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Molecular Detection and Identification of Bacteria in Urine Samples of Asymptomatic and Symptomatic Pregnant Women by 16S rRNA Gene Sequencing

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Abstract

Objectives: The purpose of this study was to identify bacteria in urine samples of pregnant women of asymptomatic and symptomatic women by 16S rRNA gene sequencing. This study aims to identify different strains of microbes causing urinary tract infection (UTI).
Methods: In the semi-quantitative culture technique, bacterial isolates such as *Escherichia coli, Klebsiella, Pseudomonas, Staphylococcus*, Coagulase-negative *Staphylococcus*, and *Proteus* were subjected to 16S rRNA gene sequencing followed by BLAST analysis and phylogenetic tree formation. The 16S rRNA gene sequencing was carried out to identify the specific strains of bacteria causing UTI.
Results: According to the BLAST analysis, sample 1 revealed a 100% similarity to *E. coli strain U5/41.* Likewise, samples 2, 3, 4, 5 and 6 exhibited a 100% similarity to *Klebsiella aerogenes strain F26, Pseudomonas entomophila strain 2014, Staphylococcus aureus strain NCTC13616, Staphylococcus saprophyticus strain FDAARGOS_355, Proteus mirabilis strain NCTC 11938*, respectively.
Conclusions: Six bacterial isolates were analyzed by 16S RNA gene sequencing followed by the construction of a phylogenetic tree construction up to the species level. This method was a valuable tool for cost-effective and accurate diagnosis of an array of uropathogens in both asymptomatic and symptomatic pregnant women.

Keywords: 16S rRNA Gene Sequencing, BLAST Analysis, Phylogenetic Tree, UTI

1. Background

Urinary tract infection (UTI) in pregnant women is a common healthcare problem, which is commonly caused by pathogens normally residing in the intestine and genital tract. Occasionally, the diagnosis of UTI is difficult, and many patients are treated based only on signs and symptoms, the error rate was 33%. The microscopy and culture method of diagnosis takes two days for identification and antibiotic sensitivity of bacteria. The patients treated empirically encounter a risk of antimicrobial resistance. The main advantage of molecular diagnosis is identifying the cause within hours up to species level. 16S real-time PCR was narrated by Lehmann et al. using probes specific to a large number of genus/species (1). All bacteria contain the 16S rRNA gene, thereby making the sequencing-based bacterial identification plausible (2). In addition, the 16S rRNA gene comprises variable regions interspersed with nucleotide sequences, which provide a species-specific signature sequence that is the hallmark of bacterial identification. The obtained sequences are compared with the known sequences in the database (3). The method is valuable in the case of a mixture of a wide range of pathogens. Also, it is useful in detecting bacteria that are difficult to grow as well as those in samples obtained from patients' post-antibiotic treatment. Strain 131 of *E. coli* has been found to be multi-drug resistant (MDR) (4). Interestingly, MDR *ST131* is resistant to fluoroquinolones such as Ciprofloxacin and aminoglycosides like Amikacin and Gentamicin. In hospitalized and community-acquired cases of UTI, strains *ST95, 73, 69* of *E. coli* are frequently isolated and persist in non-extended spectrum beta-lactamase isolates (5). Extra-intestinal pathogenic *E. coli*, including uropathogenic *E. coli*, consists of specific phylogenetic groups with different sets of virulence genes and is commonly associated with human diseases.

2. Objectives

This prospective study was carried out in the Department of Microbiology, Patna Medical College, Patna and the Department of Microbiology, GLA University, Mathura. In the following, 16S rRNA gene sequencing was carried out at the BioAxis DNA Research Centre, Hyderabad, India. Urine samples from pregnant women, both asymptomatic and symptomatic, were collected and subjected to screening methods, followed by culture on MacConkey's media. The antimicrobial sensitivity test was carried out on the nutrient agar.

3. Methods

3.1. DNA Isolation

The isolated bacteria were subjected to DNA isolation using BiopureTM kits (BioAxis DNA Research Centre).

