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Molecular Typing of Multi Drug Resistance *E.coli* that Isolates from Patient with Urinary Tract Infections in Najaf Province

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ABSTRACT

Objective: *This study aimed to detect the diversity of multidrug-resistant Escherichia coli strains isolated from urinary tract infection (UTI) patients and to assess their potential for biofilm formation.* ***Method:*** *Twenty bacterial isolates were obtained from urine cultures of UTI patients hospitalized at Najaf Teaching Hospital. The isolates were cultured on blood agar and MacConkey's agar, identified using the Vitek 2 system, and subjected to antibiotic susceptibility testing with the AST card. Genetic diversity among the isolates was further analyzed using ERIC-PCR.* ***Results:*** *The findings revealed high levels of antibiotic resistance among the E. coli isolates: 100% resistance to amoxicillin and ampicillin, 90% to ceftriaxone and ceftazidime, 70% to amikacin, and 55% to meropenem. ERIC-PCR results demonstrated genetic variation among isolates, which were grouped into five distinct clusters with similarity indices ranging from 40% to 90%.* ***Novelty:*** *This study provides insight into the alarming resistance patterns and genetic diversity of uropathogenic E. coli, underlining the critical need for tailored antibiotic stewardship and further investigation into their biofilm-forming capabilities in clinical settings.*

INTRODUCTION

Urinary tract infection (UTI) is a prevalent bacterial illness globally, mostly caused by Uropathogenic Escherichia coli (UPEC) [1]. Annually, 150 million individuals in America are affected by urinary tract infections, resulting in an expenditure of 6 billion dollars on treatment. Escherichia coli, a gram-negative bacterium, together with the normal intestinal flora, constitutes the predominant organisms identified in 75-90% of urinary tract infection cases among all urinary tract pathogens. This infection has spread at both the community and nosocomial levels, with UPEC responsible for 70–95% of UTIs [2]. It is the adhesion of E. coli to the urothelium of the urethra that marks the beginning of the infection. The following migration of the E. coli to the kidneys and bladder causes inflammation on the host, which ultimately results in cystitis and pyelonephritis [2]. The presence of virulence factors is a critical aspect in determining whether or not UPEC has the capability of forming an infection in the urinary system. In order to successfully colonise and survive in the urinary tract, E. coli makes use of a wide variety of virulence factors, including genes involved in adhesion, the iron acquisition and transport system, flagella, and toxins [3]. The virulence factors confer protection to the bacterium against urinary flow. The pathogenicity-associated island contains numerous genes and is frequently transferred horizontally between strains. Several virulence genes, including fimH (a type 1 pilus-related gene), the fimbrial adhesion gene afa, and the iron uptake system genes fyuA, ireA, iutA, and ironN, as well as the capsule genes kpsMTII, K1, and

K5, contribute to protection against complement-mediated and phagocytic killing by the host. Toxins such as α-hemolysin, encoded by the hlyA gene, and cytotoxic necrotising factor 1 (cnf1) have been implicated in the pathogenesis of urinary tract infections [4],[5]. Despite the treatability of these infections, the rising prevalence of multi-drug resistant organisms (MDR) results in complications, treatment failures, and heightened mortality and morbidity rates [6]. This study found that isolated UPEC strains from the community exhibited various virulence-associated gene patterns. These patterns were linked to patient demographics, the phylogenetic group of each UPEC strain, and a correlation was established between the abundance of virulence factors and antibiotic resistance.

RESEARCH METHOD

Sample collection

Specimens collection include 20 (urine sample) from patients suffering from urinary tract infections who were admitted to Al-Najaf teaching hospital in AL-Najaf City between July 2024 to October 2024 with age group between 30-70 years. All the specimens were labeled and transported Immediately by sterile transport swabs to the laboratory for Culture and Identification.

Bacterial culture and Antibiotics sensitivity

A single colony was extracted from each primary positive culture on blood agar and MacConkey's agar. Following bacterial isolation, E. coli isolates were cultured in Luria Bertani (LB) broth. Following 16-18 hours of incubation at 37 °C, a repeat growth was conducted to obtain a pure culture. Identification was based on morphological and cultural characteristics. Subsequently, a smear was prepared from the pure colony on a clean slide and stained with Gram's stain to observe the arrangement and reaction of the bacteria to the stain [7]. All bacteria isolated in this present study were identified using the VITEC2 system, with AST cards employed for the detection of antibiotic susceptibility testing. A bacterial strain that exhibits resistance to a minimum of three distinct classes of antibiotics is classified as multi-drug resistant (MDR).

