

## Genetic Characterization and Phylogenetic Analysis of *Escherichia Coli* Isolates from Urinary Tract Infections in Iraq

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**Abstract:** This study aimed to characterize *Escherichia coli* (E. coli) isolates from urinary tract infections (UTIs) in Iraq and compare them with international strains. Twenty midstream urine samples from UTI patients were cultured on MacConkey and nutrient agar plates. All bacterial isolates were identified using Vitek 2 system, followed by DNA extraction with the Goya Gene kit. Specific primers were used to detect E. coli, and positive samples were sequenced using the Sanger method. Phylogenetic analysis was conducted to elucidate the genetic relationships among the isolates. The results revealed a high similarity between local Iraqi E. coli isolates and national strains, with minor differences. The phylogenetic tree analysis identified three distinct strains, with an 81% similarity among most isolates, though one international strain was notably distant. The study highlights on rising the frequency of UTIs and the monetary problems they impose on both the governments and patients, emphasizing the need for active drugs that can be rapidly implemented in medical settings. This research underscores the importance of understanding local bacterial populations to inform treatment strategies, as UTIs commonly result from bacterial resistance to existing treatments. UPEC frequently targets the urethra, bladder, and kidneys, initiating infections through colonization and subsequent invasion of the bladder epithelium.

**Keywords:** UPEC, *Escherichia Coli* and Phylogenetic Tree.

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### INTRODUCTION

UTIs stand as the most predominant bacterial infections globally, impacting approximately one million individuals annually [1-3]. UTIs rank in second stage only to lower respiratory tract infections (in nosocomial infections) constituting at about 24% of cases in emerging nations [4]. This disease diagnosis necessitates the occurrence of over  $10^5$ /mL of bacteria in urine sample, manifesting with diverse signs ranging from asymptomatic cases to severe illness characterized by high fever and potential secondary bacteremia. UTIs are categorized into acute cystitis, acute pyelonephritis and asymptomatic bacteriuria [5]. The first one is a bladder infection, presents with several symptoms like dysuria, recurrent urination, and malodorous urine. While pyelonephritis, exhibits symptoms such as pyuria and bacteriuria. However, in specific populations like pregnant women, the aging, diabetics and children,

bacteria can arise to the kidneys and bladder, heightening the risk of upper UTIs.

### MATERIALS AND METHODS

Twenty midstream urine samples were collected from patients with urinary tract infections (UTIs). Initially, these samples were cultured on MacConkey and nutrient agar plates. The bacterial isolates were then identified using Vitek 2 system.

Following identification, DNA extraction was performed using the Goya Gene kit. Specific primers were used for the detection of *Escherichia coli* (E. coli): the forward primer (TATCCGGCCATGTTTTCATT) and the reverse primer (CTGTCGCCAGTCGATACCTT).

Samples that tested positive for E. coli were sequenced using the Sanger method. Finally, a

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phylogenetic tree analysis was conducted to understand the genetic relationships among the *E. coli* isolates.

## RESULT AND DISCUSSION

Given the frequency of UTIs and the monetary problems, it is crucial to develop actual drugs against this disease, that can be rapidly implemented in clinical cases [6].

