Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Molecular Identification of Uropathogenic Bacteria via 16S rRNA Gene Amplification and Sequencing

Pragnya Paramita Acharya[1], Soumya Jal[2], Gopal Krishna Purohit[3], Pradipta Kumar Pati[4]

[1]PhD Scholar, School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Odisha, India

[2]Associate Professor, School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Odisha, India

[3]Director, Heredity Biosciences, Plot No- 818, Mayfair Lagoon Road, Jayadev Vihar, Bhubaneswar, Odisha, India

[4]Assistant Professor, Christ College, Kanika Rd, near Chandi, Masik Patna Colony, Cuttack, Odisha

***Corresponding Author**

Pragnya Paramita Acharya

School of Paramedics and Allied health sciences, Centurion University of Technology and Management, Odisha, India

Email ID:pragnyaacharya8@gmail.com

Cite this paper as: Pragnya Paramita Acharya, Soumya Jal, Gopal Krishna Purohit, Pradipta Kumar Pati, (2025) Molecular Identification of Uropathogenic Bacteria via 16S rRNA Gene Amplification and Sequencing. *Journal of Neonatal Surgery*, 14 (32s), 467-475.

ABSTRACT

Urinary tract infections pose challenges to physicians, with antibiotic resistance hampering traditional approaches. Microbiological methods often fail to differentiate closely related bacterial strains, necessitating molecular resolution. This research aimed to identify uropathogenic bacteria using 16S rRNA gene amplification and sequencing.

Urine samples from 40 diagnosed patients were collected at local hospitals and diagnostics centres, Bhubaneswar. Samples were cultured on selective media for uropathogen growth and incubated at 37°C for 24–48 hours. Microbial growth was observed in 32 samples. Colonies with distinct morphology underwent Gram staining and biochemical tests for identification. Genomic DNA was extracted from purified isolates using commercial kits per manufacturer's protocols. The 16S rRNA gene was amplified using universal bacterial primers through PCR. Amplification products were analysed via agarose gel electrophoresis to confirm size. PCR products were purified and sequenced. The sequences were compared against NCBI GenBank using BLAST for species identification based on homology.

Results showed *Escherichia coli* as the predominant uropathogen, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Enterococcus faecalis*. Molecular identification provided taxonomic precision, corroborating or reclassifying phenotypic designations. Isolates displayed over 99% sequence identity to known uropathogenic strains, deposited in GenBank with accession numbers.

This research demonstrates integrating molecular diagnostics like 16S rRNA sequencing for accurate uropathogen identification. Such approaches enhance epidemiological tracking, diagnosis, and antimicrobial stewardship by linking identity and resistance. Findings contribute to understanding etiology, offering a framework for improved infection management.

Key Words: *Urinary tract infection (UTI), Uropathogenic bacteria, 16S rRNA gene sequencing, Molecular identification, Antibiotic resistance, Escherichia coli*

1. INTRODUCTION

Urinary tract infections represent a major global health burden, disproportionately affecting certain populations [1]. Over 150 million people experience UTIs yearly worldwide, with elderly individuals and those with catheters facing higher vulnerability. Within clinical settings, UTIs drive antibiotic prescriptions, especially in primary care [2]. When misdiagnosed or untreated, these infections can lead to complications including pyelonephritis and sepsis, increasing suffering and care costs [3]. Precise recognition of microbes is crucial for effective intervention and antibiotic management, particularly against

rising drug resistance.

Traditionally, identifying uropathogenic bacteria relies on culture-dependent techniques with morphological, biochemical, and serological analyses [4]. While standard in laboratories, these conventional methods are time-consuming, labor-intensive, and limited in sensitivity. Fastidious or slow-growing organisms, particularly those no longer culturable post-antibiotic exposure, may evade detection. Biochemical profiles can vary between allied species, leading to diagnostic ambiguities and treatment delays [5, 6].

To overcome these limitations, molecular diagnostic techniques have emerged as tools enabling rapid pathogen detection. Chief among them, 16S ribosomal RNA (rRNA) gene sequencing has become the gold standard for bacterial identification, differentiating organisms through conserved and hypervariable regions [7]. This gene exists across all bacteria, and its sequence variances provide taxonomic resolution to species level, even for non-culturable strains. Application of 16S rRNA gene sequencing has enhanced our understanding of microbial diversity in clinical specimens and integrated into diagnostic workflows [8, 9].

