



Original Research Paper

Isolation and Molecular Identification of Uropathogenic Organisms from Patients with UTIs

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Key Words	Abstract
Bacterial pathogens, Infection, Molecular Identification, Oxidase, Pyelonephritis, UTI	Urinary infections, commonly caused by bacterial pathogens, affect individuals across all age groups, with women, infants, and the elderly being more susceptible. This study isolated and identified bacterial strains from urine samples of 50 UTI patients under sterile conditions using different culturing media. A total of 59 distinct bacterial strains underwent gram staining and biochemical tests like Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, Urease, Catalase, Oxidase, Ammonia Production, Motility and TSI. Biochemical test results revealed that 49.15% were Gram-positive and the rest were Gram-negative strains, 89.83% were Indole-positive, 79.66% were Methyl Red-positive, 61.02% were Voges-Proskauer-positive, 72.88% were positive for citrate utilization, 25.42% of strains were urease positive, 0% Catalase-positive and motile, 18.64% were Oxidase-positive, 47.46% produced Ammonia, and 30.51% produced gas in TSI tests. These biochemical tests revealed the isolated organisms to be <i>Klebsiella oxytoca</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Salmonella typhi</i> , <i>Vibrio cholera</i> , <i>Citrobacter koseri</i> . The remaining cultures that cannot be identified were identified using molecular techniques the species were found to be <i>Clostridium sp</i> , <i>Pseudomonas sps</i> , <i>Bacillus megaterium</i> , <i>Desemzia incerta</i> , <i>Exiguobacterium sp.</i> , <i>Cellulosimicrobium funkei</i> , <i>Paraclostridium benzoelyticum</i> , <i>Proteus mirabilis</i> , <i>Lysinibacillus fusiformis</i> , <i>Lysinibacillus macroides</i> , <i>Pseudomonas sp.</i> , and Uncultured bacterium. Based on the above observation various pathogens are involved in causing urinary tract infections revealing its complexity and the need for effective management.

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1. Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections [1,2], affecting individuals of all ages, with a higher prevalence in women, infants, and the elderly [3]. UTIs can occur in various parts of the urinary system, including the urethra, bladder, ureters, and kidneys [4]. The whole urinary tract likely gets infected including kidneys, urethra, Bladder and ureters [5,6]. Several factors contribute to UTIs, including bacterial invasion, sexual activity, anatomical factors, blockages in the urinary tract, immune system issues, and the use of catheters [7,8]. UTIs are mainly caused by bacterial infections, with *E. coli* accounting for approximately 80-90% of cases acquired in the community [9,10]. Other significant bacteria include *K. pneumoniae* [11], *P. mirabilis* [12], *P. aeruginosa* [13], and *E. coli* species [14], as well as Gram-positive bacteria such as *S. saprophyticus*, *E. faecalis*, and *S. agalactiae* [15]. These bacteria are often identified through urine culture tests. The pathogenesis of UTIs involves the initial adherence of bacteria to the uroepithelium through FimH dependent manner, next their invasion into uroepithelial cells, and replication within the urinary tract, by simultaneously evading host's immune response through biofilm formation [16]. The health impacts of UTIs can vary Based on the intensity and site of the infection. Lower UTIs, or cystitis [17], typically cause signs like dysuria (painful urination) [18], frequent and urgent urination, and suprapubic pain [19]. Upper UTIs, otherwise called pyelonephritis [20], has severe symptoms including fever, flank pain, nausea and vomiting and can also lead to kidney damage or sepsis if not properly treated. Chronic and recurring UTIs can often result in chronic inflammation and scarring of the urinary tract which increases the risk of kidney dysfunction [21,22].

UTI management involves various approaches and one of the primary treatments is usage of antibiotics; however, the rise of antibiotic resistance in bacteria is making it a necessity to find other approaches in treating infections. Prevention can be done by following proper hygiene, being well hydrated which promotes regular urination. In recurrent cases, prophylactic antibiotics are used to prevent the recurring and also use other strategies

like using probiotics and cranberry supplements to prevent bacterial colonization [23]. Additionally, research is ongoing into creating vaccines against uropathogenic *E. coli* in order to provide long-term protection [24]. In order to manage the UTIs, accurate identification of the causative bacteria, appropriate antibiotic use, and preventive strategies to reduce recurrence and resistance development must be done. Several studies have highlighted how UTIs infect and the factors that are responsible in contributing to their persistence and recurrence [25-27]. There are various intrinsic factors that helps in the progression of uropathogens and some examples includes virulent factors like biofilm formation which mediates in antibiotic resistance [28]. Studies also reveals the importance of host-microbe interactions and the role of the host immune response in against infections [29,30]. Due to increase in antibiotic resistance among uropathogens, the use of antibiotics is of no use urging researchers to find alternative effective remedies. Hence, understanding the microbial causes and the pathogenesis of UTIs is essential for developing efficient treatment strategies. In this study we aim to isolate uropathogens from urine samples collected from 50 UTI patients. The samples will be serially diluted and screened using selective media, and the isolated bacteria will undergo biochemical analysis and species will be identified by molecular techniques and finally phylogenetic analysis will also be performed.

2. Methodology

2.1. Sample Collection

Urine samples were collected from 50 individuals diagnosed with UTIs. The collection process ensured sterility to prevent contamination. Each sample were collected in a sterile container and immediately transported to the laboratory under refrigerated conditions to preserve the viability of the bacteria present.