3.2. 16S rRNA Gene PCR

16S rRNA gene was amplified by PCR from the DNA isolated above. The primers used for amplification of the gene were as follows:

- Forward 27F – 5'-AGAGTTTGATCMTGGCTCAG-3'
- Reverse 1492R– 5'-CGGTTACCTTGTTACGACTT-3'

The temperature conditions of PCR were: 5 min at 94°C, 60 sec at 94°C in 35 cycles, 45 sec at 53°C, 90 sec at 68°C and 10 min at 68°C. 4°C was set as hold temperature to keep amplicons safe for the next use.

3.3. Electrophoresis

Electrophoresis of the amplified PCR product was done on 1 kb DNA ladder with 1% agarose gel and TAE as a buffer, which was subsequently visualized by staining with ethidium bromide (Figure 1).

3.4. Elution

Purification of the PCR product was done by washing with 70% ethanol and sodium acetate, followed by elution from the gel. Electroelution causes rapid and isolation of large fragments of DNA. The DNA band presented in the gel fragment was excised and dialyzed against the TAE buffer. The DNA was precipitated out by an electric current. For separation of agarose from DNA, agarose used is of low melting point agarose is commonly used as it does not denature the DNA structure. Subsequently, the sample was subjected to sequencing.

3.5. Sanger Sequencing Using Dye Terminators

The PCR amplicon was sequenced on ABI 3730XL automated DNA Sequencer. In this method, different fluorescent markers are used for labeling every dideoxynucleotide in a capillary tube. Different colored bands are produced by DNA fragments of different sizes separated in a capillary tube. For a given size DNA fragment, there is a separate band and, the colors indicate different bases at which termination of the fragment has occurred. The bases represented by short fragments, moved first in the capillary. When the light emitted by the laser falls on the capillary tube, the light emitted by the fluorescent dye was recorded on the detector. The signal from the detector of each colored band was analyzed by the sequencer and appeared on the graph as a peak. Each base had a separate peak.

3.6. BLAST Reference

The assembled DNA sequence was used to carry out BLAST with the nr database from NCBI.

3.7. Phylogenetic Tree Construction

Top ten similar sequences of BLAST were retrieved, and a phylogenetic tree was constructed using Clustal omega.

4. Results

The semi quantitative culture technique identified the following bacterial isolates: *E. coli, Klebsiella, Pseudomonas, Staphylococcus*, coagulase-negative *Staphylococcus*, and *Proteus* spp. These were subjected to 16S rRNA gene sequencing, and the results are as follows:

4.1. Sequence Obtained for Sample 1

ATGACCAGCAACACTGGAACTGAGACACGGTCCAGA-
CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG-
GGCGCAAGCCTGATGCAGCCATGCNGCGTGTATGAAGAA-
GGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGG-
GAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCA-
GAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA-
ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT-
AAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAAT-
CCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAA-
GCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAG-
CGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG-
AAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGA-
AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC-
CACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTG-
AGGCGTGGCTTCCGGANNTAACGCGTTAAGTCGACCGCC-
TGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG-
ACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTC-