This study aims to molecularly identify bacterial species causing urinary tract infections through 16S rRNA gene amplification and sequencing. Urine samples collected from local hospitals and diagnostics centres, Bhubaneswar to the symptomatic patients underwent conventional microbiological examination. DNA was extracted from pure cultures, followed by PCR amplification using universal 16S rRNA primers, and sequencing confirmed bacterial identity through reference database comparison. This molecular approach enables faster, more accurate pathogen identification, including bacteria that evade standard cultures. The methodology guides targeted antibiotic therapy, reducing treatment errors and antimicrobial resistance. Combining conventional and molecular microbiology develops a comprehensive UTI diagnostic framework, particularly valuable in high-risk settings with limited infrastructure

2. MATERIALS AND METHODS

2.1 Design of the Study and Sample Collection

In the present cross-sectional study, urine samples were obtained from patients with clinical diagnoses of urinary tract infections (UTIs) at local hospitals and diagnostics centres, Bhubaneswar. Forty participants consented, and sterile, leak-proof containers were used to collect 40 midstream urine samples from both inpatients and outpatients, aseptically. All samples were transported to the microbiology laboratory within two hours after collection and processed for microbiological examination without delay in order to avoid any decrease in the vitality and integrity of the bacterial isolates.

2.2 Culture and isolation of microorganisms

Upon arrival at the microbiology laboratory, each urine sample was processed immediately to maintain bacterial viability. Sterile inoculating loop delivering 0.001 mL of urine were aseptically inoculated on MacConkey's agar, Cystine lactose electrolyte deficient (CLED) and Blood agar media. MacConkey agar was used for selective isolation of Gram-negative enteric bacilli based on lactose fermentation. CLED agar inhibited Proteus species swarming while allowing growth of gram-negative and gram-positive uropathogens. Blood agar served as a non-selective medium to allow organism growth, detect haemolysis, and support fastidious organisms.

The plates were aerobically incubated at 37°C for 24 to 48 h, and the growth patterns, haemolysis (on blood agar), and lactose fermentation (on MacConkey and CLED agars) of bacterial colonies were noted. Colony counts were converted to colony-forming units per millilitre (CFU/mL). $\geq 10^5$ CFU/mL was considered significant bacteriuria indicating urinary infection according to standard clinical microbiology criteria.

2.3 Biochemical Identification

Biochemical identification was performed on all bacterial isolates to determine their species based on standard microbiological tests. Initial observations included colony morphology, colour, odor, and haemolysis on blood agar, followed by Gram staining to classify bacteria as Gram-positive or Gram-negative. Each isolate was then subjected to key biochemical tests, including catalase, oxidase, indole production, citrate utilization, urease activity, and triple sugar iron (TSI) reactions. These tests helped differentiate between common uropathogens such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis*. The results guided the selection of representative strains for further molecular analysis.

2.4 Genomic DNA Extraction and 16S rRNA Gene Amplification

Genomic DNA was extracted from pure bacterial isolates cultured overnight in Luria-Bertani (LB) broth using the MPure Bacterial DNA Extraction Kit (MP Biomedicals, India), in accordance with the manufacturer's instructions. The quantity and purity of the extracted DNA were evaluated using a Nanodrop spectrophotometer (Thermo Scientific, USA), measuring absorbance at 260/280 nm, while DNA integrity was confirmed by electrophoresis on a 1% agarose gel.

For molecular identification, the 16S rRNA gene was targeted using universal bacterial primers. Amplification was carried

out using primer pair 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Each PCR reaction consisted of a total volume of 25 µL, including 12.5 µL of genes to protein PCR Master Mix, 1 µL of each primer (10 µM concentration), 2 µL of genomic DNA template, and 8.5 µL of nuclease-free water. The thermal cycling protocol was as follows: an initial denaturation step at 95°C for 5 minutes; 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute; followed by a final extension step at 72°C for 10 minutes. The amplified PCR products were then analysed by agarose gel electrophoresis to confirm the presence and expected size (~1500 bp) of the 16S rRNA amplicons.

2.5 Gel Electrophoresis and Visualization

Amplified PCR products were resolved on 1.2% agarose gel stained with green florescent dye (Barcoad Biosciences, India) and visualized under UV illumination using a gel documentation system (Bio-Rad, USA). A 100 bp DNA ladder was used as a molecular size marker.

2.6 Sequencing and Bioinformatics Analysis

Positive amplicons were purified using the PCR Purification Kit (Genes to protein, India) and share the sample to Heredity Biosciences, Bhubaneswar for Sanger sequencing. After receiving the sequencing results, the quality was verified using Chromas software, and BLAST analysis was performed against the NCBI GenBank database for species-level identification. Sequences with ≥99% identity were considered definitive matches. Identified sequences were submitted to GenBank, and accession numbers were obtained.

2.7 Statistical Analysis

Descriptive statistics were used to summarize bacterial prevalence. Sequence identity percentages were calculated, and the diversity of isolates was compared using phylogenetic tree construction via MEGA X software.

