International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 26-03-2021; Accepted: 19-04-2021: Published: 28-04-2021
www.botanyjournals.com
Volume 6, Issue 2, 2021, Page No. 632-642

Characterization of urinary tract Infectious bacteria from in and outdoor patients of Duta Deepti Satsang charitable hospital, Deoghar, Jharkhand, India

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Abstract
Urinary Tract Infection (UTI) has been a common disease of people of all age group, especially in women. Lack of keen knowledge regarding the use of antibiotics, most of the cases shows multidrug resistance. This study aims to screen the sensitivity of UTI bacteria obtained from in and outdoor patients of Duta Deepti Satsang Charitable Hospital, Deoghar in Jharkhand (India). A total of 350 urine samples collected, out of which 79 numbers were found to be culture positive, thus showing significant bacterial growth. The most common isolates were identified to be *E. coli* 56 (70.9%), *Klebsiella* sp. 11 (13.9%), *Proteus* sp. 7 (8.9%) and *Pseudomonas* sp. 5(6.3%). Most of the isolates were found to be resistant to ampicillin, amoxycillin, nalidixic acid, ofloxacin and ciprofloxacin. Three bacterial isolates i.e., *Escherichia* sp. (DDSCH54), *Klebsiella* sp. (DDSCH62) and *Proteus* sp. (DDSCH72) that showed multidrug resistance were further subjected to species level identification through 16S rRNA sequences and phylogenetic analysis. Based on 16S rRNA sequence analysis, the selected three bacteria were identified up to species level as *Escherichia coli* (MW857549), *Klebsiella pnemonae* (MW857551) and *Proteus mirabilis* (MW857554). The present findings could depict few predominant UTI bacteriathat showed MDR respectively which were identified as most prevalent infectious UTI bacteria showing broad range of antibiotic resistance from the patient of Deoghar, Jharkhand, India.

Keywords: urinary tract infection (UTI), antibiotic sensitivity, out and indoor patient, antibiotic efficacy, 16s rRNA, phylogenetic analysis

Introduction

Urinary tract infections (UTI) are most common human bacterial infections that affects people of all age groups in general and particularly most prevalent in women. Majority of such infection are caused by Enterobacteriaceae and mostly these are known to acquire drug resistance easily. In most of the cases there is a need to start a prophylactic therapy before culture and sensitivity results are available (Srinivasan *et al* 2015; Janda and Abbott 2007; Johnson *et al* 2019; Winand *et al* 2020; Peker *et al* 2019) [1, 2, 3, 4, 5]. Area specific monitoring studies aimed at obtaining knowledge about the type of bacteria responsible for UTIs and their resistance patterns may help clinicians to choose the right empirical treatment (Flores-Mireles *et al* 2015) [6].

Worldwide, about 150 million people are diagnosed with UTI each year, and UTI are classified as uncomplicated or complicated (Nuutinen and Uhari 2001) [7]. Uncomplicated UTIs occur in sexually active healthy female patients with structurally and functionally normal urinary tracts (Hooten *et al* 1996) [8]. Complicated UTIs are those that are associated with co-morbid conditions that prolong the need for treatment or increase the chances for therapeutic failure (Ronald and Ludwig 2001) [9]. These conditions include abnormalities of the urinary tract that impede urine flow, the existence of a foreign body (e.g., indwelling catheter, stones) or infection with multidrug resistant pathogens. UTIs in male patients are considered complicated. Despite involvement of the upper urinary tract, pyelonephritis can be considered uncomplicated when it occurs in a healthy patient (Gharbi *et al* 2019; MacNeily 2001; Schlager 2003; Layton 2003; Hooten 2003) [10, 11, 12, 13, 14].

Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency and occasionally suprapubic tenderness. Acute pyelonephritis describes the clinical syndrome characterized by flank pain or tenderness, or both, and fever, often associated with dysuria, urgency, and frequency. More than 95% of urinary tract infections are caused by a single bacterial species (Chen *et al* 2013) [15]. *E. coli* is the most frequent of the infecting organisms in acute infection. *Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas,* and *Enterococci* species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population. *Corynebacterium urealyticum* has been recognized as an important nosocomial pathogen. Anaerobic organisms are rarely pathogens in the urinary tract. Coagulase-negative *Staphylococci* are a common cause of urinary tract infection in some reports. *Staphylococci saprophyticus* tends to cause infection in young women of a sexually active age (Fisher *et al* 2011; Tanti and Buragohain 2013; Kommedal *et al* 2014; Milani *et al* 2013; Yergeau *et al* 2012) [16, 17, 18, 19, 20].

