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Original Research Article

MOLECULAR IDENTIFICATION OF *ESCHERICHIA COLI* STRAIN ISOLATED FROM URINARY TRACT INFECTIONS AT A TERTIARY CARE HOSPITAL IN SIALKOT, PAKISTAN

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ABSTRACT

Background: *Urinary tract infections (UTIs) caused by Escherichia coli remain a primary global health concern, with increasing multidrug resistance posing significant diagnostic and therapeutic challenges. Molecular techniques such as 16S rRNA sequencing provide a reliable approach for accurate identification and phylogenetic characterization of resistant strains.* ***Objective:*** *To isolate and identify multidrug-resistant (MDR) Escherichia coli strains from urinary tract infection patients in District Sialkot, Pakistan, using 16S rRNA sequencing.* ***Study Design:*** *Cross-sectional molecular study.* ***Setting:*** *Department of Microbiology, tertiary care hospital in Sialkot, Pakistan.* ***Duration of Study:*** *From July 2023 to December 2023.* ***Methods:*** *Twenty E. coli isolates were obtained from urine samples of patients with clinically diagnosed UTIs. Identification was performed using standard microbiological techniques. Antibiotic susceptibility testing was conducted using the Kirby–Bauer disc diffusion method against commonly used antibiotics. The isolate exhibiting multidrug resistance was subjected to molecular identification via 16S rRNA gene sequencing and phylogenetic analysis. Sequence data were compared with reference strains using BLAST and submitted to GenBank.* ***Results:*** *Antibiotic susceptibility testing revealed high levels of resistance to ceftriaxone (95.2%), cefoperazone (93.5%), ciprofloxacin (86.5%), cefotaxime (84.6%), and cefixime (86%). Molecular analysis confirmed the isolate as Escherichia coli, with phylogenetic analysis showing close homology to previously reported E. coli strains. The sequence of the multidrug-resistant strain was submitted to GenBank under the accession number Escherichia coli strain KS8861 (PP897401.1).* ***Conclusion:*** *16S rRNA sequencing serves as a robust and accurate method for identifying multidrug-resistant E. coli strains associated with UTIs. Incorporating molecular diagnostics into routine microbiological workflows can enhance early detection and guide effective antimicrobial therapy.*

Keywords: Urinary Tract Infection, Antibiotic Resistance, Molecular identification, 16S rRNA sequencing, Sialkot

INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent microbial infections, posing a significant public health concern by affecting 150 million people worldwide annually. (1, 2). Uropathogenic *Escherichia coli* (UPEC) strains have been reported to account for about 80% cases of UTIs (2, 3). Antibiotics have been used for a long time as the modern and primary practice to treat infections like UTIs (4), but the rise of antibiotic resistance among virulent pathogens presents a significant challenge to public healthcare settings (3).

The urine culture method has been termed the Gold standard for the identification of bacterial isolates. However, the procedure requires a considerable time and yields false negatives in about 20% of the symptomatic UTI patients. Therefore, empirical therapy with recommended antibiotics must be initiated to safeguard patient safety. (5). There has been an increase in the use of DNA-based methods for effective and rapid identification of various pathogens worldwide (6). Bacteria have a 16S rRNA gene with conserved regions among species, making 16S rRNA gene sequencing a highly sensitive technique in the Diagnosis of pathogenic bacteria (7, 8). However, such methods are utilized less frequently, particularly in developing countries (5). Several recent studies have reported the identification of *Escherichia coli* using the molecular approach. However, there has been minimal work done in Sialkot regarding the use of the 16S rRNA sequencing technique in the Diagnosis of the pathogenic *E. coli* strains. Therefore, the present study was conducted to perform molecular identification of the *E. coli* strain.