3. RESULTS

3.1 Bacterial Isolation and Culture

Out of 40 urine specimens collected, 32 samples showed significant bacterial growth (≥10^5 CFU/mL). *Escherichia coli* was the predominant isolate (37.5%), followed by *Klebsiella pneumoniae* (21.875%), *Pseudomonas aeruginosa* (18.75%), *Proteus mirabilis* (12.5%), and *Enterococcus faecalis* (9.375%) Table-1 & Figure-1. Colony morphology on selective media, along with Gram staining, provided preliminary identification. Biochemical profiles confirmed these isolates, which were selected for molecular identification. Samples with mixed growth or contaminants were excluded from sequencing.

Table 1: Frequency Distribution of Uropathogenic Isolates

Bacterial Species	Number of Isolates (n)	Percentage (%)
Escherichia coli	12	37.5%
Klebsiella pneumoniae	7	21.875 %
Pseudomonas aeruginosa	6	18.75%
Proteus mirabilis	4	12.5%
Enterococcus faecalis	3	9.375 %
Total	32	100%

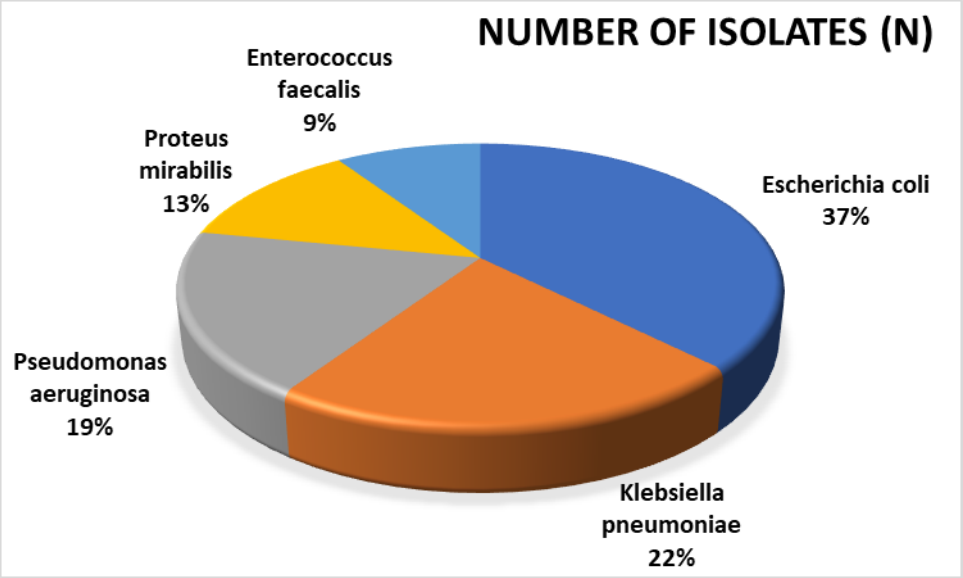

Figure 1: Distribution of predominant uropathogens isolated from urine samples, showing relative frequencies of bacterial species identified through culture and biochemical tests.

3.2 Biochemical Characterization

Standard biochemical tests were done as a routine procedure for the initial identification of all 32 significant uropathogens isolated from urine cultures. Escherichia coli was the predominant organism, positive for indole and lactose fermentation; it was negative for citrate and urease. K. pneumonia was positive for citrate and urease fermentation and indole-negative. *Pseudomonas aeruginosa* was identified by the oxidase positive, non-lactose fermenting organism. *Proteus mirabilis* was strongly active for urease, utilization of citrate, and swarming. The strain is catalase negative and fermentative of lactose, and is a Gram-positive coccus (*Enterococcus faecalis*). Key biochemical tests and their results for each organism are outlined in (**Table 2**) **(Figure-2, Figure 3, Figure 4, Figure 5 and Figure 6**).

