Short Communication

Decoding Uropathogenic *Klebsiella pneumoniae*: A Biochemical and Genotypic Insight into Isolates from Western Tamil Nadu

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Abstract

Urine tract infections (UTIs) are among the most common bacterial infections, and are often caused by multidrug-resistant pathogens. The purpose of this study was to separate, characterize, and confirm inspiring microorganisms from clinical UTI samples. Clinical isolates were collected from infected patients and subjected to biochemical and morphological characterization. Based on biochemical analysis, the isolates were identified as Klebsiella pneumoniae. To confirm this identity, molecular analysis was performed by extracting genomic DNA from the isolates, followed by purification using the Hi-pura purification kit. The traditional PCR 16s rRNA was used to target genes, and after the PCR analysis, the agarose gel was subjected to electrophoresis (1.5%) as well as 100 BP markers. There were about 1.2 kB in the amplified product size, and sequencing of pure PCR products validated the identity of the organism. This study confirmed the presence of Klebsiella pneumoniae in UTI patients from the western region of Tamil Nadu, highlighting the importance of molecular techniques for accurate microbial identity and epidemiological monitoring.

Keywords- *Urinary Tract Infection (UTI), Klebsiella pneumonia, Biochemical Characterization, 16S rRNA Gene Sequencing, Molecular Identification, Multidrug-Resistant Pathogens*

Introduction

Urine tract infections (UTI) are one of the most prevalent bacterial infections affecting millions of individuals worldwide. They are especially common in women, elderly patients, and individuals with underlying medical conditions, such as diabetes or immunization [1]. UTIs can be classified as straight or complex depending on factors such as physical abnormalities,

catheterization, or antibiotic resistance. Klebsiella, a gram-negative, faculty-anaerobic bacterium, is one of the leading motivational agents of pneumonia, especially in hospital-deficit infections [2]. The growing emergence of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains has raised serious concerns regarding treatment efficacy and clinical management [3].

Klebsiella pneumoniae is an opportunistic pathogen that collects in the gastrointestinal tract and can spread to the urinary tract, causing severe infection [4]. It contains several viral factors, including capsular polysaccharides, side rides, and adhesives, which facilitate immune theft and biofilm formation [5]. The ability to acquire resistance genes through horizontal gene transfer further complicates this ability of *Klebsiella pneumoniae* [6]. Extended-spectrum beta-lactamase (ESBL)-Producing *Klebsiella pneumoniae* strains have shown resistance to cephalosporin and other beta-lactam antibiotics, and an exact pathogen identity (2005) requires a molecular-based diagnostic approach. Importance of biochemical and molecular characterization Traditional microorganism methods Bacterial pathogens [7] play an important role in early identification, including biochemical and morphological identity.

Biochemical trials, such as citrate utilization tests, vogas-promoter tests, and urase tests, help to separate *Klebsiella pneumoniae* from other enterobacteria [8]. However, biological characteristics alone may not be sufficient for accurate pathogen identification because of the phenotypic similarity between bacterial species. Molecular technology, especially 16S rRNA gene sequencing, provides more accurate identification of bacterial species [8]. The 16s rRNA genes are highly conserved among the species of bacteria but contain overgrowth areas that allow discrimination at the genus and species levels [9]. The 16s rRNA has been widely used for the identification of bacteria in clinical microbiology [8] via sequencing after PCR-based amplification of genes. This approach ensures rapid and reliable identification of *Klebsiella pneumoniae* in patients with UTIs, facilitating targeted treatment strategies and epidemiological monitoring. Study importance and objectives given the increasing occurrence of MDR *Klebsiella pneumoniae* infections, understanding their prevalence and molecular characteristics in UTI patients is essential for effective infection control. This study aimed to separate and characterize *Klebsiella pneumoniae* from clinical UTI samples using biochemical and molecular methods. Molecular confirmation through 16s rRNA gene sequencing will provide insights into the epidemiology of *Klebsiella pneumoniae* infection in the western region of Tamil Nadu.