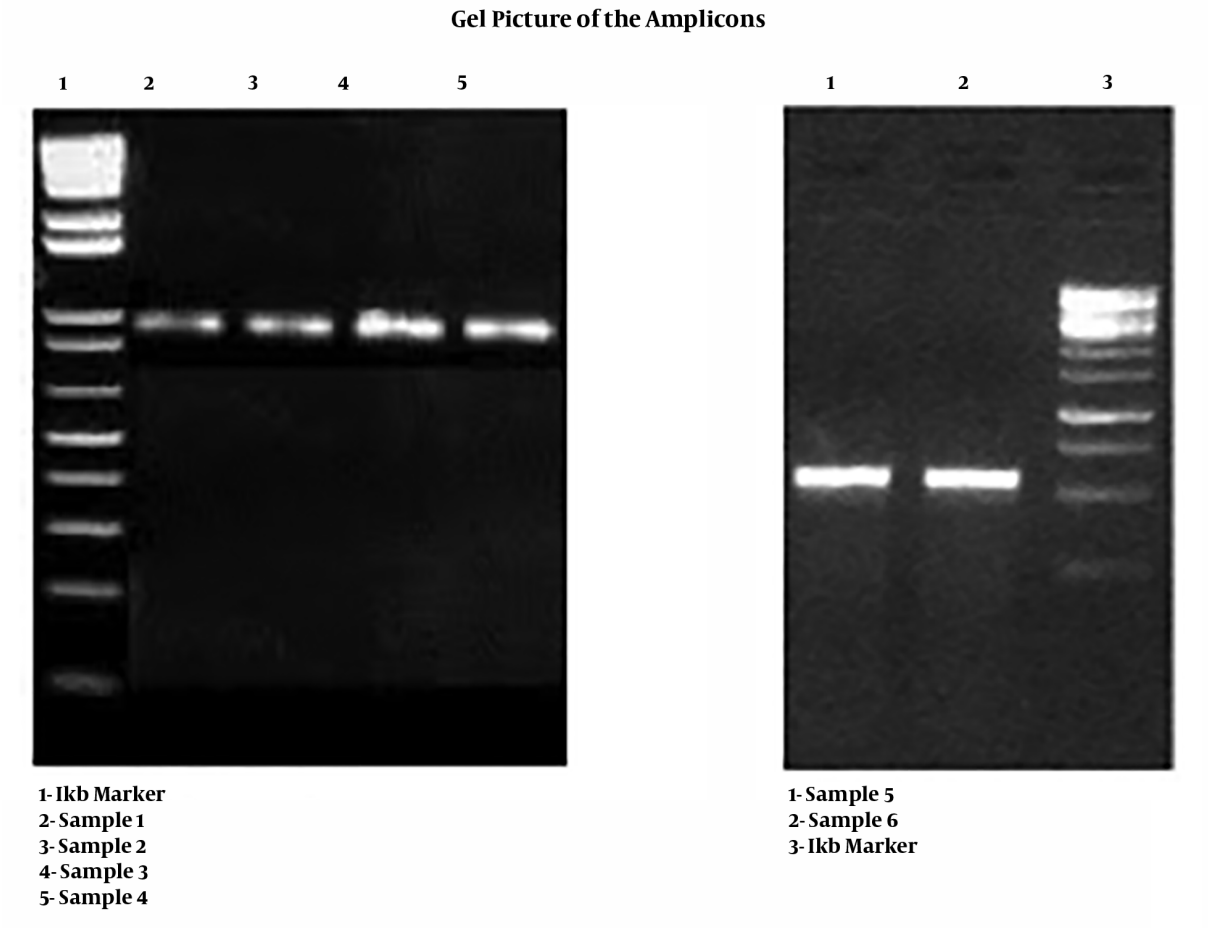

Figure 1. Agarose gel electrophoresis

GATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCA

4.1.1. Inference

The sequence obtained was 100% identical to the partial gene sequence of 16S rRNA of *Escherichia coli* strain *U5/41* (Figures 2 and 3).

4.2. Sequence for Sample 2

ATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCGTTAAGGTTAATAACCTTGGCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTG

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Escherichia coli strain U 5/41 16S ribosomal RNA gene, partial sequence	1481	1481	100%	0.0	100.00%	NR_024570.1
Escherichia fergusonii strain ATCC 35469 16S ribosomal RNA, complete sequence	1471	1471	100%	0.0	99.40%	NR_074902.1
Escherichia fergusonii strain NBRC 102419 16S ribosomal RNA gene, partial sequence	1471	1471	100%	0.0	99.40%	NR_114079.1
Escherichia coli strain JCM 1649 16S ribosomal RNA gene, partial sequence	1471	1471	100%	0.0	99.40%	NR_112558.1
Escherichia fergusonii strain ATCC 35469 16S ribosomal RNA gene, partial sequence	1471	1471	100%	0.0	99.40%	NR_027549.1
Shigella flexneri strain ATCC 29903 16S ribosomal RNA gene, partial sequence	1471	1471	100%	0.0	99.40%	NR_026331.1
Escherichia coli strain NBRC 102203 16S ribosomal RNA gene, partial sequence	1467	1467	100%	0.0	99.28%	NR_114042.1
Shigella boydii strain P288 16S ribosomal RNA gene, partial sequence	1466	1466	100%	0.0	99.28%	NR_104901.1
Shigella sonnei strain CECT 4887 16S ribosomal RNA gene, partial sequence	1466	1466	100%	0.0	99.28%	NR_104826.1
Brenneria alni strain pvfi20 16S ribosomal RNA gene, partial sequence	1466	1466	100%	0.0	99.28%	NR_116340.1