2.2. Isolation of bacteria

To isolate bacteria from the urine samples, serial dilutions were performed. The samples were diluted from 10⁻¹ to 10⁻⁵ to reduce the concentration of bacterial load and obtain single colonies. Dilutions 10⁻² to 10⁻⁵ were then spread on three different types of agar media: Nutrient Agar, MacConkey

Agar and Mannitol Salt Agar [31]. After incubation, morphologically different colonies were observed and selected. These colonies were inoculated onto a new media to isolate pure cultures. The strains were labeled U1 to U59.

2.3. Microbiological Identification: Gram Staining and Biochemical Tests

2.3.1. Gram Staining

Gram staining is a technique that classifies bacteria as Gram-positive or Gram-negative based on their cell wall structure. The heat fixed bacteria culture is stained with different stains step by step using crystal violet, iodine, decolorized with alcohol, and counterstained with safranin. The gram-positive stains are observed in –purple and the gram-negative ones in pink [32].

The selected colonies underwent a set of biochemical tests to assess their metabolic and enzymatic properties.

2.3.2. Indole Test

The Indole test is conducted to evaluate a bacterium's capacity to convert the amino acid tryptophan into indole. Bacteria are introduced into tryptophan broth and incubated at 37°C for 24-48 hours. Following incubation, Kovac's reagent is added to the culture. A positive result is identified by the appearance of a red ring at the top of the medium, confirming indole production [33].

2.3.3. Methyl Red Test

MR test evaluates the ability of bacteria to produce stable acids through glucose fermentation. Bacteria are cultured in MR broth and incubated at 37°C for 24-48 hours. A few drops of methyl red indicator are added after incubation. A red color confirms a positive result, indicating the production of stable acids and a decrease in the medium's pH [34].

2.3.4. Voges-Proskauer Test

The VP test identifies the production of acetoin through glucose fermentation. Bacteria are introduced into VP broth and incubated at 37°C for 24-48h. After incubation, Barritt's reagents A and B are added to the culture. A red color that appears after shaking indicates a positive result, confirming acetoin production. [34].

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2.3.5. Citrate Utilization Test

The Citrate utilization test assesses whether bacteria can utilize citrate as their only carbon source. Bacteria are cultured into Simmons Citrate Agar slants and incubated at 37°C for 24-48h. A shift in color from green to blue in the medium signifies a positive result, indicating that the bacteria can utilize citrate and generate alkaline byproducts. [35].

2.3.6. Urease Test

Urease test is performed to check whether the bacteria can hydrolyse urea into ammonia and CO₂. Bacterial colonies were inoculated into urea broth supplemented with 40% urea and incubated at 37°C for 24-48 hours. A color change to pink in the medium indicated a positive result, which confirms the production of urease and the hydrolysis of urea [36].

2.3.7. Catalase Test

The Catalase test is done to determine whether the bacteria is able to produce catalase. A small amount of bacterial culture is placed onto a glass slide, and few drops of Hydrogenperoxide will be added. The positive result is indicated by formation of bubbles [37].

2.3.8. Oxidase Test

The Oxidase test is used to identify bacteria that can produce cytochrome c oxidase. A small amount of bacterial culture is placed on filter paper soaked with tetramethyl-p-phenylenediamine reagent. A positive result will be observed if there is a color change to dark purple within 30 seconds, confirming the presence of cytochrome c oxidase [38].

2.3.9. Ammonia Production Test

The Ammonia production test will assess the production of ammonia from various nitrogenous compounds. The screened bacterial cultures will be inoculated into the peptone broth and incubated at 37°C for 24-48 h. after incubation Nessler's reagent will be added, which produces a yellow to brown color if the bacteria are able to produce ammonia indicating positive result [39].

2.3.10. Motility Test

The Motility test determines whether bacteria are capable of movement. Loopful of 24-hour-old

culture was used and Stab into a SIM medium. the tubes were incubated at 37°C for 7 days. Diffuse growth radiating from the stab line or cloudiness throughout the medium indicates a positive result, confirming bacterial motility [40].

2.3.11. Triple Sugar Iron (TSI) Agar Tests

To check the bacteria ability to utilize three different sugar molecules (Glucose, sucrose and lactose) and ability to produce H₂S and release of gas due to carbohydrate fermentation, TSI test is performed. The media changes color due to the presence of phenol red indicator and the results are interpreted based on this color change of the media, H₂S production is confirmed by the appearance of black color in the media. If the bacteria are able to produce gas during the fermentation process the media cracks and starts to lift upwards [41].

2.4. Identification through Molecular Techniques

Unknown bacterial cultures were further identified using molecular technique. The genomic DNA of the bacteria was isolated using phenol chloroform method. in this method the cells were initially lysed, then separated and finally the DNA is precipitated. The isolated genomic DNA will then be subjected to amplify specific DNA sequences by PCR (Polymerase Chain Reaction). Primers targeting conserved regions, such as 16S rRNA genes [42], were utilized to ensure broad coverage across various bacterial species. The PCR conditions included a denaturation step of 94°C for 30 seconds, annealing at 57°C for 30 seconds and extension at 72°C for 30 second. Along with initial denaturation step in the first cycle at 95°C for 5 minutes and final extension step at the last cycle at 72°C for 5 minutes.