Methods

Sample collection and separation Urine samples were collected from patients diagnosed with urinary tract infection (UTI) in hospitals and clinical centers in the western region of Tamil Nadu. The samples were unevenly collected in sterile containers and transferred to the microbiology laboratory for further analysis. Standard microbiological processes were followed for bacterial isolation, including McConkey if vaccination on plates, followed by incubation at 37 ° C for 24 hours at 37 ° C [10]. Colonies displaying mucoid and lactose-concentration characteristics specific to *Klebsiella pneumoniae* were chosen for further analysis. Biochemical characterization: The proposed isolates were subjected to biochemical testing to confirm their identity. Standard biochemical trials include citrate utilization tests, urase tests, indole tests, methyl Red-Vogs Prosures (MR-VP) test, and the Triple Sugar Iron (TSI) test [8]. The ability of the isolates to ferment glucose, lactose, and sucrose was evaluated using a TSI slant, while their ability to use citrate as the sole carbon source was determined

using semon citrate agar. In addition to the absence of hydrogen sulfide production, positive reactions in the vogs-promoter and urace tests confirmed the presence of *Klebsiella pneumoniae* [11]. Genomic DNA was administered and purified following the manufacturer's instructions to genomic DNA, Klebsiella pneumonia was isolated using the Hy-pura bacterial genomic DNA refinement kit (HIMEDIA, India). Briefly, bacterial cultures were grown in Luria-Bartani (LB) broth overnight at 37 °C, and the cells were cut by centrifugation.

The bacterial pellet was lysed using lysis buffer and proteinus K treatments, followed by treatment with pure genomic DNA [12] after the rain and washing stages. DNA purity and concentration with an absorption ratio of 260/280 Nm were used to confirm that DNA quality was evaluated using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). Polymerase Chain Reaction (PCR) was performed to confirm the identification of *Klebsiella pneumoniae* using the 16S rRNA gene as a molecular marker. Reacted using universal 16s rRNA primers: Forward Primer: 5′--AGAGTTTTGATGACTGGACTCAG ATCAGA-3 ' • Reverse Primer: 5′-GGTTACTTTGTTACGACTT-3’

The PCR reaction mixture included 50 ng genomic DNA, 10 × PCR buffer, 2.5 mm $MGCL_2$, 0.2 mm DNTPS, 10 picoMols of each primer, and a final amount of 25 µl of TAQ DNA polymerase in the final amount of TAQ DNA polymerase. The thermal cycling conditions included an initial denaturation step at 95 °C for 5 min, followed by 30 s at 94 °C. After 30 cycles at 55 °C for 30 s, expansion at 72 °C for 1 min, and a final extension at 72 °C for 5 min (Classes 2004). Gel Electrophoresed and DNA Squeeze PCR products were analysed using 1.5% agarose gel electrophoresis in 1 × TAE buffer. The gel was stained with ethidium bromide and visualized using a UV Transilluminator. A 100 bp DNA ladder (Thermo Fisher Scientific, USA) was used as a molecular marker. A required amplicon size of approximately 1.2 KB confirmed the presence of Klebsiella pneumonia [8]. The PCR products were purified and sequenced using a PCR refinery kit (Qiagen, Germany).

Sanger sequencing was performed for molecular confirmation and phylogenetic analysis of a commercial sequencing feature. To confirm the species, the sequences obtained using BLAST (basic local alignment search equipment) against the NCBI GenBank database were analyzed. Many sequence alignments and phylogenetic analyses have been performed to compare isolates with reference strains using mega software [13]. The phylogenetic tree was constructed using a neighborhood method with bootstrap support of 1000 replicates. Statistical analysis All experiments were performed in triplicate, and data were analyzed using statistical software. The prevalence of *Klebsiella pneumoniae* was determined and antimicrobial resistance patterns were evaluated using standard statistical methods, including classified data analysis (GraphPad Prism, version 9) for a curry-circulation test.