Figure 2. BLAST reference of *Escherichia coli*

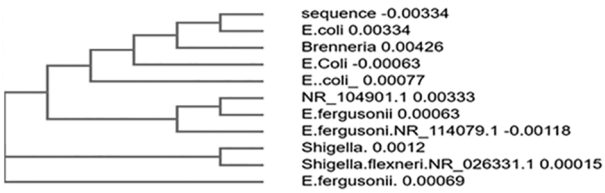

Figure 3. Phylogenetic tree of *Escherichia coli*

4.2.1. Inference

The sequence obtained was 100% identical to the partial gene sequence of 16S rRNA of *Klebsiella aerogenes* strain *F26* (Figures 4 and 5).

4.3. Sequence for Sample 3

ATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTC

4.3.1. Inference

The sequence obtained was 100% identical to *Pseudomonas entomophila* strain 2014 (Figures 6 and 7).

4.4. Sequence for Sample 4

ATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTC-

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Klebsiella aerogenes strain F26 16S ribosomal RNA gene, partial sequence	1504	1504	100%	0.0	100.00%	MK559555.1
Klebsiella aerogenes strain NCTC10006 genome assembly, chromosome: 6	1504	8950	100%	0.0	100.00%	LR134126.1
Klebsiella aerogenes strain NCTC10006 genome assembly, plasmid: 2	1504	1504	100%	0.0	100.00%	LR134122.1
Klebsiella aerogenes strain FDAARGOS_513 chromosome, complete genome	1504	12012	100%	0.0	100.00%	CP033817.1
Klebsiella aerogenes strain CB46I 16S ribosomal RNA gene, partial sequence	1504	1504	100%	0.0	100.00%	MK014300.1
Klebsiella aerogenes strain FDAARGOS_327 chromosome, complete genome	1504	12008	100%	0.0	100.00%	CP031756.1
Klebsiella aerogenes strain DAS43 16S ribosomal RNA gene, partial sequence	1504	1504	100%	0.0	100.00%	MH819718.1
Klebsiella aerogenes strain gol2 16S ribosomal RNA gene, partial sequence	1504	1504	100%	0.0	100.00%	MK426816.1
Klebsiella aerogenes strain NCTC9735 genome assembly, chromosome: 1	1504	11945	100%	0.0	100.00%	LR134475.1

Figure 4. BLAST reference of *Klebsiella*

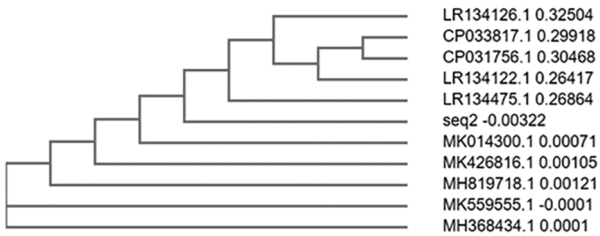

Figure 5. Phylogenetic tree of *Klebsiella aerogenes*

GGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGC-TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG-CAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACT-CT

4.4.1. Inference

The sequence obtained was 100% identical to *Staphylococcus aureus* strain *NCTC13616* (Figures 8 and 9).