DNA extraction *ERIC – PCR* amplification

The alkaline lysis technique was used to extract DNA. Nanodrop was used to measure the concentration of DNA. One microlitre of each primer, ten microlitres of the master mix, 1.5 microlitres of MgCl2, two microlitres of template DNA, and 3.5 microlitres of deionised water were used in 20 microlitre ERIC-PCR procedures. The ERIC-PCR reaction protocol included an initial denaturation at 94 °C for 1 minute, followed by 30 cycles comprising a denaturation step at 94 °C for 30 seconds, annealing at 52 °C for 35 seconds, extension at 72 °C for 4 minutes, and a final extension at 72 °C for 5 minutes. PCR products underwent electrophoresis on a 1.5% agarose gel (Merck) at 80 V for 180 minutes, followed by visualisation under UV light using a gel documentation system [8]. Table [1], [2]:

Table 1. Primers used in Eric-PCR amplification

***Eric 1*genes**	**Primer sequences**	**References**
Eric-1 (F)	5- CACTTAGGGGTCCTCGAATGTA-3	(Xia et al.,. 2012)
Eric-1 (R)	5-AAGTAAGTGACTGGGGTGAGCG-3	

Table 2. Components of PCR cycle

No	**Components**	**Volume (µl)**
1	PCR Master mix	10.0
2	Distilled water DNA-free	3.5
3	Forward primer (10 pmol/µl)	1.0
4	Reverse primer (10pmol/µl)	1.0
5	DNA template	2.0
6	MgCl2	1.5
	Total	20

RESULTS AND DISCUSSION

Results

Antibiotics sensitivity

In this study, the results showed an increase in the rate of *E. coli* resistance to most of the antibiotics used, according to Table (3), This test was done by using the VITEK 2 system.

Table 3. Antibiotics sensitivity patterns of E. coli

No of Isolate	**Antibiotics**	**AST %**	**No of Isolate**	**Antibiotics**	**AST%**
E1	Amoxicillin	100%	**E11**	Ciprofloxacin	95%
E2	Amoxicillin–clavulanic acid	100%	**E12**	Levofloxacin	75%
E3	Piperacillin-tazobactam	100%	**E13**	Norfloxacin	100%
E4	Ampicillin–sulbactam	100%	**E14**	Moxifloxacin	95%
E5	Ampicillin	100%	**E15**	Tetracycline	90%
E6	Cefotaxime	95%	**E16**	Tobrmycin	90%
E7	Ceftazidime	90%	**E17**	Amikacin	70%
E8	Ceftriaxone	90%	**E18**	Gentamicin	90%
E9	Cefepime	75%	**E19**	Meropenem	55%

No of Isolate	Antibiotics	AST %	No of Isolate	Antibiotics	AST%
E10	Cefazolin	95%	**E20**	Trimethoprim–sulfamethoxazole	95%

***ERIC – PCR* amplification Analysis**

A dendrogram, a type of hierarchical clustering analysis used to visualize the genetic similarity among 20 E. coli isolates based on ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction) fingerprinting. Similarity Scale (Y-Axis): The vertical axis represents similarity, with values ranging from 0 to 1. Isolates that are more genetically similar cluster together at higher similarity levels. There are several major clusters, indicating different genetic groups among the E. coli isolates. Some isolates (e.g., 11 and 14) show high similarity, clustering at a high similarity level. Others, like 9 and 10, also form a close genetic group. Some isolates are more distinct, joining clusters at lower similarity levels. The dendrogram suggests that the E. coli isolates exhibit a range of genetic diversity, with some groups being more closely related than others, figure (1):

