, Rainey PB, Bell SC. Comparison of three molecular techniques for typing Pseudomonas aeruginosa isolates in sputum samples from patients with cystic fibrosis. J Clin Microbiol. 2011 Jan;49(1):263-8. doi: 10.1128/JCM.01421-10. Epub 2010 Nov 17. PMID: 21084517; PMCID: PMC3020435.

24- Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Żaczek A, Dziadek J. Methodological and Clinical Aspects of the Molecular Epidemiology of Mycobacterium tuberculosis and Other Mycobacteria. Clin Microbiol Rev 2016 Apr;29(2): 239-90. doi: 10.1128/CMR.00055-15. PMID: 26912567; PMCID: PMC4786889.