Results and Discussion

Microbial Isolation and Biochemical Characterization

Urinary tract infections (UTI) are one of the most prevalent bacterial infections, especially in women, due to physical and physical factors [14]. In this study, urine samples were collected from patients diagnosed with UTI in private and government hospitals. Three urine samples were collected in sterile containers and transported to the laboratory under cold storage conditions for further analysis. The samples were stored at 4 °C to preserve microbial viability for biochemical and molecular characterization [15]. Initially, the collected urine samples were vaccinated

in Luria broth and incubated overnight at 37 °C. Subsequently, cultures without antibiotics were mounted on plates and incubated overnight at 37 °C. The colonies that developed on the plates were placed on the plates if fresh lb was further covered and stored at 4 °C for future processing. Village staining results showed that most of the isolates were gram-positive $COCI_2$, which agrees with the findings of previous studies, indicating that gram-positive bacteria, especially Staphylococcus SPP., Escherichia coli [16] with general liability agent with gram-negative bacteria. Bacterial isolates were further subjected to a series of biochemical trials, including Indol, Voges-Proscair, Methyl Red, Critical Utilization, Catalase, Gelatin Degradation, Carbohydrate Formation Test, Triple Sugar Irne (TSI) test, and antibiotics. Biochemical analysis showed that the isolates were non-Miley, catalyst-positive, oxide-negative, and capsulated rod-shaped bacteria, which suggested their affiliation with Klebsiella pneumonia, a well-known UTI pathogen, as shown in Figure 1 and Table 1 [17].

Genomic DNA Isolation and PCR Analysis

Genomic DNA was extracted from bacterial isolates, and the volume was determined using a UV spectrophotometer at an absorption ratio of 260/280 Nm to assess protein and RNA contamination, as shown in Figure 2. The presence of protein contaminants interferes with DNA deforestation in PCR reactions, which requires a purification step using a spin column-based DNA purification kit (Hipura, Chennai, India). After purification, the DNA quality was resolved to ensure suitability for downstream applications. PCR amplification of the 16s rRNA genes, a widely used molecular marker, was used for bacterial identification [8] using specific primers. The response mixture included 50–100 ng of genomic DNA, 1.5 mm $MgCl_2$, 30 pmol primers, and 1 unit of Taq DNA polymerase. The thermal cycling conditions included an initial denaturation phase at 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 58 °C (annealing) for 1 min, and 72 °C for 2 min (extension), 72 °C for 72 °C, 72 °C for 10 min, and 10 min for 10 min. PCR products were analyzed on 1.5% ethidium bromide pre-relaxed agarose gel using electrophoresis and conceived under a UV Transilluminator. The 1.2 kB piece of the 16s rRNA genes was successfully amplified (Figure 3), confirming the presence of bacterial DNA. The amplified PCR product was subjected to Sanger sequencing to determine the exact genetic identity of the isolates.

Implications and Future Perspectives

Implications and Future Perspectives Accurate microbial identity in UTI cases is vital for steering appropriate antibiotic remedies and stopping headaches, including pyelonephritis and recurrent infections [18]. The successful amplification of the 16S rRNA gene provides a foundation for phylogenetic evaluation and species-degree identity. Further sequencing and comparative evaluation with publicly available databases, including NCBI BLAST, will offer a definitive taxonomic category for bacterial isolates. This observation highlights the significance of integrating biochemical, microscopic, and molecular processes for complete microbial characterization of UTIs. Future studies should include antibiotic resistance profiling and entire-genome sequencing to better understand the pathogenic mechanisms and emerging resistance trends in UTI-related bacteria [19].