The amplified DNA fragments were separated by base pair size through gel electrophoresis, to confirm the amplification. The next step involved sequencing the PCR products to determine the exact nucleotide sequences of the targeted regions. These sequences were subsequently compared against the NCBI database [43] using the BLAST [44] to identify the closest matches. This comparison enabled the determination of the bacterial species based on genetic similarity to known sequences, providing a robust and accurate identification of the unknown strains.

2.5. Phylogenetic Tree Construction

Phylogenetic analysis was done to understand the evolutionary relationships among the isolated bacterial strains. The sequence obtained was first aligned with other closely related bacterial sequences by using MEGA 11 software [45], after alignment a phylogenetic tree was constructed using neighbor-Joining method with a boot strap value of 1000. The resulting phylogenetic tree provided a visual representation of the evolutionary history of the isolated strains and their closeness to the known species.

3. Results

3.1. Sample collection

Urine samples were collected from 50 patients diagnosed with UTI in sterile containers. The patients across different age groups and genders are summarized in the Table 1. The data indicates that the majority of the UTI patients are female, with highest concentration of patients in the 18-30 age groups.

Table 1 Distribution of UTI Patients by Age and Gender

Age Group	Gender		Total
	Male	Female	
18-30	2	13	15
31-45	5	9	14
45-65	3	9	12
>65	3	6	9
Total	13	37	50

3.2. Isolation of bacteria

Serial dilutions were done using urine samples collected from 50 UTI patients to isolate bacterial strains. From each sample, 100 µL of solution from dilution factors ranging from 10⁻² to 10⁻⁵ were spread onto three different agar media: NA, MacConkey agar, and Mannitol Salts agar each. Growth of different bacterial cultures were observed after incubation and differentiated based on their morphological characteristics. From these plates, morphologically distinct bacterial strains were selected and subcultured. A total of 59 distinct strains were observed and were labeled as U1 to U59.

3.3. Microbiological Identification: Gram Staining and Biochemical Tests

In the analysis of biochemical tests conducted on 59 bacterial strains isolated from urine samples,

several key results were observed. Among the bacterial strains 49.15% of strains were identified as Gram-positive, while 50.85% were Gram-negative. The Indole test revealed 89.83% of strains tested positive. In case of MRVP test 79.66% of strains tested positive Methyl Red and 61.02% were positive for VP test. Citrate utilization was positive in 72.88% of cultures. The Urease test revealed that 25.42% were positive for urease activity. Both the Catalase and Motility tests yielded 0.00% positive results. For the Oxidase test, 18.64% of strains were positive for oxidase production. Ammonia production was observed in 47.46% of strains. The TSI test revealed 11.86% positive results for H₂S production, 30.51% of strains were positive for gas production, 74.58% tested positive for glucose fermentation, 32.20% were positive for sucrose fermentation 32.20% positive results for lactose fermentation (Table 2,3).

Table 2 Biochemical Tests Results- This table displays the outcomes of various biochemical tests conducted on 59 different samples, identified by their sample number (U1 to U59)

S.No	Name of the Sample	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1.	U1	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+
2.	U2	+	+	+	+	-	-	+	+	-	-	-	+	+	+	+
3.	U3	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+
4.	U4	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+
5.	U5	+	+	-	+	-	-	-	+	-	-	-	+	+	+	-
6.	U6	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+
7.	U7	+	+	-	+	+	-	+	+	-	-	+	+	-	-	-
8.	U8	+	+	-	+	+	-	+	-	-	-	-	+	-	-	-
9.	U9	+	+	-	+	+	-	+	+	-	-	+	+	-	-	-
10.	U10	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-
11.	U11	+	+	-	+	-	-	+	+	-	-	-	+	+	+	+
12.	U12	+	+	-	+	-	-	+	-	-	-	-	+	+	+	+
13.	U13	+	+	-	+	-	-	+	-	-	-	+	+	+	+	+
14.	U14	+	+	+	-	-	-	-	+	-	-	-	+	-	-	+
15.	U15	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+
16.	U16	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+
17.	U17	+	+	+	-	-	-	-		-	-	-	-	-	-	+
18.	U18	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+
19.	U19	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-
20.	U20	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
21.	U21	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-

22.	U22	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-
23.	U23	-	+	+	+	-	-	-	+	-	-	-	+	-	-	+
24.	U24	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-
25.	U25	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-
26.	U26	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
27.	U27	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-
28.	U28	+	-	+	+	-	-	-	-	-	+	+	+	-	-	+
29.	U29	+	-	+	+	-	-	-	-	-	+	+	+	-	-	+
30.	U30	-	-	-	+	+	-	-	+	-	+	-	+	-	-	-
31.	U31	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-
32.	U32	+	-	+	-	+	-	-	+	-	+	-	+	-	-	-
33.	U33	+	+	+	+	-	-	-	+	-	-	+	+	+	+	-
34.	U34	+	-	+	-	-	-	-	+	-	-	+	+	-	-	-
35.	U35	+	-	-	+	-	-	-	+	-	-	+	+	-	-	-
36.	U36	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
37.	U37	+	+	-	+	-	-	-	-	-	-	-	+	+	+	-
38.	U38	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
39.	U39	+	+	+	-	+	-	-	-	-	-	-	+	-	-	+
40.	U40	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+
41.	U41	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-
42.	U42	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+
43.	U43	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+
44.	U44	+	+	+	+	-	-	-	+	-	-	-	+	-	-	+
45.	U45	+	+	-	+	-	-	-	-	-	-	+	+	+	+	-
46.	U46	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+
47.	U47	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+
48.	U48	+	+	-	+	-	-	-	+	-	-	-	+	-	-	-
49.	U49	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
50.	U50	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+
51.	U51	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
52.	U52	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+
53.	U53	+	+	+	+	-	-	-	+	-	-	+	+	+	+	+
54.	U54	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+
55.	U55	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
56.	U56	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-
57.	U57	+	-	+	+	+	-	-	-	-	-	+	+	+	+	-
58.	U58	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-
59.	U59	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+