4.5. Sequence for Sample 5

TTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGC-TGGCGGCGTGCCTAATACATGCAAGTCGAGCGAA

CAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGG-ACGGGTGAGTAACACGTGGGTAACCTACCTATAA

GACTGGGATAACTTCGGGAAACCGGAGCTAATACCG-GATAACATTTGGAACCGCATGGTTCTAAAGTGAA

AGATGGTTTTGCTATCACTTATAGATGGACCCGCGC-CGTATTAGCTAGTTGGTAAGGTAACGGCTTACCA

AGGCGACGATACGTAGCCGACCTGAGAGGGTGATCG-GCCACACTGGAACTGAGACACGGTCCAGACTCCT

ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGC-GAAAGCCTGACGGAGCAACGCCGCGTGAGTGATG

AAGGGTTTCGGCTCGTAAAACTCTGTTATTAGGGAA-GAACAAATGTGTAAGTAACTGTGCACGTCTTGAC

GGTACCTAATCAGAAAGCCACGGCTAACTACGTGCC-AGCAGCCGCGGTAATACGTGTGGCAAGCGTTATC

CGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT-CTTAAGTCTGATGTGAAAGCCCACGGCTCAACCG

TGGAGGGTCATTGGAAACTGGGCTTGAGTGCAGAAG-AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG

CGCAGAGATATTAGTGGAGGAACACCAGTGGCGAAG-GCGACTTTCTGGTCTGTAACTGACGCTGATGTGC

GAAAGCGTGGGGATCAAACAGGATTAGATACCCTGG-TAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT

AGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCA-TTAAGCACTCCGCCTGGGGAGTACGACCGCAAGG

TTGAAACTCAAAGGAATTGACGGGGACCCGCACAAG-CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCG

AAGAACCTTACCAAATCTTGATGAAAACTCTAGAGA-TAGAGCCTTCCCCTTC

4.5.1. Inference

The sequence obtained was 98.47% identical to *Staphylococcus saprophyticus* strain *FDAARGOS_355* (Figures 10 and 11).

4.6. Sequence for Sample 6

TGGGGTTGATCATGGCTCAGATTGAACGCTGGCGGC-AGGCCTAACACATGCAAGTCGAGCGGTAACAGGA

GAAAGCTTGCTTTCTTGCTGACGAGCGGCGGACGGG-TGAGTAATGTATGGGGATCTGCCCGATAGAGGGG

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pseudomonas entomophila strain 2014 chromosome, complete genome	1611	11279	100%	0.0	100.00%	CP034337.1
Pseudomonas entomophila strain 1257 chromosome, complete genome	1611	11139	100%	0.0	100.00%	CP034338.1
Bacterium strain E70 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MH998436.1
Pseudomonas putida strain CK223 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MH889110.1
Pseudomonas sp. strain AZ5 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MK125505.1
Pseudomonas guariconensis strain njensis 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MK318649.1
Pseudomonas sp. strain BYT-1 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MH539878.1
Pseudomonas sp. WCHPs060039 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MH428810.1
Pseudomonas guariconensis strain MR149 16S ribosomal RNA gene, partial sequence	1611	1611	100%	0.0	100.00%	MG674358.1

Figure 6. BLAST reference of *Pseudomonas*

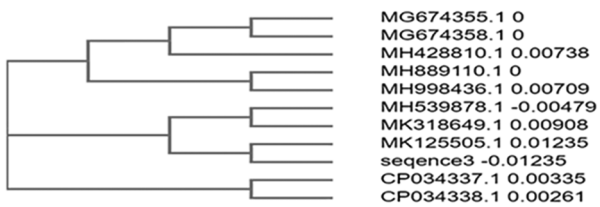

Figure 7. Phylogenetic tree of *Pseudomonas entomophila*

GATAACTACTGGAAACGGTGGCTAATACCGCATAATGTCTACGGACCAAAGCAGGGGCTCTTCGGACCTT

GCACTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCTCT

AGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCTACGGGAGGCAGCAGT

GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGTTG

TAAAGTACTTTCAGCGGGGAGGAAGGTGATAAGGTTAATACCCTTGTCAATTGACGTTACCCGCAGAAGA

AGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAGGCGTTAATCGGAATTACTGGG

CGTAAAGCGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGAGCTTAACTTGGGAATTGCATCT

GAAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATG

TGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAG

CAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCTTGAACC

GTGGCTTCTGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAATGCGAAGAACCTTACCTAC

TCTTGACATCCAGCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGG

CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGC

CAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAA

GTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCAGATACAAAGAGAAGCGACCTCGC

GAGAGCAAGCGGAACTCATAAAGTCTGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCG

GAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC

ACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGAT

TCATGACTGGGGTGAAGTCGTAACAAGGTAACC

4.6.1. Inference

The sequence obtained was 100% identical to the partial gene sequence of 16S rRNA of *Proteus mirabilis* strain *NCTC 11938* (Figures 12 and 13).

