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MOLECULAR IDENTIFICATION AND PHYLOGENETIC CHARACTERIZATION OF UTI CAUSING BACTERIA USING THE 16S RRNA GENE

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ABSTRACT Antimicrobial resistance has emerged as a critical health concern worldwide. It has threatened the effectiveness of clinical treatments and public health safety. Accurate identification of resistant pathogens is crucial for correct diagnosis, effective surveillance, epidemiological tracking and therapeutic decision-making. Recent advances in molecular sequencing and bioinformatics have revolutionized bacterial taxonomy, enabling the detection of resistant genes and phylogenetic diversity with high precision. The integration of phenotypic screening with 16S rRNA-based genetic profiling represents a robust strategy for identifying multidrug-resistant pathogens in clinical, environmental, and agricultural contexts. This study reviews contemporary methodologies for isolating antibacterial-resistant bacteria and genetically characterizing them via the 16S ribosomal RNA (rRNA) gene.

KEYWORDS : 16S rRNA Gene, Genetic Identification, Taxonomy, UTI, Pathogens, etc.

INTRODUCTION

Rapid emergence of antimicrobial resistant and multidrug resistant bacteria constitutes a major global public health crisis. According to the World Health Organization (WHO, 2024), antimicrobial resistance (AMR) is the reason for approximately 1.27 million deaths every year. Biochemical and culture-based morphological identification methods are time consuming and have limitation in differentiating between closely related species or detecting new, unculturable bacteria (Janda & Abbott, 2021; Patel, 2001). However, since morphology-based identification methods have limited application in efficient diagnosis, molecular or genetic approaches—especially PCR amplification and DNA sequencing of the 16S rRNA gene have gained importance and momentum in identifying and categorizing bacterial species associated with antimicrobial resistance.

The 16S rRNA gene, is a highly conserved domain of the prokaryotic genome scattered with variable regions, that enables accurate taxonomic identification, evolutionary and phylogenetic analysis (Salvà Serra, 2023). The combination of Polymerase Chain Reaction (PCR) and sequencing technologies like the Next-Generation Sequencing (NGS), the 16S rRNA gene offers a universal target sequence for detecting bacterial lineages along with the integration of drug resistance gene profiling (de Bastiani et al., 2024; Cason et al., 2022).

MATERIALS AND METHODS

Isolation of pathogenic bacteria: Urine samples from female patients diagnosed with UTIs were collected aseptically. The samples were cultured on CLED (Cystine-Lactose-Electrolyte Deficient) agar and MacConkey agar plates to isolate the microorganisms. The plates were incubated at 37°C for 18-24 hours. Pure culture of bacteria was obtained by streaking the culture on Luria-Bertani media plates followed by single colony culture. Three pathogens were found to occur frequently in the urine samples as previously reported (Prasad and Mishra, 2024) with resistance to multiple drugs currently used as first line of treatment.

DNA Extraction and PCR amplification: Genomic DNA was extracted using commercial kit (Bunshi Bioscience). The 16S rRNA gene was then amplified using universal primers—commonly 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′)—to generate ~1.5 kb fragments (Ahmed et al., 2023). PCR conditions are optimized for annealing temperature and cycle number to minimize non-specific amplification.

Sequencing and Phylogenetic analysis: Amplicons were purified and outsourced to Bunshi Bioscience for sequencing via Sanger sequencing. Resulting sequences were compared to the NCBI Database for taxonomic identification using BLASTn. Phylogenetic trees were constructed using NCBI's Tree Viewer feature bioinformatics tools employing neighbor-joining method.

RESULTS AND DISCUSSIONS

Sequence analysis of the 16S rRNA gene using the NCBI BLAST tool identified three bacterial species: Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae, each showing over 98-100% sequence similarity with reference strains. The phylogenetic relationships confirmed close clustering with known resistant lineages. Similar findings have been reported by Karungamye et al. (2023) and Sivaraman et al. (2021), emphasizing the reliability of 16S rRNA sequencing for accurate species identification. The results highlight 16S rRNA analysis as an effective molecular approach for detecting and characterizing resistant bacterial pathogens.

Figure - 1: PCR Amplification – Lane 1: Marker; Lane 2, 3 and 4: 1.5kb Amplification of Clinical Isolates; Lane 5: Negative Control; Lane 6: Positive Control

Figure -2: Klebsiella Pneumoniae Phylogenetic Tree

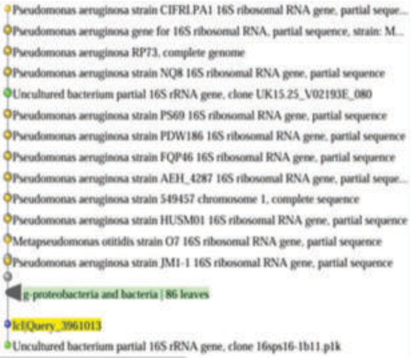

Figure -3: Pseudomonas Aeruginosa Phylogenetic Tree

Figure - 4: E.coli Phylogenetic Tree

CONCLUSION

Three UTI causing bacteria — Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae—were identified using 16S rRNA gene sequencing. The results confirm that 16S rRNA analysis is a reliable molecular tool for accurate bacterial identification and resistance monitoring. Future studies should incorporate whole-genome sequencing and resistance gene profiling to further elucidate the genetic mechanisms driving antimicrobial resistance.

REFERENCES

1. 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.
2. Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Molecular diagnosis, 6(4), 313-321.
3. Salvà Serra, F. (2023). Bacterial whole-genome sequencing for establishment of reference sequences, comparative genomics, biomarker discovery and characterization of novel taxa.
4. de Bastiani, D. C., Silva, C. V., Christoff, A. P., Cruz, G. N. F., Tavares, L. D., de Araújo, L. S. R., ... & Pereira, A. J. (2024). 16S rRNA amplicon sequencing and antimicrobial resistance profile of intensive care units environment in 41 Brazilian hospitals. Frontiers in Public Health, 12, 1378413.
5. World Health Organization. (2024). Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP): Supplementary Protocols. World Health Organization.
6. Cason, C., D'Accolti, M., Soffritti, I., Mazzacane, S., Comar, M., & Caselli, E. (2022). Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. Frontiers in microbiology, 13, 969863. https://doi.org/10.3389/fmicb.2022.969863
7. Prasad, K., & Mishra, A. (2024). Antibacterial Effect of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) Medicinal Plants Against Urinary Tract Infections (UTIs) Pathogens Isolated from Clinical Samples of Female Patients. Frontiers in Health Informatics, 13(8).
8. Karungamye, P., Rugaika, A., Mtei, K., & Machunda, R. (2023). Antibiotic resistance patterns of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa isolated from hospital wastewater. Applied microbiology, 3(3), 867-882.
9. Sivaraman, G. K., Rajan, V., Vijayan, A., Elangovan, R., Prendiville, A., & Bachmann, T. T. (2021). Antibiotic resistance profiles and molecular characteristics of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae isolated from shrimp aquaculture farms in Kerala, India. Frontiers in Microbiology, 12, 622891.