A-Indole test, B-Methyl Red test, C-Voges Prokauer's test, D-Citrate utilization test, E-Urease test, F-Catalase test, G-Oxidase test, H-Ammonia

production test, I-Motility test, J-TSI-H₂S Production test, K-TSI-Gas Production test, L-TSI-Glucose fermentation test, M-TSI-Sucrose

fermentation test, N-Lactose fermentation test, O-Gram staining

Positive results are indicated by "+", and negative results are indicated by "-".

Table 3 This table shows the distribution of positive and negative results across each biochemical test conducted on the bacterial strains

Biochemical Tests	Positive (%)	Negative (%)
Indole test	89.83	10.17
Methyl Red test	79.66	20.34
Voges Proskauer test	61.02	38.98
Citrate utilization test	72.88	27.12
Urease test	25.42	74.58
Catalase test	0.00	100.00
Oxidase test	18.64	81.36
Ammonia production test	47.46	50.85
Motility test	0.00	100.00
TSI-H ₂ S Production test	11.86	88.14
TSI-Gas Production test	30.51	69.49
TSI-Glucose fermentation test	74.58	25.42
TSI-Sucrose fermentation test	32.20	67.80
Lactose fermentation test	32.20	67.80
Gram staining	49.15	50.85

Out of the 59 isolates 24 isolates were found to be *K. oxytoca* (U5, U6, U7, U8, U9, U10, U56, U57), *E. coli* (U19, U22, U58), *S. typhi* (U20, U31), *C. koseri* (U21, U37, U38, U45, U48, U49), *V. cholerae*

(U24, U26, U35) and *P. aeruginosa* (U30, U34, U36) (Table - 4). The remaining 35 unidentified bacterial colonies will be subjected to molecular identification.

Table 4 This table shows the frequency rates of each bacterial strain identified through biochemical test from the samples U1-U59

Bacterial Strain	Frequency	Sample Names
Unknown	35	U1, U2, U3, U4, U6, U11, U12, U13, U14, U15, U16, U17, U18, U23, U25, U27, U28, U29, U32, U33, U39, U40, U41, U42, U43, U44, U46, U47, U50, U51, U52, U53, U54, U55, U59
<i>Klebsiella oxytoca</i>	7	U5, U6, U7, U8, U9, U10, U56, U57
<i>Escherichia coli</i>	3	U19, U22, U58
<i>Salmonella typhi</i>	2	U20, U31
<i>Citrobacter koseri</i>	6	U21, U37, U38, U45, U48, U49
<i>Vibrio cholerae</i>	3	U24, U26, U35
<i>Pseudomonas aeruginosa</i>	3	U30, U34, U36
TOTAL	59	

3.4. Identification through Molecular Techniques

Molecular identification techniques were employed in order to identify the remaining 35 bacterial strains which were unable to be identified with biochemical tests. Genomic DNA was extracted from the bacterial cells, and by using 16S rRNA primers, the target gene were amplified through PCR. The amplified PCR products were confirmed using gel electrophoresis and then sequenced. The resulting sequences were used to identify the bacteria by using the BLAST search tool

Table 5. Shows the results for the 35 unknown bacterial strains identified through 16s rRNA PCR. The table includes the bacterial strain identified, the frequency of each strain, and the specific sample names in which they were found.

Sample Name	Species Identified	Frequency
U1, U4, U6	<i>Bacillus megaterium</i>	3
U2, U3	<i>Desemzia incerta</i>	2
U11, U12, U13	<i>Exiguobacterium</i> sp.	3
U14, U16, U17, U18	<i>Cellulosimicrobium funkei</i>	4
U15	<i>Paraclostridium benzoelyticum</i>	1
U25, U27, U33	<i>Proteus mirabilis</i>	3
U39	<i>Lysinibacillus fusiformis</i>	1
U40	<i>Lysinibacillus macroides</i>	1
U41, U51	<i>Pseudomonas</i> sp.	2
U42, U43, U44, U46, U47, U50, U52, U53, U54, U55, U59	<i>Clostridium</i> sp.	11
U23, U28, U29, U32	Uncultured bacterium	4
	Total	35