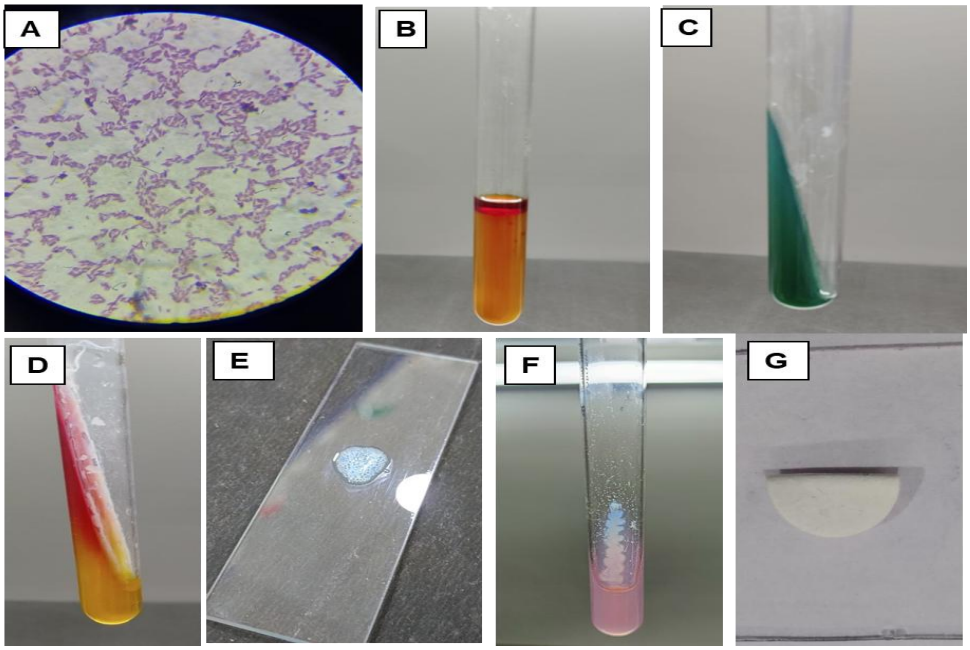

Figure 2. Standard biochemical tests identified *Escherichia coli*. The isolate showed: Gram-negative rod morphology, positive Indole test, negative Citrate utilization, negative Urease test, positive Catalase test, positive Lactose fermentation on MacConkey agar, and negative Oxidase test. These results confirmed the bacterial isolate as E. coli.

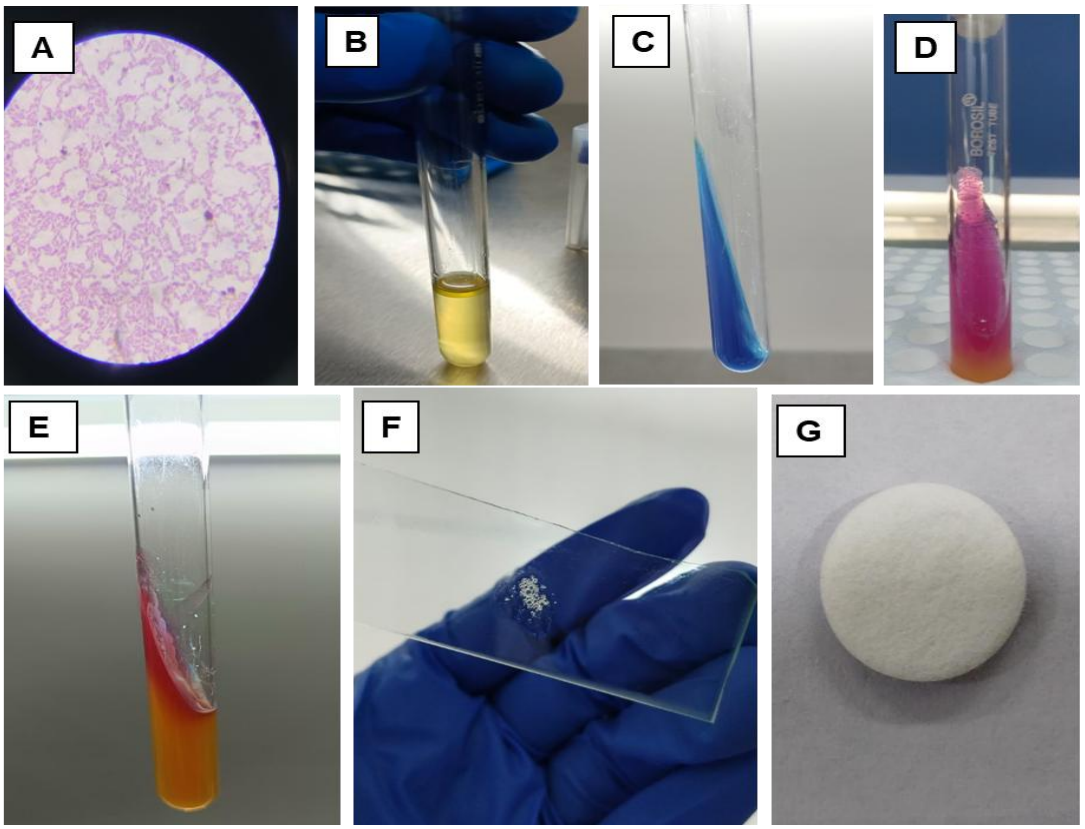

Figure 3 Biochemical characterization of *Klebsiella spp*. was performed as routine diagnostic procedures. The isolate showed: (A) Gram-negative bacilli morphology; (B) Indole test negative; (C) Citrate utilization positive; (D) Urease test positive; (E) Catalase test positive; (F) Lactose fermentation positive on MacConkey agar; (G) Oxidase test negative.