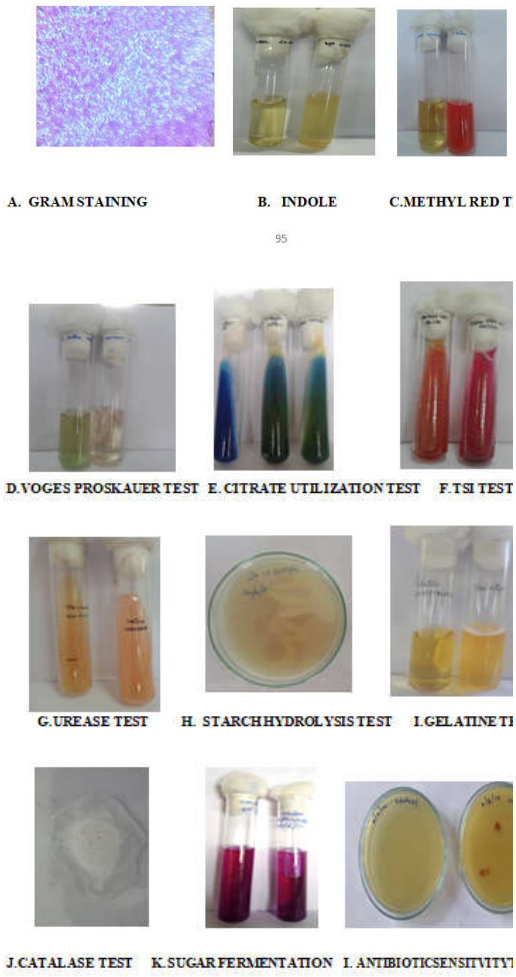

Figure 1. Various biochemical reactions for *Klebsiella pneumoniae* identification

Figure 2. Genomic DNA from clinical bacterial isolates. Lane 1 – DNA from pus sample; Lane 3 – DNA from UTI patient; Lane 2 – Empty.

Figure 3. PCR amplification of the 16S rRNA gene. Lane 1 – 1kb marker; Lane 4 – 1.2kb PCR product (pus sample); Lane 5 – 1.2kb PCR product (urine sample); Lane 2 – Empty; Lane 3 – Spill. Lower bands indicate primer dimers due to excess primers

S.NO	BIOCHEMICAL TEST	Results
A	Gram staining	Positive
B	Indole test	Negative
C	Methyl red(MR)	Positive
D	Voges proskauer (VP)	Negative
E	Citrate utilization test	Positive
F	Triple sugar iron test	Positive
G	Urease test	Positive
H	Starch hydrolysis	Negative
I	Gelatine test	Positive
J	Catalase test	Positive
K	Carbohydrate – Acid production	Negative
L	Carbohydrate – gas production	Positive

Table 1- Shows the cumulative Various Biochemical Results

Summary

Urinary tract infections (UTIs) are some of the most common bacterial infections, with girls being more susceptible to UTIs due to anatomical and physiological factors. This study aimed to isolate, symbolize, and carry out molecular analyses of bacterial pathogens in patients with UTI. Urine samples were collected from patients at non-public and government hospitals, transported under bloodless storage, and cultured in Luria broth. Cultured samples were spread onto LB agar and incubated at 37°C overnight. Isolated colonies were sub-cultured, stored at 4°C, and subjected to Gram staining, which revealed that most isolates were gram-positive cocci. Further biochemical examinations, together with catalase, oxidase, citrate usage, and triple sugar iron assessments, suggested the presence of Klebsiella pneumoniae, a known UTI pathogen. Genomic DNA was extracted, purified using a spin column approach, and quantified the usage of UV spectrophotometry. PCR amplification of the 16S rRNA gene was accomplished with unique primers, and a 1.2 kb amplicon turned into efficaciously received. Agarose gel electrophoresis revealed the presence of bacterial DNA, and the PCR product was sent for sequencing to identify the bacterial lines.

This study demonstrates the importance of integrating biochemical and molecular techniques for the identification of UTI pathogens. Accurate microbial characterization is important for directing effective antibiotic therapy and monitoring the development of resistance. Further sequencing and phylogenetic analysis will provide information on UTI-inflicting microorganisms, potentially contributing to better diagnostic and therapeutic strategies. Future research needs to awareness on entire-genome sequencing and antibiotic resistance profiling to deal with the increasing occurrence of multidrug-resistant bacterial traces in UTIs

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