3.5. Phylogenetic Tree Construction

Phylogenetic analysis of the bacterial strains was performed using the Neighbor-Joining method in MEGA 11 Software [45, 46]. A consensus tree based on 1000 bootstrap replicates was constructed to represent the evolutionary connections among the analyzed taxa. Branches with bootstrap support lower than 50% were omitted, and the bootstrap values reflect the proportion of replicates in which the taxa were grouped together [47]. Evolutionary distances were estimated using the Maximum Composite Likelihood approach [48], employing 24 nucleotide sequences with ambiguous positions

which compared against known sequences. This molecular approach helped us in identifying *Bacillus megaterium* in samples U1, U4, and U6; *Desemzia incerta* in U2 and U3; *Exiguobacterium* sp. in U11, U12, and U13; *Cellulosimicrobium funkei* in U14, U16, U17, and U18; *Paraclostridium benzoelyticum* in U15; *Proteus mirabilis* in U25, U27, and U33; *Lysinibacillus fusiformis* in U39; *Lysinibacillus macroides* in U40; *Pseudomonas* sp. in U41 and U51; *Clostridium* sp. in U42, U43, U44, U46, U47, U50, U52, U53, U54, U55, and U59; and an uncultured bacterium in U23, U28, U29, and U32 (Table 5).

removed (pairwise deletion), resulting in a final dataset of 916 positions. The tree exhibited distinct clustering patterns, where the *Lysinibacillus* species were closely related with one other and the close relationship between *E. faecalis*, *S. saprophyticus*, and *S. aureus*. *P. aeruginosa* which were grouped closely with *Pseudomonas* sp., were observed and *Proteus mirabilis* showed a close relationship with *Escherichia coli*. *Salmonella typhi* and *Citrobacter koseri* form a separate clade with high bootstrap support, which indicated a distinct lineage within the Enterobacteriaceae family. The uncultured bacteria were scattered, indicating diverse origins.

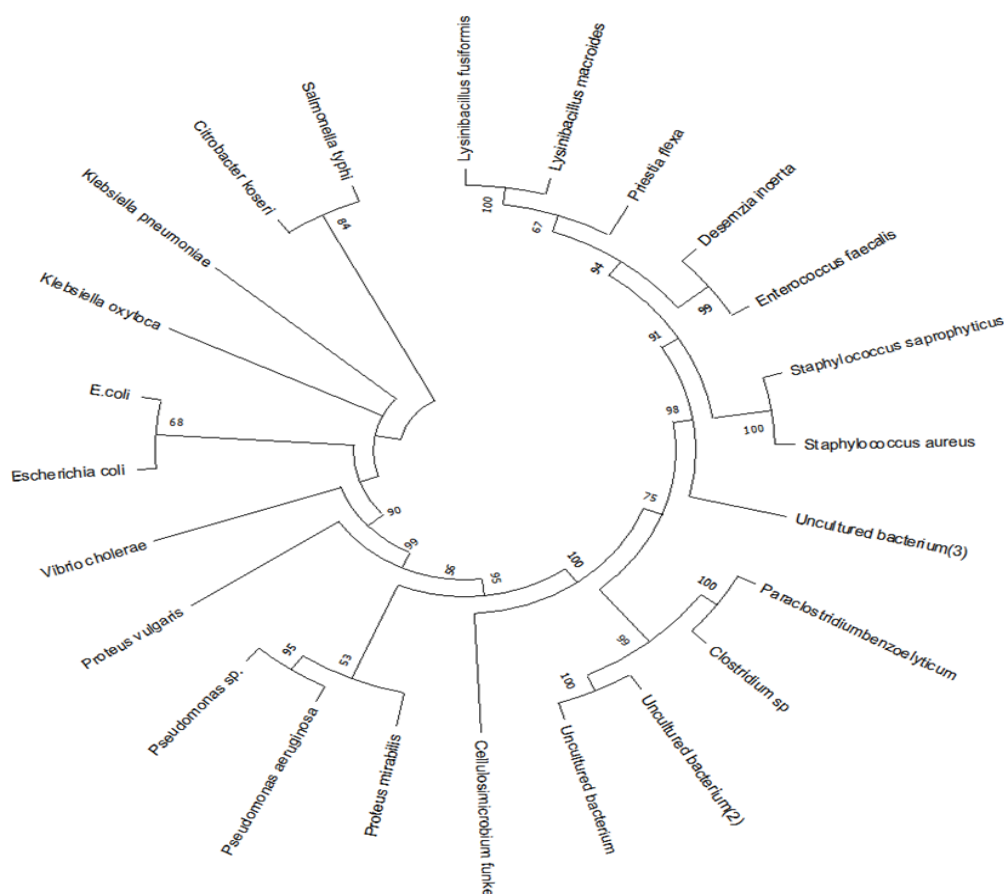


Figure 1. Phylogenetic tree of bacterial strains isolated from urinary tract infections.

4. Discussion

This study aimed to isolate and identify bacterial strains from urine samples of UTI patients, by utilizing biochemical tests and molecular techniques. The results revealed a various bacterial species with significant metabolic and enzymatic activities. A comparison with previous studies highlights several points of similarities and differences. In this work, we have isolated 59 distinct bacterial strains and identified from 50 urine samples of UTI patients, with an approximate equal distribution of Gram-positive and Gram-negative bacteria. With the use of biochemical tests, along with molecular techniques (16S rRNA gene PCR and sequencing), helped in the identification of various pathogens like *Escherichia coli*, *Klebsiella oxytoca*, *Citrobacter koseri*, *Bacillus megaterium*, and *Vibrio cholerae*. In coherence to our studies, other studies have consistently reported *E. coli* as the predominant UTI pathogen, responsible for 80-90% of community-acquired infections [49-55]. Other studies have also identified significant proportions of *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *Enterobacter* sp. [56-57]. However, 1393