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus aureus strain NCTC13616 genome assembly, chromosome: 1	1439	8632	100%	0.0	100.00%	LR134193.1
Staphylococcus aureus strain NCTC4163 genome assembly, chromosome: 1	1439	8621	100%	0.0	100.00%	LR134139.1
Staphylococcus aureus strain NCTC11965 genome assembly, chromosome: 1	1439	8621	100%	0.0	100.00%	LR134093.1
Staphylococcus aureus strain NCTC4137 genome assembly, chromosome: 1	1439	7176	100%	0.0	100.00%	LR134091.1
Staphylococcus aureus strain NCTC9555 genome assembly, chromosome: 1	1439	8615	100%	0.0	100.00%	LR134090.1
Staphylococcus aureus strain NCTC5660 genome assembly, chromosome: 1	1439	7187	100%	0.0	100.00%	LR134088.1
Staphylococcus aureus strain NCTC7121 genome assembly, chromosome: 1	1439	7181	100%	0.0	100.00%	LR134087.1
Staphylococcus aureus strain NCTC13142 genome assembly, chromosome: 1	1439	8637	100%	0.0	100.00%	LR134086.1
Staphylococcus aureus strain NCTC12233 genome assembly, chromosome: 1	1439	8632	100%	0.0	100.00%	LR134085.1

Figure 8. BLAST reference of *Staphylococcus aureus*

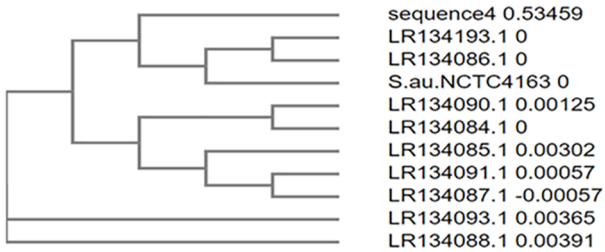

Figure 9. Phylogenetic tree of *Staphylococcus aureus*

5. Discussion

In the present study, a comprehensive molecular characterization of six bacterial isolates was carried out. We observed that the sequence in sample 1 was 100% identical to the partial gene sequence of 16S rRNA of *E. coli* strain *U5/41*. Phylogenetically strains included *U5/41, E. fergusonii* strain *ATCC35469, E. fergusonii* strain *NBRC102419, E. coli* strain *JCM1649, E. fergusonii* strain *ATCC35469, Shigella flexneri* strain *ATCC29903, E. coli* strain *NBRC102203, Shigella boydii* strain *P288, Shigella sonnei* strain *CECT4887*, and *Brenneriaalni strain pvfi20*. Campos et al. also conducted a study in Brazil, of *E. coli* isolated from urine samples of hospitalized patients and identified strains 131 and 69 as the most frequently found *E. coli* strains (6). Strain 69 was found to be associated with both community-acquired and healthcare-associated UTIs (7). The MDR manner of these strains was attributed due to the dfrA17-aadA5 gene, which makes these strains resistant to Trimethoprim, aminoglycosides. The other ST groups identified by Campos et al. included *ST648, ST405, ST73*, and *ST10*.

The sequence of sample 2 was 100% identical to that of the partial gene sequence of 16S rRNA of *Klebsiella aerogenes* strain *F26*. The phylogenetically identified strains were *F26, K. aerogenes* strain *NCTC10006, K. aerogenes* strain *NCTC10006, K. aerogenes* strain *FDAARGOS_513 chromosome, K. aerogenes* strain *CB46l, K. aerogenes* strain *FDAARGOS_327 chromosome, K. aerogenes* strain *DAS43, K. aerogenes* strain *gol2, K. aerogenes* strain *NCTC9735*, and *K. aerogenes* strain *CX-122*.