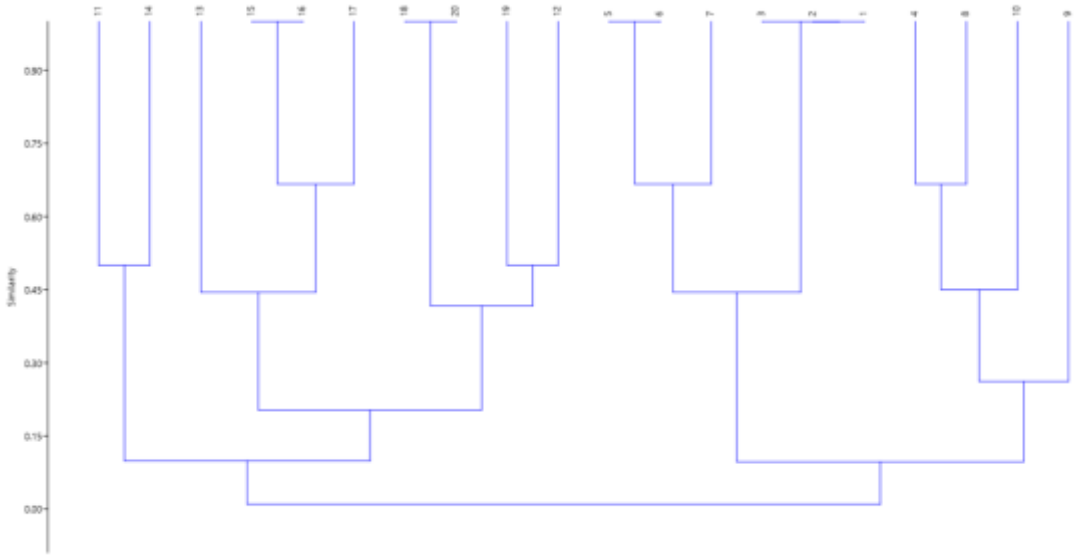

Figure 1. Dendrogram drawn for the strains of E. coli

Based on the dendrogram analysis:

1. High Similarity Isolates (Closely Related):
   a. Isolates 11 and 14 cluster together at a similarity level above 90%, meaning they are nearly identical.
   b. Isolates 15 and 16 also show high similarity, clustering above 85-90%.
   c. Isolates 4, 8, 10, and 9 form a tight cluster at a high similarity level (~85-90%).
   d. Isolates 5, 6, and 7 show close genetic relatedness (above 80% similarity).
2. Low Similarity Isolates (Genetically Diverse):
   a. Isolate 13 appears to be more distinct, joining the main cluster at a lower similarity level (~40-50%).

b. Isolate 12 and 19 cluster separately before merging with other isolates at a lower similarity (~30-40%).
c. The most genetically diverse isolate is likely at the bottom-most branch, which joins all other isolates at the lowest similarity (~15-20%).
d. In this study , we consider a similarity threshold of ~50-60%, there for we can divide the isolates into 4-5 major clusters :Cluster 1: (11, 14), Cluster 2: (13, 15, 16, 17, 18, 20), Cluster 3: (19, 12), Cluster 4: (5, 6, 7, 3, 2, 1) and Cluster 5: (4, 8, 10, 9).

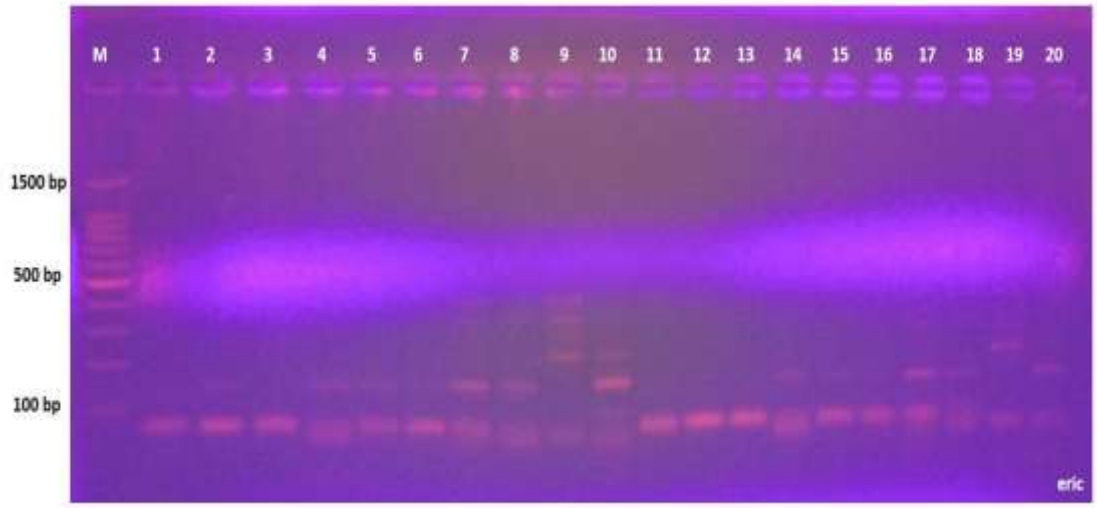

Figure 2. Results of ERIC PCR gel electrophoresis image showing DNA banding patterns for 20 E. coli isolates. The bands represent amplified repetitive sequences, and their presence or absence is used to determine genetic similarity.