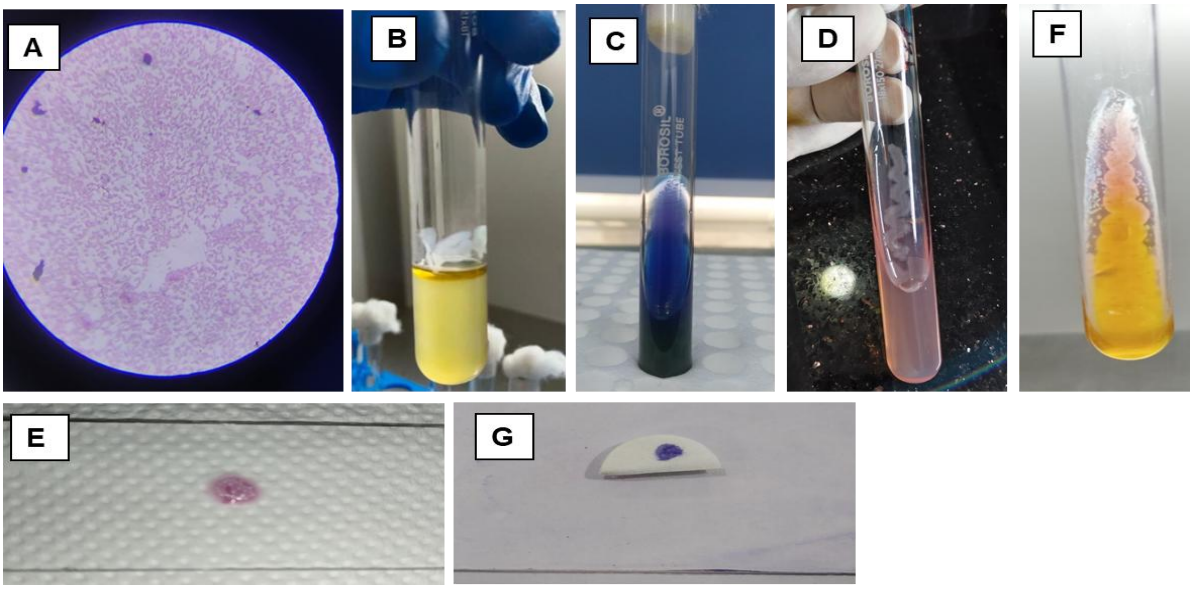

Figure 4 Biochemical characterization of *Pseudomonas species* was performed during routine diagnostic procedures. (A) Gram staining showed Gram-negative bacilli. The isolate was negative for Indole production (B), positive for Citrate utilization (C), and positive for Urease activity (D). Catalase activity was positive (E), as was lactose fermentation (F), while the oxidase test was negative (G). These biochemical profiles support identification of the isolate as Pseudomonas spp.