In sample 3, the sequence was 100% identical to that of *Pseudomonas entomophila* strain *2014*. The phylogenetically identified strains were *P. entomophila* strain *2014, P. entomophila* strain *1257 chromosome, Bacterium* strain *E70 16S ribosomal RNA* gene, *P. putida* strain *CK223, P.* spp strain *AZ5, P. guariconensis* strain *njensis, P.* spp strain *BYT-1, P.* spp *WCHPs060039, P. guariconensis* strain *MR149*, and *P. guariconensis* strain *MR144*.

In sample 4, the sequence was 100% identical to that of *Staphylococcus aureus* strain *NCTC13616*. Phylogenetically identified strains included *S. aureus* strain *NCTC13616, S. aureus* strain *NCTC4163, S. aureus* strain *NCTC11965, S. aureus* strain *NCTC4137, S. aureus* strain *NCTC9555, S. aureus* strain *NCTC5660, S. aureus* strain *NCTC7121, S. aureus* strain *NCTC13142, S. aureus* strain *NCTC12233, S. aureus* strain *NCTC13552*.

In sample 5, the strain identified was *Staphylococcus saprophyticus* strain *FDAARGOS_355*. The other phylogenetically identified strains were *S. saprophyticus* strain *FDAARGOS_355, S. saprophyticus* strain *FDAARGOS_137, S. saprophyti-*

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Staphylococcus saprophyticus strain FDAARGOS_355 chromosome, complete genome	1825	10914	100%	0.0	98.47%	CP022093.2
Staphylococcus saprophyticus strain FDAARGOS_168 chromosome, complete genome	1825	10942	100%	0.0	98.47%	CP014113.2
Staphylococcus saprophyticus strain FDAARGOS_137, complete genome	1825	10925	100%	0.0	98.47%	CP014057.2
Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305 16S ribosomal RNA, comp	1825	1825	100%	0.0	98.47%	NR_074999.2
Staphylococcus saprophyticus strain RJ17 16S ribosomal RNA gene, partial sequence	1825	1825	100%	0.0	98.47%	KJ540934.1
Staphylococcus saprophyticus subsp. saprophyticus ATCC 15305 DNA, complete genome	1825	10914	100%	0.0	98.47%	AP008934.1
Staphylococcus sp. S04009 16S ribosomal RNA gene, partial sequence	1821	1821	99%	0.0	98.46%	MH643903.1
Staphylococcus saprophyticus subsp. saprophyticus strain NCTC7666 genome assembly, ch	1820	10898	100%	0.0	98.37%	LR134089.1
Staphylococcus saprophyticus strain FDAARGOS_336 chromosome, complete genome	1820	10909	100%	0.0	98.37%	CP022056.2

Figure 10. BLAST reference of *Staphylococcus saprophyticus*

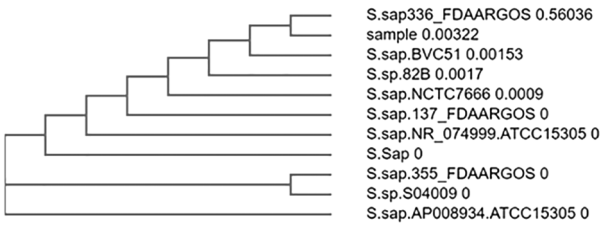

Figure 11. Phylogenetic tree of *Staphylococcus saprophyticus*

cus sub spp. *saprophyticus ATCC 15305, S. saprophyticus* strain *RJ17, S. saprophyticus* sub spp. *saprophyticus ATCC 15305, Staphylococcus* spp. *S04009, S. saprophyticus* sub spp. *saprophyticus* strain *NCTC7666, S. saprophyticus* strain *FDAARGOS_336, S.* spp *82B, S. saprophyticus* strain *BVC51.*

The sequence of the sample was 100% identical to that of the partial gene sequence of 16S rRNA of *Proteus mirabilis* strain *NCTC 11938.* The phylogenetically identified strains were *P. mirabilis* strain *NCTC 11938, P. mirabilis* strain *PmSC1111, P. mirabilis* strain *NCTC4199, P. mirabilis* strain *AR_0029, P. mirabilis* strain *AR379, P. mirabilis* isolate *GN2, P. mirabilis* strain *AR_0156, P. mirabilis* strain *AR_0159, P. mirabilis* strain *AR_0059*, and *P. mirabilis* strain *AOUC-001.*