Due to the lack of knowledge and improper use of antibiotics most of these applications trend to show multidrug resistance. Study of UTI among the population of Deoghar district can help to understand the efficacy of the antibiotics, used by the physicians, for the treatment of UTI. This will help to prevent unnecessary use of antibiotics. The present study has been carried out with the following objectives: 1. Survey of suspected UTI in IPD and OPD patients of Duta Deepti Satsang Charitable Hospital,

Deoghar in Jharkhand (India), 2. Isolation and identification of bacterial pathogens from the collected samples, 3. Determination of sensitivity of the isolated pathogens to commonly used antibiotics, and 4. Identification of selected MDR bacteria up to species level based on 16S rRNA analysis and construction of phylogenetic tree.

Materials and Methods

Selection of patients

Patients like pregnant women and those having other diseases like diabetes, renal disease, etc. were included except those who are already under antibiotic therapy or discontinued their antibiotic doses.

Collection of urine sample and culture conditions

Each patient was given a questionnaire enquiring their medical and personal details before collecting the urine sample.Urine samples were collected by clean catch midstream method. Samples were immediately cultured using appropriate media and pure cultures were obtained using standard methods.

The urine specimens were inoculated on to CLED and Mac-Conkey agar culture media and incubated. After overnight incubation at 37^{o}C, plates were read and colony count noted. Only the specimens which showing colony counts of $>10^5$ were included in the study.

Atibiotic efficacy assay

Identification and antibiotic susceptibility testing (AST) were done with a panel of antibiotics on Mueller-Hinton agar following standard procedures. AST was done by disc diffusion test and results interpreted according to Clinical laboratory Standards Institute (CLSI) guidelines. Antibiogram of Gram negative bacilli were tabulated and resistance profile for various antibiotics was compared. Differences between inpatient and outpatient sensitivity percent were noted. Antibiotic susceptibility tests were conducted using a panel of antibiotics following standard procedure (Bauer *et al* 1966; Murray *et al* 1995; CLSI 2009; Das *et al* 2020) [21, 22, 23, 24].

Identification

Isolates were identified on the basis of their standard cultural, morphological and biochemical characteristics (Aneja 2014; Bergey and Holt 1993; Cappuccino and Sherman 2017) [25, 26, 27]. 16S rRNA sequence based analysis was used for confirmation of identity of some of the potent MDR isolates (MacFaddin 1985; 2000) [28, 29].

Genomic DNA isolation, PCR conditions, amplification of 16S rRNA gene and sequencing

DNA isolation from bacterial samples was done using the EXpure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd following the manufacture's protocol. DNA concentrations were measured by Qubit 3.0.

PCR amplification of the 16SrRNA gene of the isolated bacteria was performed with the universal primers 27F (5'-AGAGTTTGATCTGGCTCAG-3') and 1492R (5'-TACGGTACCTTGTTACGACTT-3′), which amplified approximately 1400 bp.

All PCR reactions were carried out in 25 µL volumes containing 2.5 µL of 10× PCR buffer with $MgCl_2$, 2 µL of a mixture containing each of the dNTP's at a concentration of 2.5 mol/L, each primer at a final concentration of 5.0 pM and 1 U of Taq DNA polymerase. The amplification was run for 30 cycles and each cycle comprised of 30 sec of denaturation at 94°C, 30 sec of annealing at 55°C, and a 2 min of extension step at 72°C. The PCR amplification was preceded by incubation at 95°C for 7 min. After 30 cycles, there was a final 5 min extension at 72°C. The PCR amplifications were performed using a Perkin Elmer Thermal Cycler (GeneAmp PCR2400, Perkin Elmer, USA). The PCR products were purified by following manufacture's protocol with Montage PCR Clean up kit (Millipore) (Petti 2007; Junemann *et al* 2012; Salipante *et al* 2013; Salipante *et al* 2013; Rosenthal *et al* 2014) [30, 31, 32, 33, 34]. The PCR product was sequenced using the same primers. Sequencing reactions were performed using a ABI PRISM®BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (AppliedBiosystems).

Further, the contig sequences of 16S rRNA of all the three UTI bacterial isolates were submitted to NCBI-GenBank and obtained accessions.

Phylogenetic analysis

The 16SrRNA sequence was blast using NCBI blast similarity search tool. The phylogenetic analysis among the related sequence of blast results was performed followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequences. The resulting aligned sequences were cured using the program Gblocks 0.91b. This Gblocks eliminates poorly aligned positions and divergent regions (removes alignment noise).Finally, the program PHYLIP package was used to construct the phylogenetic tree based on maximum-likelihood.

Results and Discussion

A total of 350 urine samples were collected from both OPD and IPD patients respectively. Out of the total collected samples, 79 numbers of samples were culture positive that showed significant pathogenic growth. Among the samples with positive growth, 52 were of female patients (48 adults and 4 children) and 27 male patients (adults).

Through standard morphological, physiological and biochemical analyses, all the 52 isolates were identified up to genus level that represented by *Escherichia* sp. -56 (70.9%), *Klebsiella* sp. -11 (13.9%), *Proteus* sp.- 7 (8.9%) and *Pseudomonas* sp. - 5 (6.3%). Most of the isolates were found to be resistant to ampicillin, amoxycillin, nalidixic acid, ofloxacin and partially resistant to norfloxacin and ciprofloxacin (Fig 1; Table 1).