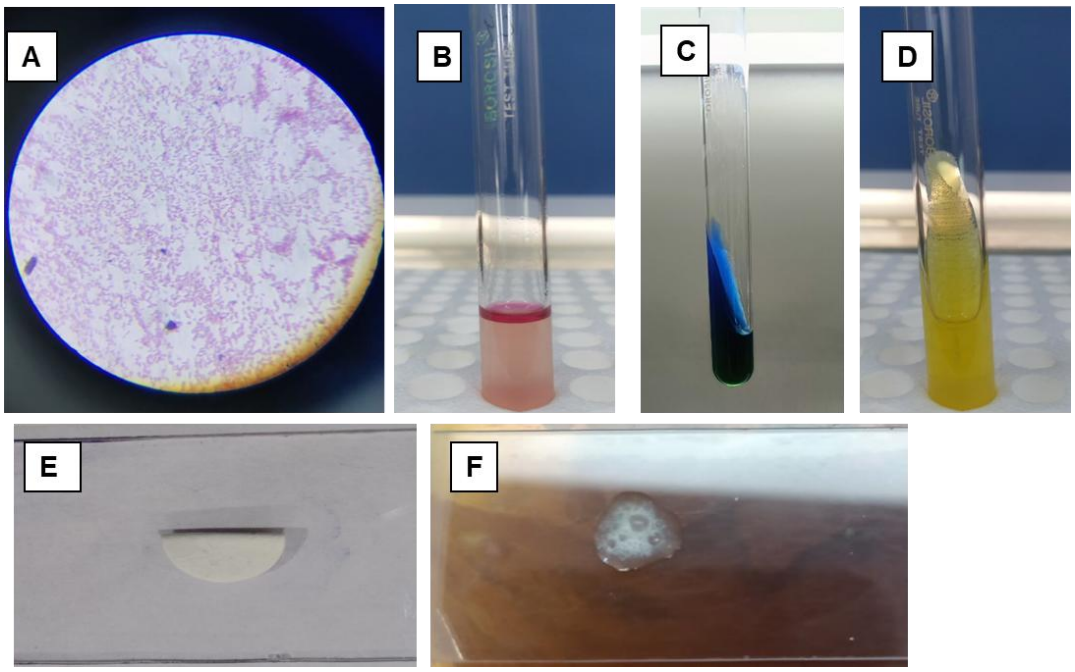

Figure 5. Biochemical characterization of *Proteus spp*. isolates based on diagnostic assays. (A) Gram staining showed Gram-negative rods. (B) Indole test was positive, indicating tryptophanase activity. (C) Citrate utilization test was positive, showing the organism utilizes citrate as carbon source. (D) Lactose fermentation was positive, indicating metabolic activity on lactose. (E) Oxidase test was negative, consistent with Proteus species profile. (F) Citrate test was positive, confirming growth on citrate media. These results confirm the identity and metabolic traits of Proteus spp.

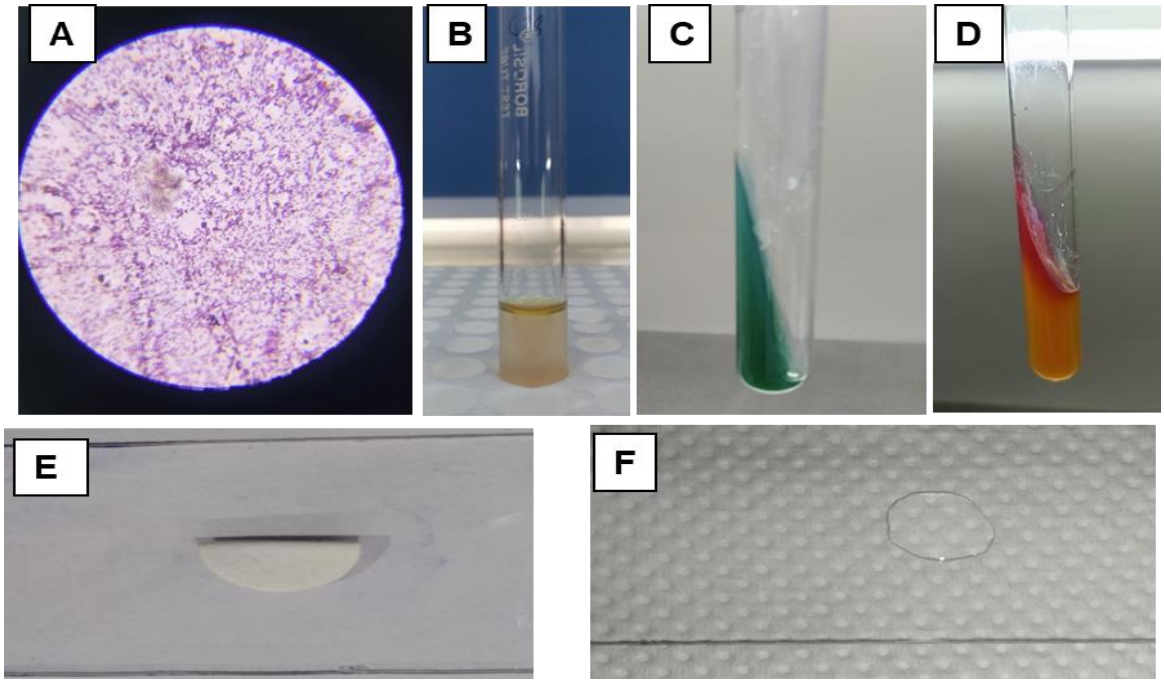

Figure 6: Biochemical characterization of the Enterococcus isolate was performed to support phenotypic identification. (A) Gram staining showed a Gram-negative reaction. (B) The indole test was negative, indicating no tryptophanase activity. (C & F) The isolate was negative for citrate utilization. (D) Positive lactose fermentation was observed. (E) The oxidase test was negative, confirming absence of cytochrome c oxidase. These profiles are consistent with Proteus species and support its role in urinary tract infections.

Table 2: Biochemical Characteristics of Predominant Uropathogens

Test	*E. coli*	*Klebsiella* pneumonia	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Enterococcus faecalis*
Gram Stain	–	–	–	–	+
Indole	+	–	–	+	–
Citrate Utilization	–	+	+	+	–
Urease	–	+	–	+	+
Oxidase	–	–	+	–	–
Lactose Fermentation	+	+	–	–	+
Catalase	+	+	+	+	–
Motility	+	–	+	+ **(crowded)**	–

3.3 PCR Amplification and Gel Electrophoresis

PCR amplification of the 16S rRNA gene using universal primers (27F/1492R) yielded clear bands of approximately 1500 bp in all 32 selected isolates. Gel electrophoresis confirmed successful amplification with no nonspecific products. Amplicons were suitable for sequencing and were purified for downstream processing.