METHODOLOGY

Isolation and Identification of *E. coli* Strains

The present study included clinical *E. coli* isolates from UTI patients at a tertiary care hospital in Sialkot from July to December 2023. The Urine samples were processed in accordance with the standard guidelines. The isolates were subjected to standard microbiological procedures and biochemical testing methods. The samples were cultured on Cystine Lactose-Electrolyte-Deficient Agar (CLED agar) medium, followed by incubating the plates for 18- 24 hours at 37 °C. The plates with bacterial growth were then processed for further testing, such as colony characteristics, Gram staining, and biochemical tests. (9).

Antibiotic Sensitivity Testing

The Antibiotic sensitivity of selected *E. coli* strains (n=20) was assessed against a panel of antibiotics (Oxoid, UK) employing the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) protocol. (10).

Genomic DNA Extraction

E. coli strain (n=1) that showed a high level of multidrug resistance was then subjected to identification using the 16S rRNA sequencing technique. A pure bacterial colony of the *E. coli* strain was inoculated into 10 mL of Trypticase Soy broth and incubated at 37 °C overnight to isolate the purified strain. The genomic DNA was then extracted

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using QIAamp DNA mini extraction kits (Qiagen, Cat. No.:51306) according to the manufacturer's instructions.

Identification of E. coli by PCR

The identification of *E. coli* was achieved by DNA sequencing of the 16S rRNA gene using the universal primers of 27F (5′ AGAGTTTGATCCTGGCTCAG 3′) and 1492R (5′ TACGGCTACCTTGTTACGACTT 3′). The thermal cycling procedure involved an initial cycle at 94°C for 5 minutes, followed by 30 cycles, each one at 94°C for 30 seconds, 52.7°C for 30seconds, 72°C for 2 minutes, concluding with a final extension step at 72°C for 7 minutes. About 6 µL of the resulting amplification product was then assessed through electrophoresis on 1% agarose gel, which was visualized under UV light and photographed (11). The obtained products were subjected to purification by Gene Jet gel extraction kit (Cat. No.: K0691) in accordance with the manufacturer's instructions and stored at -20°C. The purified products were then dispatched to Apical Scientific Sdn Bhd, Malaysia, for DNA sequencing. The resulting nucleotide FASTA sequence was submitted to the NCBI GenBank with accession number (PP897401.1). Analysis of the sequence was then carried out with MEGA 11 software to assess the evolutionary relationship employing the Maximum Likelihood Tree Method with a maximum sequence difference of 0.01.

RESULTS

The present study included a total of 20 *Escherichia coli* strains isolated from UTI patients admitted to Allama Iqbal Memorial Hospital, Sialkot, Pakistan, from July to December 2023.

Antibiotic Susceptibility Testing

Table 1 demonstrates the antibiotic susceptibility patterns of *E. coli* isolates. The testing revealed the highest levels of resistance of isolates for ceftriaxone, cefoperazone, and ciprofloxacin with 95.2%, 93.5% and 86.5%, respectively. Moreover, the isolates also demonstrated elevated levels of resistance to amikacin (77.9%), fosfomycin (76%), amoxicillin-clavulanic acid (74.5%), gentamicin (71.3%), and nitrofurantoin (74.9%). Fortunately, the isolates showed better sensitivity levels to imipenem and meropenem, with 70.8% and 71.9%, respectively.

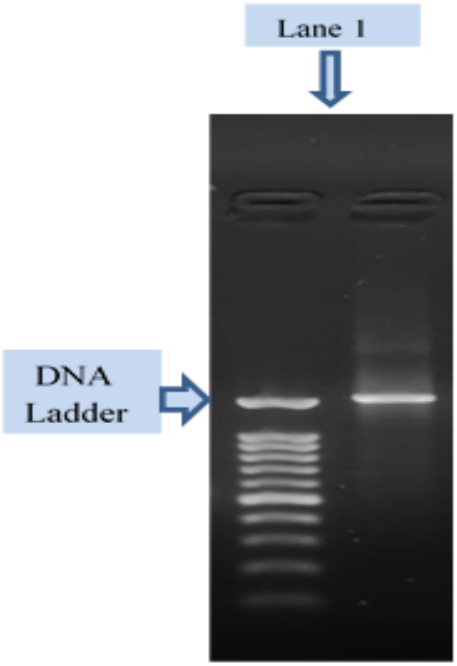

Figure 1. PCR products of *E. coli* strain (Lane 1) from the amplification of 16S rRNA gene using agarose gel electrophoresis