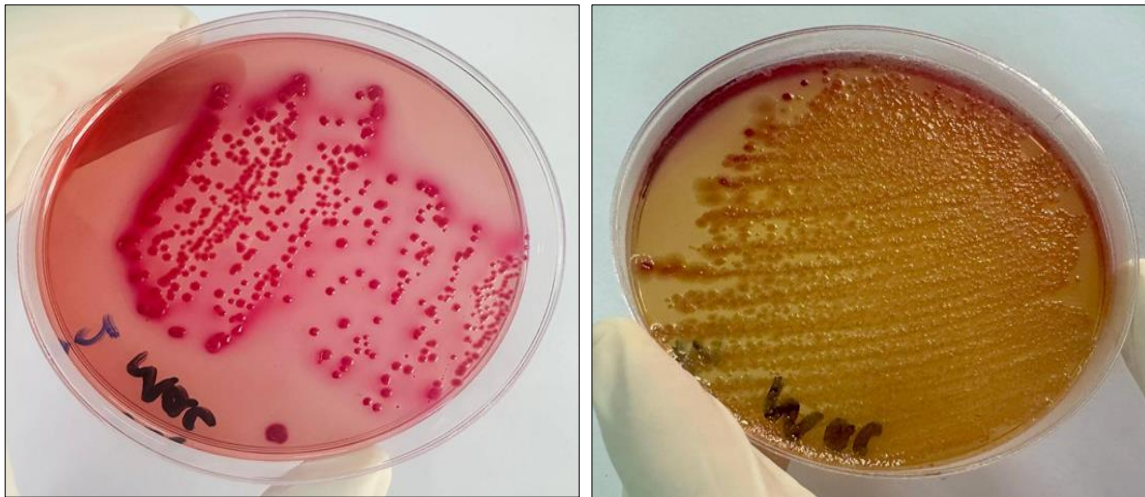


Figure 1: Bacterial Cultures on MacConkey Agar (left) and Nutrient Agar (right)

Organism Quantity:		Selected Organism : <i>Escherichia coli</i>															
BP Infection Site:		Collected:															
Source:																	
Comments:																	
Identification Information		Analysis Time: 3.87 hours	Status: Final														
Selected Organism		99% Probability <i>Escherichia coli</i>															
ID Analysis Messages		Bionumber: 0405610450526610															
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 2: The Vitek 2 system confirmed the presence of *Escherichia coli* in the bacterial cultures

Species/Abbrv	DNA Sequences	Translated Protein Sequences
1. seq1 E.Coli	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
2. seq2 E.coli	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
3. seq3 E.Coli	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
4. LR743515.1:71378-71725 <i>Escherichia coli</i> plasmid p5848A3 other	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
5. LC501688.1:13328-13675 <i>Escherichia coli</i> 25-Ec-C-116 plasmid p26C116-2 sequence_id: contig4	GCTTGGTCA	GGAGGCACAGC
6. CP043033.1:117751-118098 <i>Escherichia coli</i> strain XDL chromosome complete genome	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
7. CP041300.1:116945-117292 <i>Escherichia coli</i> O1:H42 strain CLSC36 chromosome complete genome	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG
8. CP041304.1:118568-118915 <i>Escherichia coli</i> strain MSHS 133 chromosome complete genome	ATGTTTTCATTTTCCGGGGCCGCAGCGGCAGTCA	GGTTAAACTGCTGTGGTCCACCGGTGACGG

Figure 3: The multiple sequence alignment analysis was conducted on partial sequences of the *E. coli* from UTI, including IraqIQD-No.1 to IQD-No. Isolates