3.4 16S rRNA Sequencing and Identification

Sequencing results confirmed the identity of all isolates with ≥98% similarity to reference sequences in the NCBI GenBank database. All sequences were submitted to GenBank, and accession numbers were obtained (**Table-3**). Minor sequence variations were observed, reflecting strain-level diversity.

Table 3: 16S rRNA Sequencing Results and NCBI GenBank Accession Numbers

Isolate Code	Identified Species	% Identity	GenBank Accession No.
UTI-01	*Escherichia coli*	99.8%	PV715786
UTI-02	*Klebsiella pneumoniae*	99.5%	PV715794
UTI-03	*Pseudomonas aeruginosa*	99.7%	PV715787
UTI-04	*Proteus mirabilis*	99.1%	PV715788
UTI-05	*Enterococcus faecalis*	99.20%	PV715797

4. DISCUSSION

This study employed both conventional and molecular techniques to identify uropathogenic bacteria from patients with suspected urinary tract infections (UTIs). The findings highlight the predominance of *Escherichia coli* among isolates, consistent with numerous reports identifying it as the primary etiological agent of UTIs, particularly in women and catheterized patients [10,11]. The presence of other Gram-negative organisms, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, supports previous epidemiological observations linking these species to nosocomial and complicated UTIs [12,13].

Traditional biochemical tests were used for initial identification, which aligned well with results obtained from 16S rRNA gene sequencing. However, culture-based methods alone may be insufficient, especially when bacterial loads are low, or when patients have received prior antibiotic therapy that suppresses growth [14]. Several fastidious or slow-growing organisms can evade detection using conventional techniques, leading to underdiagnosis or misidentification. Thus, molecular tools are increasingly essential in clinical microbiology.

The amplification and sequencing of the 16S rRNA gene provided definitive identification at the species level, with all samples showing ≥98% similarity to known reference strains. This result confirms the reliability of the 16S rRNA gene as a

molecular marker due to its highly conserved regions interspersed with variable domains that allow discrimination between closely related bacterial taxa [15]. The successful submission of representative sequences to NCBI GenBank enhances the availability of local strain data for future reference and phylogenetic studies.

The antibiotic susceptibility data revealed alarmingly high resistance rates to commonly used antibiotics, such as ampicillin and ciprofloxacin, particularly among *E. coli* and *Pseudomonas* isolates. These findings echo global concerns regarding the increasing prevalence of multidrug-resistant (MDR) uropathogens [16]. Imipenem showed 100% sensitivity across all tested isolates, consistent with its classification as a broad-spectrum carbapenem typically reserved for severe or resistant infections [17]. However, the indiscriminate use of such last-resort antibiotics must be monitored to prevent resistance development.

Nitrofurantoin and gentamicin also demonstrated moderate effectiveness, particularly against *E. coli* and *Enterococcus faecalis*, and may remain viable options for empirical treatment of uncomplicated UTIs. These results support the need for regular antimicrobial surveillance to inform evidence-based empirical treatment guidelines [18]. Molecular identification techniques such as 16S rRNA sequencing should be integrated into routine diagnostics, especially in cases with atypical presentations, recurrent infections, or therapeutic failures [19].

One limitation of this study is the sample size, which, although adequate for molecular confirmation, may not reflect the full microbial diversity encountered in different geographic or demographic populations. Additionally, 16S sequencing cannot distinguish pathogenic strains from commensals within the same species and does not provide functional or resistance gene data, which could be addressed through whole genome sequencing or metagenomic approaches in future studies.

5. CONCLUSION

This study demonstrates the value of integrating conventional microbiological methods with molecular techniques, specifically 16S rRNA gene sequencing, for the accurate identification of uropathogenic bacteria in urinary tract infections. While traditional culture and biochemical tests provide initial insight, molecular identification offers superior specificity, particularly for distinguishing closely related species and detecting fastidious organisms. The high prevalence of *Escherichia coli* and multidrug-resistant Gram-negative bacteria underscores the urgent need for targeted antimicrobial stewardship. The 16S sequencing results not only confirmed bacterial identity but also facilitated the creation of a local sequence database for future epidemiological monitoring. Incorporating molecular diagnostics into routine UTI screening could significantly enhance clinical decision-making and help curb the growing threat of antibiotic resistance.