Table 1 Antibiotic Resistance Pattern (%) of *Escherichia coli* isolates

Antibiotics	*Escherichia coli* (N = 20)	
	R (%)	S (%)
Amoxicillin-Clavulanate	74.5	25.5
Cefoperazone-Sulbactam	62.1	37.9
Cefoperazone	93.5	12.7
Piperacillin-Tazobactam	45.3	54.7
Ceftriaxone	95.2	6.5
Imipenem	29.2	70.8
Meropenem	28.1	71.9
Ciprofloxacin	86.5	13.5
Amikacin	77.9	22.1
Gentamicin	71.3	28.7
Nitrofurantoin	74.9	25.1
Fosfomycin	76	24
Cefixime	86	14
Sulfamethoxazole-Trimethoprim	67.3	32.7
Amoxicillin	82	18
Cefotaxime	84.6	15.4

Identification of *E. coli* through 16S rRNA Sequencing

Genomic DNA was extracted from the isolated *E. coli* strain designated as KS8861. The 16S rRNA gene was amplified using PCR and yielded an approximate sequence of 1500bp by using sequencing primers (Figure 1). This obtained sequence has been registered in Genbank under the accession number PP897401.1. The BLAST tool was used to further analyze this sequence by comparing it against the collection of sequences available at GenBank. The results revealed that the test sequence shared 98.95% similarity with strains such as *Escherichia fergusonii* ATCC 35469 (NR_027549.1), *Shigella sonnei* CECT 4887 (NR_104826.1), and 98.60% with *Escherichia coli* strain NBRC 102203 (NR_114042.1) and *Escherichia fergusonii* ATCC 35469 (NR_074902.1 indicating a strong evolutionary link, which also highlights their high degree of similarity on a genetic level.

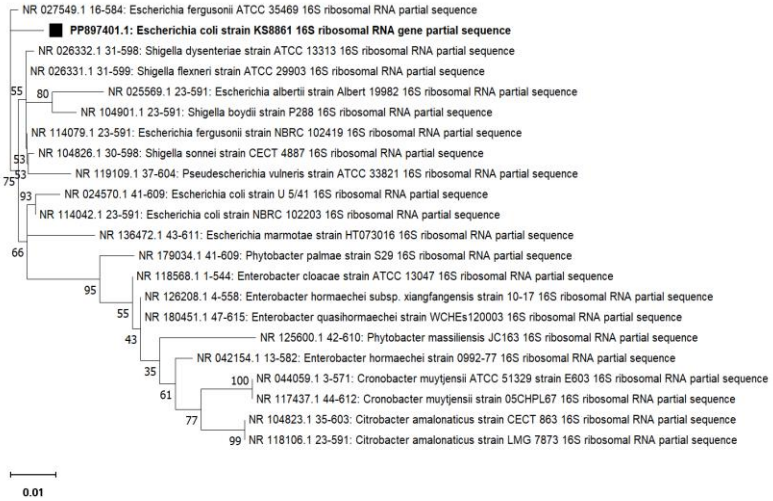

Figure 2. The phylogenetic tree of E. coli KS8861 (PP897401.1) depicts the strain's relationship with the closest genera based on 16S rRNA nucleotide sequences. The phylogenetic tree was constructed using the Maximum likelihood tree method with 1000 bootstrap replicates, and the bar denotes the Kimura 2-parameter distance of 0.01.Figure 2. The phylogenetic tree of E. coli KS8861 (PP897401.1) depicts the strain's relationship with the closest genera based on 16S rRNA nucleotide sequences. The phylogenetic tree was constructed using the Maximum likelihood tree method with 1000 bootstrap replicates, and the bar denotes the Kimura 2-parameter distance of 0.01

A phylogenetic tree based on the 16S rRNA sequence was further constructed, showing a close relationship between KS8861 and *E. coli*

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strains. These findings confirmed that it belonged to the family Enterobacteriaceae (Figure 2).