the identification of species like *Vibrio cholerae* and *Lysinibacillus fusiformis* in this study helps in understanding the diversity of UTI pathogens. The biochemical tests in this study showed that 89.83% of them were Indole positive, 79.66% were positive for Methyl Red, 61.02% were Voges-Proskauer positive, 72.88% were positive for citrate utilization, Urease activity was detected in 25.42% of strains, 0% were positive for catalase and motility, Oxidase activity was observed in 18.64% of strains, and 47.46% were able to produce ammonia and Gas production in TSI tests was observed in 30.51% of strains. Similar biochemical profiles have also been reported in earlier studies [58]. However, the absence of Catalase-positive strains in this study is contradictory with that of previous findings as catalase activity is commonly observed in many UTI pathogens, indicating potential differences in bacterial communities or methodologies [59].

While this study didn't analyze the antibiotic resistance profiles, but checking the prevalence of UTI bacteria is similar to that of other reports which has also mentioned the MDR presence in the

commonly isolated bacteria. Drug-resistant UTI—especially Multidrug resistant (MDR) strains are becoming a widespread issue in recent years [60, 61]. The use of 16S rRNA gene sequencing and phylogenetic analysis provided detailed insights into the evolutionary relationships of the isolated strains. This work highlights the complexity of UTI-associated bacterial populations and emphasized the necessity for precise identification for effective management strategies. Molecular techniques have become important in UTI research for accurate bacterial identification and understanding of microbial diversity. Studies have demonstrated the utility of 16S rRNA sequencing in identifying both well-known and novel uropathogens, which aligns with the findings of this study [62-66]. This study helped in understanding the prevalence of UTI pathogens using Biochemical test and molecular identification.

5. Conclusion

This study successfully isolated 59 bacterial strains from urine samples collected from 50 UTI infected patients and initially screened them using biochemical tests and the unidentified organism were screened through molecular techniques. The biochemical tests revealed the metabolic pathways of different UTI pathogens. The molecular identification of the unknown strain also helped us in understanding the prevalence of UTI pathogens and their closest relation with known organism by phylogenetic analysis. These findings help in understanding the prevalence of UTI pathogens which is crucial for accurate diagnosis and treatment. Future studies will be done on antibiotic susceptibility of the isolated microbes which might help in management of UTI.

References

- [1] Sheerin, N. S. (2011). Urinary tract infection. *Medicine*, 39(7), 384-389.
- [2] Foxman, B. (2010). The epidemiology of urinary tract infection. *Nature Reviews Urology*, 7(12), 653-660.
- [3] Foxman, B. (2002). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *The American journal of medicine*, 113(1), 5-13.
- [4] Najar, M. S., Saldanha, C. L., & Banday, K. A. (2009). Approach to urinary tract infections. *Indian journal of nephrology*, 19(4), 129-139.
- [5] Barnett, B. J., & Stephens, D. S. (1997). Urinary tract infection: an overview. *The American journal of the medical sciences*, 314(4), 245-249.
- [6] Azhagu Madhavan S., Pandian A. (2025). Antihyperglycemic activity of polyphenolic metabolites and biosynthesized silver nanoparticles from *Pedaliu murex*: Characterization and application of antioxidant and uropathogenic antimicrobial activities. *Microbial Pathogenesis*. 205: 107620.
- [7] Vasudevan, R. (2014). Urinary tract infection: an overview of the infection and the associated risk factors. *J Microbiol Exp*, 1(2), 00008.
- [8] Foxman, B., & Brown, P. (2003). Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. *Infectious Disease Clinics*, 17(2), 227-241.
- [9] Gilani, S. Y. H., Shah, S. R. A., Ahmad, N., & Bibi, S. (2016). Antimicrobial resistance patterns in community acquired urinary tract infections. *Journal of Ayub Medical College Abbottabad*, 28(3), 572-574.
- [10] Azhagu Madhavan S., Pandian P. (2024). Identification and characterization of silver nanoparticles from *Erythrina indica* and its antioxidant and Uropathogenic antimicrobial properties. *Microbial Pathogenesis*, 190: 106635.
- [11] Nagarajan, S., T. Mohan Das, Pandian A., Raaman N. (2009). Design, synthesis and gelatin studies of 4,6-O-butylidene- α,β -unsaturated- β -C-glycosidic ketones: application to plant tissue culture. *Journal of Materials Chemistry*, 26: 4587-4596.
- [12] Nagarajan, S., Pandian A., Raaman N., Mohan Das. T. (2010). Regioselective facile one-pot Friedländer synthesis of sugar-based heterocyclic biomolecules. *Carbohydrate Research*, 345(14): 1988-1997.
- [13] Nagarajan, S., Pandian A., Raaman N., Anup S., Elizabeth Sobhia M., Mohan Das. T. (2012). Stereoselective synthesis of sugar-based β -lactum derivatives: Docking studies and its biological evaluation. *Tetrahedron*, 68(4): 3037-3045.
- [14] Satheeshkumar, C., M. Ravivarma, Pandian A., Silambarasan V., N. Raaman, D. Velmurugan, C. Songs and P. Rajakumar. (2015). Synthesis, anti-microbial activity and molecular docking studies on triazolylcoumarin derivatives. *Journal of Chemical Sciences*, 127(3): 565-574.
- [15] Vinothini K., M. Sri Devi, Veronica Shalini, Sudharshan Sekar, RB. Semwal, Pandian A, DK. Semwal. (2017). *In vitro* micropropagation total phenolic content and comparative antioxidant activity of different extracts of *Sesbania grandiflora* (L.) Pers. *Current Science*, 113(6): 1142-1147.