Jenkins et al. obtained pus samples and joint fluids from 23 patients using 1,343 bp PCR. Of 38 samples using 762/598 bp PCR, 33 samples were negative by both culture and PCR. Moreover, 16S rDNA was identified in 8/17 culture-positive samples (8). The bacteria identified were *S. aureus, Streptococcus pneumoniae, Streptococcus viridians, Prevotella pleuritidis*, and *Prevotella oulorum*. Gene sequencing helped in identifying anaerobes in samples positive on culture. As the Gram-positive cell wall is disrupted readily during the extraction process, it is not identified as easily as Gram-negative bacteria (9-12). However, Jenkins et al. found that for both Gram-negative and -positive bacteria, there was no bias as they could not be identified by PCR (8).

Van der Zee et al. demonstrated that PCR-based detection can replace the culture-based diagnosis except in the case of antibiotic sensitivity testing that might be essential for the adequate treatment of patients. These results were confirmed by 16S PCR. However, the limitation of this study was that only a few strains were tested, and hence, the presence and homology of target genes need further substantiation (13).

Tajbakhsh et al. isolated and detected Gram-negative bacteria, causing UTI in patients from Shahrekord Hospitals, Iran. The study used PCR, which was found to be an effective method for diagnosis of bacteria causing UTI, especially Gram-negative ones and also other infections (14).

Abulmeshah carried out a study to identify organisms causing UTI by doing 16S rRNA gene sequencing and BLAST analysis and found that *E. coli, K. pneumoniae, S. aureus, P. mirabilis* and *P. aeruginosa* were the most prevalent organisms (15).

5.1. Conclusions

Six bacterial isolates were analyzed by 16S rRNA gene sequencing, followed by the construction of a phylogenetic tree formation up to the species level. This method was a valuable tool for a cost-effective and accurate diagnosis of an array of uropathogens in both asymptomatic and symptomatic pregnant women. If the samples presented MDR on sensitivity testing, the specific strain isolated by PCR would provide guidelines for the management of UTI in pregnant women in the future.

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Proteus mirabilis strain NCTC 11938 16S ribosomal RNA, partial sequence	2776	2776	100%	0.0	100.00%	NR_043997.1
Proteus mirabilis strain PmSC1111 chromosome, complete genome	2750	19183	99%	0.0	99.80%	CP034090.1
Proteus mirabilis strain NCTC4199 genome assembly, chromosome: 1	2750	19194	99%	0.0	99.80%	LR134205.1
Proteus mirabilis strain AR_0029 chromosome, complete genome	2750	19211	99%	0.0	99.80%	CP029725.1
Proteus mirabilis strain AR379 chromosome, complete genome	2750	19222	99%	0.0	99.80%	CP029133.1
Proteus mirabilis isolate GN2 chromosome, complete genome	2750	19200	99%	0.0	99.80%	CP026581.1
Proteus mirabilis strain AR_0156, complete genome	2750	19205	99%	0.0	99.80%	CP021852.1
Proteus mirabilis strain AR_0159, complete genome	2750	19205	99%	0.0	99.80%	CP021550.1

Figure 12. BLAST reference of *Proteus mirabilis*

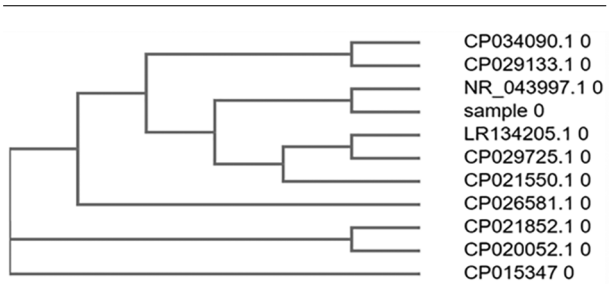

Figure 13. Phylogenetic tree of *Proteus mirabilis*

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Footnotes

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