DISCUSSION

UTIs with *E.coli* as the causative pathogen are a critical clinical issue, affecting millions of people globally (Behzadi et al., 2020). However, research detailing the antibiotic resistance patterns of E. coli associated with UTIs and the molecular identification, specifically in Sialkot, Pakistan, is still limited.

The current study included a total of 20 *E. coli* isolates from UTI patients. *E. coli* was found to be predominant in causing UTI, consistent with several previous studies (5, 11, 12). Our findings indicate that the E. coli isolates showed maximum resistance to cephalosporins, penicillins, and fluoroquinolones, and also demonstrated increased resistance to amikacin and gentamicin. However, the highest sensitivity was observed to carbapenems. The high resistance rates of *E. coli* isolates against the cephalosporins, penicillins, and ciprofloxacin agree with previous studies conducted in other regions (13). The overuse of antibiotics is more likely to be the cause of this elevated resistance (14). In contrast to this, Mlugu et al. (2023) reported that isolates exhibited high sensitivity against these antibiotics. The current study further indicated that the bacterial isolates were susceptible to imipenem (70.8%), meropenem (71.9%), and piperacillin-tazobactam (54.7%), suggesting that carbapenems are more effective than other antibiotics. These findings are consistent with previous studies conducted in India and Pakistan, respectively (15).

Our study further employed a molecular approach to identify the *E. coli strain* via 16S rRNA sequencing followed by phylogenetic analysis. The phylogenetic analysis, corroborated by BLAST results, showed that the isolated *E. coli* strain from UTI patients showed the highest degree of relatedness with *Shigella* and *Escherichia* species. The strong sequence similarities between *E. coli* and *Shigella* have been reported by several previous studies. (11, 16). Similar to the current study, Lakshmi et al. (2020) identified the microbial strains in urine samples from UTI patients by 16S rRNA gene sequencing. (16). A previous study that compared the conventional urine culture method with 16S rRNA gene sequencing reported the higher accuracy of 16S rRNA sequencing in identifying bacterial strains causing UTIs (5). In another study conducted in Iraq, 50 isolates of UPEC isolated from UTI patients were successfully identified via 16S rRNA sequencing. (11).

It is noteworthy that our study has limitations: the sample size is too small, and only a single strain was subjected to molecular analysis due to insufficient resources. Therefore, the results cannot be generalized. Future research is thus recommended to integrate techniques like 16S rRNA gene sequencing for rapid identification of pathogenic bacteria and continuous surveillance of the antibiotic-sensitive patterns to manage such critical bacterial infections efficiently. Moreover, it highlights the need for future investigations at the genetic level through whole-genome sequencing to gain deeper insights into virulent genes, antibiotic-resistant strains, and overall genetic diversity, particularly in developing countries.

CONCLUSION

This study highlights the role of the 16S rRNA gene sequencing method in identifying bacteria causing UTIs, underscoring the need to adopt modern diagnostic techniques, particularly in middle and lower-middle-income countries, for effective management of Urinary tract infections.

DECLARATIONS

Data Availability Statement

All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication

Approved

Funding

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

ANWAL SHEHZADI
Conceived the study, collected data, performed analysis and prepared the first draft of the manuscript

ROOMA ADALAT*
Supervised the study, coordinated among authors, provided expert guidance, finalized the manuscript and approved the final version

UZMA NISAR
Assisted in data collection, literature review and manuscript editing

SADAF ILYAS
Contributed to methodology design, statistical analysis and data interpretation

HAFSA AMJAD
Helped in patient recruitment, data organization and manuscript formatting

HIRA EHSAN
Contributed to referencing, proofreading and final revisions of the manuscript

FAISAL IQBAL
Assisted in data validation, result interpretation and manuscript review

SOBIA ALYAS
Contributed to final editing, quality checking and approval of the final manuscript

All authors read and approved the final version of the manuscript.

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