According to this results, most lanes (1–20) show multiple bands, indicating successful amplification. Some isolates have more distinct and intense bands (e.g., lanes 9, 10, 19, 20). Some lanes have fewer visible bands, suggesting lower amplification or genetic differences (e.g., lanes 3, 5, 12) The banding pattern varies between isolates, explaining the clustering in the dendrogram.

The most bands appear in lanes 9, 10, 19, and 20, meaning these isolates have more diverse ERIC sequences, while fewer bands in lanes 3, 5, and 12 suggest possible genetic variation or lower amplification. Isolates 9, 10, 19, and 20 have multiple strong bands across all size ranges, indicating high genetic diversity. Some isolates (e.g., 3, 5, 12) have fewer bands, mostly in the smaller size range (100-500 bp), suggesting lower diversity (figure 2).

Discussion

Our results indicate a higher prevalence of bacteria associated with urinary tract infections, consistent with global trends [9], [10]. The emergence of multidrug resistance (MDR) resulting from the production of β-lactamases in Gram-negative bacteria presents a significant global challenge, restricting treatment options and elevating hospital costs, as well as morbidity and mortality rates. The rise of high antibiotic resistance rates and multidrug-resistant phenotypes in bacteria associated with urinary tract infections poses a significant public health issue globally. Prior research has indicated comparable

findings [11]. This study corroborates the findings of Bouchillon et al. [12], indicating that UPEC isolates exhibit over 90% resistance to ciprofloxacin and levofloxacin antibiotics. Resistance rates to antimicrobial drugs were higher in isolates from male patients compared to those from female patients [13]. Fluoroquinolones are commonly utilized for treating urinary tract infections in male patients [14]. Male infections may be more challenging to eliminate due to elevated antibiotic resistance rates in strains isolated from males, potentially resulting in recurrent infections. These observations align with findings from earlier studies [12],[14],[15]. It is advisable to conduct susceptibility analysis of isolates to antibiotics before selecting a treatment. The prevalence of multidrug-resistant strains in this study (90%) was significantly higher than the 16.4% reported in another study conducted in Mexico [16]. The study by Paniagua-Contreras et al. [11] demonstrated that 97% of the strains were multidrug-resistant (MDR). Susceptibility patterns are known to vary across geographical regions and may change over time [14].

The ERIC PCR technique identified their types. The isolates were categorised into four primary groups, E1 to E5. The survey results indicated that the ERIC PCR method serves as a viable alternative to molecular techniques like RAPD PCR and RFLP [17]. Meacham et al. from the University of Michigan assessed the genotyping of numerous E. coli samples and concluded that multiple ERIC-PCR methods should be employed to determine genetic similarities through genotyping [18]. In 2006, Lindsay et al. examined the distribution of ERIC sequences across the complete genomes of intestinal bacteria, specifically E. coli and Shigella. In 2011, Ramazanzadeh et al. employed ERIC-PCR to assess the genetic diversity of E. coli obtained from hospital samples. The study analysed 230 clinical samples, identifying 205 that could be genotyped into 20 clusters, predominantly within phylogenetic group D. The significant genetic diversity observed in the isolated E. coli suggests a low infection rate within the hospital setting [19]. This study indicates that some E. coli isolates are closely related, suggesting a potential common source, such as a shared environmental or clinical origin. Others exhibit moderate to low similarity, suggesting genetic diversity within the population, potentially arising from various sources or evolutionary distinctions. The isolates in our study exhibited genetic diversity based on ERIC-PCR fingerprinting. The anticipated outcome arises from the random collection of isolates from various sources and samples, indicating that transmission may have occurred from clones of diverse origins. These findings are consistent with other studies [20],[21],[22]. Loncaric et al. [23] demonstrated that the spread of antibiotic resistance is attributable not only to the dissemination of various β-lactamases but also to clonal transmission.

CONCLUSION

Fundamental Finding : This study highlights that the genetic diversity of *Escherichia coli* isolates, particularly those causing urinary tract infections (UTIs), contributes significantly to their virulence and resistance against commonly used antibiotics. **Implication :** The presence of multidrug-resistant strains, compounded by

hospital-acquired environmental pressures, underscores the urgent need for more stringent infection control policies and the development of novel antimicrobial strategies. **Limitation :** However, the study is limited by its relatively small sample size and the confinement of data collection to a single hospital setting, which may affect the generalizability of the findings. **Future Research :** Further investigations should incorporate larger, multi-center cohorts and molecular analyses to better understand resistance mechanisms and to identify potential therapeutic targets.

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