Ethical Consideration

Ethical approval was not required as sample collection followed routine diagnostic procedures. Samples were obtained from pathology centres with patient consent for research. All procedures followed clinical protocols and ethical guidelines, maintaining patient confidentiality throughout the research.

Source of Funding

None

Conflict of Interest

None

Authors' Contributions

All authors contributed to the conception, design, and writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors are thankful to Heredity Biosciences, Bhubaneswar, India for extending their research facilities. We also thank the Heredity Biosciences team for the ongoing support and cooperation to conduct the study and analyze data. Their support in this regard in terms of infrastructure, technical help and scientific input was useful in execution of this work

REFERENCES

[1] Sharma, K., & Verma, R. (2023). Urinary tract infections: A review. World Journal of Biology Pharmacy and Health Sciences, 13(2), 189–192. https://doi.org/10.30574/wjbphs.2023.13.2.0099

[2] Ussai, S., & Rizzo, M. (2016). Antibiotic Treatment of Urinary Tract Infections (UTIs) In Primary Care: An Italian Pilot Study. Journal of Pharmacovigilance, 4(4). https://doi.org/10.4172/2329-6887.1000215

[3] Wagenlehner, F. M. E. (2023). Urinary tract infections including pyelonephritis. Medizinische Klinik, Intensivmedizin Und Notfallmedizin, 118(6), 464–469. https://doi.org/10.1007/s00063-023-01034-7

[4] Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson

DE, Brubaker L. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012 Apr;50(4):1376-83. doi: 10.1128/JCM.05852-11. Epub 2012 Jan 25. PMID: 22278835; PMCID: PMC3318548.

[5] Jorgensen, J. H., & Ferraro, M. J. (2000). Antimicrobial susceptibility testing: special needs for fastidious organisms and difficult-to-detect resistance mechanisms. Clinical Infectious Diseases, 30(5), 799–808. https://doi.org/10.1086/313788

[6] Ayelign, B., Abebe, B., Gelaw, A., Shibabaw, T., Meshesha, S., Shibeshi, A., Addis, Z., & Dagnew, M. (2018). Bacterial isolates and their antimicrobial susceptibility patterns among pediatric patients with urinary tract infections. Türk Üroloji Dergisi/Turkish Journal of Urology, 44(1), 62–69. https://doi.org/10.5152/tud.2017.33678

[7] Aziz, R., & Sameer, E. (2021). Molecular detection of antibiotic resistance genes and biofilm production among uropathogenic bacteria. Systematic Reviews in Pharmacy, 11(10), 1018–1038. https://doi.org/10.31838/srp.2020.10.152

[8] Lu, X., Wu, W., Wang, M., & Huang, Y. (2008). 16S rRNA gene sequencing for pathogen identification from clinical specimens. National Medical Journal of China, 88(2), 123–126. https://doi.org/10.3321/j.issn:0376-2491.2008.02.014

[9] Rampini, S. K., Bloemberg, G. V., Böttger, E. C., Büchler, A. C., Dollenmaier, G., Speck, R. F., & Keller, P. M. (2011). Broad-Range 16S rRNA Gene Polymerase Chain Reaction for Diagnosis of Culture-Negative Bacterial Infections. Clinical Infectious Diseases, 53(12), 1245–1251. https://doi.org/10.1093/cid/cir692

[10] Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010;7(12):653–660.

[11] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269–284.

[12] Wagenlehner FM, Naber KG. Treatment of bacterial urinary tract infections: presence and future. Eur Urol. 2006;49(2):235–244.

[13] Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrob Chemother. 2003;51(1):69–76.

[14] Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory. J Clin Microbiol. 2007;45(9):2761–2764.

[15] Sune D, Rydberg H, Augustinsson ÅN, Serrander L, Jungeström MB. Optimization of 16S rRNA gene analysis for use in the diagnostic clinical microbiology service. J Microbiol Methods. 2020 Mar;170:105854. doi: 10.1016/j.mimet.2020.105854. Epub 2020 Jan 23. PMID: 31982454.

[16] Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis. Clin Infect Dis. 2011;52(5):e103–e120.

[17] Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. Front Microbiol. 2017;8:587.

[18] Kahlmeter G. The ECO.SENS project: a prospective, multinational, multicentre epidemiological survey of the prevalence and antimicrobial susceptibility of urinary tract pathogens–interim report. J Antimicrob Chemother. 2000;46(Suppl A):15–22.

[19] Acharya P, & Jal S. (2024). The Ethno-pharmaceuticals Fighting UTI-Resistant Bacteria: Synergistic Potential Exploration. International Journal of Advancement in Life Sciences Research, 7(3), 24-36. https://doi.org/https://doi.org/10.31632/ijalsr.2024.v07i03.003.