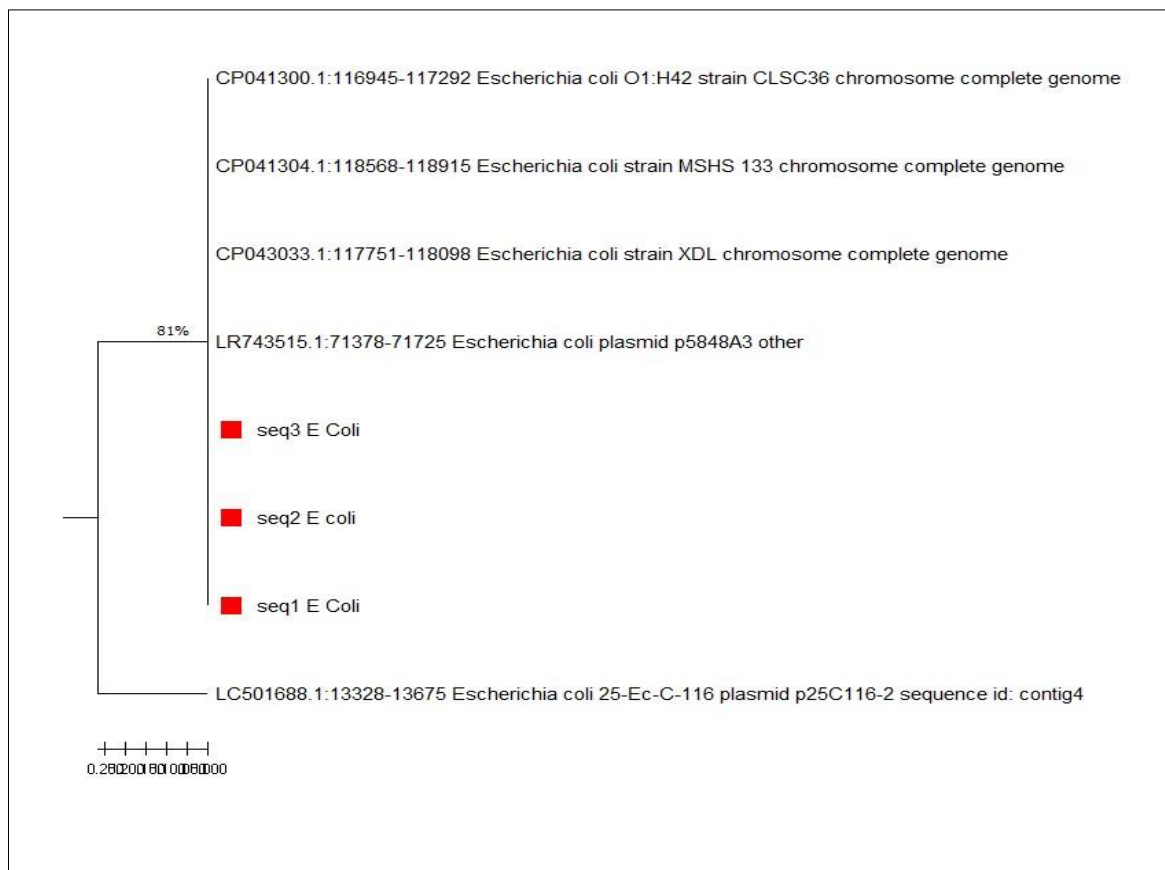
	1	2	3	4	5	6	7
1. seq1 E Coli							
2. seq2 E coli	0.000000000						
3. seq3 E Coli	0.000000000	0.000000000					
4. LR743515.1:71378-71725 Escherichia coli plasmid p5848A3 other	0.000000000	0.000000000	0.000000000				
5. LC501688.1:13328-13675 Escherichia coli 25-Ec-C-116 plasmid p25C116-2 sequence id: contig4	0.529900200	0.529900200	0.529900200	0.529900200			
6. CP043033.1:117751-118098 Escherichia coli strain XDL chromosome complete genome	0.000000000	0.000000000	0.000000000	0.000000000	0.529900200		
7. CP041300.1:116945-117292 Escherichia coli O1:H42 strain CLSC36 chromosome complete genome	0.000000000	0.000000000	0.000000000	0.000000000	0.529900200	0.000000000	
8. CP041304.1:118568-118915 Escherichia coli strain MSHS 133 chromosome complete genome	0.000000000	0.000000000	0.000000000	0.000000000	0.529900200	0.000000000	0.000000000

**Figure 4: Compute pairwise distance calculations for all local and international strains to understand their relationships within the alignment and phylogenetic tree**

Phylogenetic Analysis of *E Coli* isolate from UTI.

Phylogenetic analysis of *E Coli* from UTI revealed the presence of high similarity between Iraq and international strain. Three distinct strains were identified.

The phylogenetic tree (Figure 4 and 5) illustrates the relationships among these strains, which share similarity percentages 81%. However, International was notably distant and phylogenetically separated from the other strains.



**Figure 5: Phylogenetic tree analysis was conducted based on the partial sequences of the E coli from UTI. Three isolates from Iraq, designated as IQD-No.1 to IQD-No.3. Marked with red sequar. Compaered with International strains were used for genetic species typing analysis. The phylogenetic tree was made by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in MEGA version 6.0**

The results of the research indicate that local Iraqi isolates are closely related to national isolates, with only minor differences [7, 8]. It is known that urinary tract infections are common due to bacterial resistance to commonly used treatments. The study aimed to

understand the relationship between local *Escherichia coli* isolates and other strains to provide a comprehensive overview. As vital organs like kidneys, the urethra and bladder are frequently targeted by UPEC. The development of a urinary tract infection (UTI) begins

with colonizing of the urethral area by UPEC. Subsequently, UPEC invades the epithelium of bladder [9-11].

## CONCLUSION

The research indicates that local Iraqi E. coli isolates are closely related to national isolates, with minor differences. UTIs are common due to bacterial resistance to treatments, and the study aimed to provide a comprehensive overview of the relationships between local E. coli isolates and other strains. UPEC often targets the urethra, bladder, and kidneys, initiating UTIs by contaminating and colonizing the urethral area, followed by invasion of the bladder epithelium.

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