- [16] Klein, R. D., & Hultgren, S. J. (2020). Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies. *Nature Reviews Microbiology*, 18(4), 211-226.
- [17] Anderson, R. U. (1999). Management of lower urinary tract infections and cystitis. *Urologic Clinics of North America*, 26(4), 729-735.
- [18] Rosen, J. M., & Klumpp, D. J. (2014). Mechanisms of pain from urinary tract infection. *International Journal of Urology*, 21, 26-32.
- [19] Ferry, S. A., Holm, S. E., Stenlund, H., Lundholm, R., & Monsen, T. J. (2004). The natural course of uncomplicated lower urinary tract infection in women illustrated by a randomized placebo controlled study. *Scandinavian journal of infectious diseases*, 36(4), 296-301.
- [20] Roberts, J. A. (1999). Management of pyelonephritis and upper urinary tract infections. *Urologic Clinics of North America*, 26(4), 753-763.
- [21] Keren, R., Shaikh, N., Pohl, H., Gravens-Mueller, L., Ivanova, A., Zaoutis, L., ... & Hoberman, A. (2015). Risk factors for recurrent urinary tract infection and renal scarring. *Pediatrics*, 136(1), e13-e21.
- [22] Sivalingam AM., Pandian P. (2024). Cerebellar Roles in Motor and Social Functions and Implications for ASD. *Cerebellum*, 23(6):2564-2574.
- [23] Koradia, P., Kapadia, S., Trivedi, Y., Chanchu, G., & Harper, A. (2019). Probiotic and cranberry supplementation for preventing recurrent uncomplicated urinary tract infections in premenopausal women: a controlled pilot study. *Expert review of anti-infective therapy*, 17(9), 733-740.
- [24] Brumbaugh, A. R., & Mobley, H. L. (2012). Preventing urinary tract infection: progress toward an effective *Escherichia coli* vaccine. *Expert review of vaccines*, 11(6), 663-676.
- [25] Franco, A. V. M. (2005). Recurrent urinary tract infections. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 19(6), 861-873.
- [26] Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology*, 13(5), 269-284.
- [27] Mysorekar, I. U., & Hultgren, S. J. (2006). Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract. *Proceedings of the National Academy of Sciences*, 103(38), 14170-14175.
- [28] Soto, S. M., Smithson, A., Martinez, J. A., Horcajada, J. P., Mensa, J., & Vila, J. (2007). Biofilm formation in uropathogenic *Escherichia coli* strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. *The Journal of urology*, 177(1), 365-368.
- [29] Lacerda Mariano, L., & Ingersoll, M. A. (2020). The immune response to infection in the bladder. *Nature Reviews Urology*, 17(8), 439-458.
- [30] Dasari Venkateswarlu, Arjun P., Samiraj R. (2021). Detection of β -lactamase in Clinical samples of Prakasam District, Andhra Pradesh. *Research Journal of Pharmacy and Technology*, 14(6): 2995-2998.
- [31] Said, A., El-Gamal, M. S., Abu-Elghait, M., & Salem, S. S. (2021). Isolation, identification and antibiotic susceptibility pattern of urinary tract infection bacterial isolates. *Lett. Appl. NanoBioSci*, 10, 2820-2830.
- [32] Coico, R. (2006). Gram staining. *Current protocols in microbiology*, (1), A-3C.
- [33] Alves, M. S., Dias, R. C. D. S., de Castro, A. C. D., Riley, L. W., & Moreira, B. M. (2006). Identification of clinical isolates of indole-positive and indole-negative *Klebsiella* spp. *Journal of clinical microbiology*, 44(10), 3640-3646.
- [34] McDevitt, S. (2009). Methyl red and voges-proskauer test protocols. *American Society for Microbiology*, 8.
- [35] MacWilliams, M. P. (2009). Citrate test protocol. *American Society for Microbiology*, 1-7.
- [36] Toda, T., Hagihara, Y., & Takeya, K. (1961). A simple urease test for the classification of mycobacteria. *American Review of Respiratory Disease*, 83(5), 757-761.
- [37] Bonnichsen, R. K., Chance, B., & Theorell, H. (1947). Catalase activity. *Acta chem. scand*, 1, 685-709.
- [38] Raitio, M., Jalli, T., & Saraste, M. (1987). Isolation and analysis of the genes for cytochrome c oxidase in *Paracoccus denitrificans*. *The EMBO Journal*, 6(9), 2825-2833.
- [39] Eschenlauer, S. C. P., McKain, N., Walker, N. D., McEwan, N. R., Newbold, C. J., & Wallace, R. J. (2002). Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. *Applied and environmental microbiology*, 68(10), 4925-4931.
- [40] Turnbull, L., & Whitchurch, C. B. (2014). Motility assay: twitching motility. *Pseudomonas Methods and Protocols*, 73-86.

- [41] Lehman, D. (2005). Triple sugar iron agar protocols. American Society for Microbiology: Washington, DC, USA, 1-7.
- [42] Wilson, K. H., Blitchington, R. B., & Greene, R. C. (1990). Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. Journal of clinical microbiology, 28(9), 1942-1946.
- [43] NCBI, <https://www.ncbi.nlm.nih.gov/>, 2024
- [44] NCBI, BLASTn https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome
- [45] Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution <https://doi.org/10.1093/molbev/msab120>.
- [46] Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.
- [47] Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791
- [48] Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.
- [49] Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic Escherichia coli. Nature Reviews Microbiology, 2(2), 123-140. <https://doi.org/10.1038/nrmicro818>
- [50] Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284. <https://doi.org/10.1038/nrmicro3432>
- [51] Ayyadurai, T., Moola, A.K., Mohan, P.K., Thiruppathi, S.K., Shanmugam, A. and Bollipo Diana, R.K., 2022. Ameliorative effects of Guilandina bonduc L. aqueous seed extract on letrozole induced polycystic ovary syndrome in female wistar albino rats. Advances in Traditional Medicine, 22(4), pp.885-903.
- [52] Jeyaram, M., Jeyaram, Y., Ayyadurai, T., Gurusamy, M. (2026). Exploration of Streptomycetes as Biocontrol Agents Against Plant Pathogens. In: Kaur, T., Kumar, H., Manhas, R.K. (eds) Streptomycetes: Biological Candidates for Sustainable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-95-5803-2_6.
- [53] Meenaloshini, P., Thirumurugan, A., Achiraman, S. and Kumar, T.S., 2025. Protective effect of hydroalcoholic Crateva religiosa G. Forst. bark extract on oxidative stress, hormonal imbalance and gene expressions (CYP19A1 and PPAR γ) in letrozole-induced polycystic ovarian syndrome rats. Journal of Ethnopharmacology, 353, p.120354.
- [54] Ayyadurai, T., Mohan, P. K., Annamalai, A., Kamaraj, C., & Waheeb, M. Q. (2026). Biogenic silver nanoparticles synthesis from Guilandina bonduc L. seed kernel extract with antibacterial, antioxidant and anticancer potential against ovarian Cancer cells. Results in Chemistry, 103246.
- [55] Thirumurugan, A., Padmanaban, M., Kumar, D. S., Govindharaju, R., Padmavathy, S., Karthikeyan, V., ... & Karthik, S. (2026). Phytochemical and antioxidant properties of Kedrostisfoetidissima and its larvicidal potential against mosquitoes. South African Journal of Botany.
- [56] Jancyhelan, J., Thirumurugan, A., Alagumanian, S., & Vignesh, K. (2026). Eco-friendly biosynthesis of silver nanoparticles using marine algal extract and their biomedical applications. Results in Chemistry, 103606.
- [57] Sakthivel V, Ilanthiraya S, Jeevanantham G, Aravindhan T, Priya K, Thirumurugan A, Larvicidal Activity Of Marine Macroalgae: A Comprehensive Review. Int J Drug Deliv Technol. 2026;16(4s): 908-917; DOI: 10.25258/ijddt.16.4s.106
- [58] Ayyadurai, T., Ravi, S., & Annamalai, A. (2026). Steroidogenic Enzyme Dysregulation in Polycystic Ovary Syndrome: Mechanistic Insights and Emerging Therapeutic Strategies. The Journal of Steroid Biochemistry and Molecular Biology, 107088.
- [59] Anbazhagan, V., Natrajan, A., Arul, J. et al. Strategic development of a buffalo farming framework and optimization of the foraging behaviour using mathematical models. Trop Anim Health Prod 58, 424 (2026). <https://doi.org/10.1007/s11250-026-05211-6>
- [60] Meda SSR, Joseph NM, Lakshmikanthan M, Ayyadurai T, Muthu S. Comparative Efficacy of Mandelic Acid Peel versus Topical Kojic Acid 2% in the Treatment of Periorbital Melanosis. Int J Drug Deliv Technol. 2026;16(15s): 118-123. DOI: 10.25258/ijddt.16.15s.14.
- [61] Meda SSR, Srinivasan VR, Lakshmikanthan M, Ayyadurai T, Muthu S. Topical Prostaglandin Analogues in Androgenetic Alopecia: A Systematic Review of Clinical Evidence. Int J Drug Deliv Technol. 2026;16(15s): 108-117. DOI: 10.25258/ijddt.16.15s.13.
- [62] Anger, J., Lee, U., Ackerman, A. L., Chou, R., Chughtai, B., Clemens, J. Q., ... & Chai, T. C. (2019). Recurrent uncomplicated urinary tract infections in women: AUA/CUA/SUFU guideline. The Journal of urology, 202(2), 282-289.

- [63] Kline, K. A., & Lewis, A. L. (2017). Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Urinary tract infections: molecular pathogenesis and clinical management*, 459-502.
- [64] Critchley, I. A., Cotroneo, N., Pucci, M. J., Jain, A., & Mendes, R. E. (2020). Resistance among urinary tract pathogens collected in Europe during 2018. *Journal of Global Antimicrobial Resistance*, 23, 439-444.
- [65] Stamm, W. E. (2002). Scientific and clinical challenges in the management of urinary tract infections. *The American journal of medicine*, 113(1), 1-4.
- [66] Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761-2764. <https://doi.org/10.1128/JCM.01228-07>