Table 1: Antibiotic resistance analysis for the UTI bacterial isolates

Antibiotics used	Test organisms			
	Escherichia sp.(56 plates)	*Klebsiella* sp. (11 plates)	*Proteus* sp. (7 plates)	*Pseudomonas* sp. (5 plates)
Ampicillin	48	9	6	5
Amoxycillin	32	9	7	5
Nalidixic acid	52	10	7	5
Ofloxacin	45	7	5	3
Norfloxacin	30	7	5	4
Ciprofloxacin	33	6	6	3

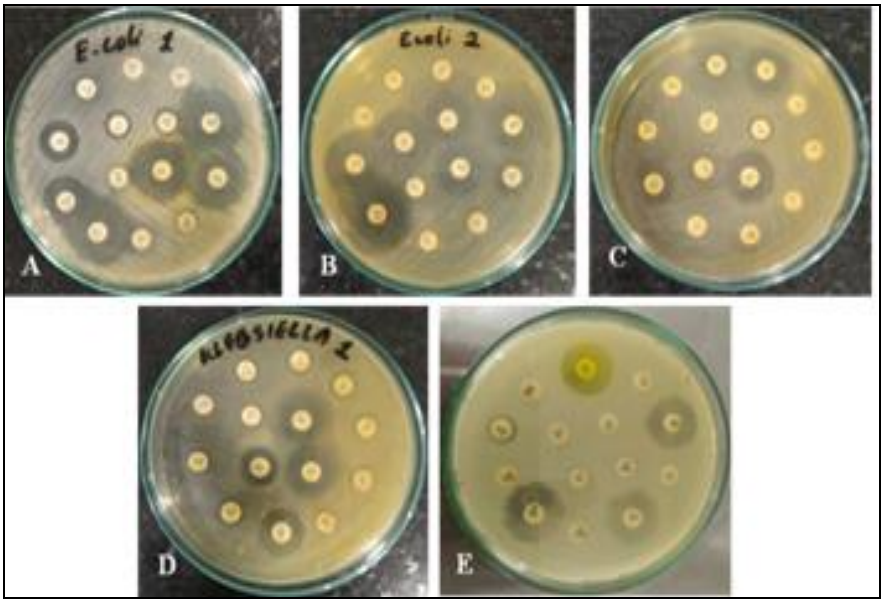

Fig 1: Images of five antibiotic sensitivity tests of the selected isolates: A. *E. coli* (DDSCH09),\ B. *E. coli* (DDSCH33), C. *E. coli* (DDSCH54), D. *Klebsiella* sp. (DDSCH62), and E. *Proteus* sp. (DDSCH72)

Out of these five isolates, three isolates *viz.*, *Escherichia* sp. (DDSCH54), *Klebsiella* sp. (DDSCH62) and *Proteus* sp. (DDSCH72) from all the four groups that showed highly resistant against the panel of antibiotics were finally selected for species level identification and phylogenetic analysis.

PCR amplified 16S rDNA bands were excised from all the samples and purified for further sequence analysis. Partial sequences were used to determine the phylogenetic analysis of the bacteria, which revealed the sequences of approximately ~1335bp. The sequences were submitted to NCBI Genbank and obtained accession number i.e., *Escherichia* sp. (MW857549), *Klebsiella* sp. (MW857551) and *Proteus* sp. (MW857554).The sequences were further used for phylogenetic analysis and identification up to species level.

The application of PCR based bacterial identification for defined clinical syndromes contribute mostly to our understanding of these illnesses. Out of 79 bacterial UTI isolates, five could be identified for the broad range of antibiotic resistance that caused life-threatening illnesses. While 16S rDNA PCR and sequencing identified as *Escherichia* sp. (MW857549), *Klebsiella* sp.(MW857551) and *Proteus* sp.(MW857554) respectively [Fig 2-5].

Table 2-4 represents the identity scores from the 16S rDNA differentiation of microorganism database for *Escherichia* sp. (MW857549), *Klebsiella* sp. (MW857551) and *Proteus* sp. (MW857554) respectively. The query isolate shares 100% identity with their respective group with the score assesses relatedness on the basis of the number of aligned bases and percent identity.

>Contig – *Escherichia* sp. (MW857549): 16S rDNA sequences

CGGTAGCTAATACCGCATAACGTCGCAAGACCAAA
GAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTG
CCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGC
TCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGG
ATGACCAGCCACACTGGAACTGAGACACGGTCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGT
ATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAG
CGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTC
ATTGACGTTACCCGCAGAAGAAGCACCGGCTAACT
CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGC
AGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATCTGATACTGGCAAGCT
TGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTA
GCGGTGAAATGCGTAGAGATCTGGAGGAATACCG
GTGGCGAAGGCGGCCCCCTGGACGAAGACTGACG
CTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGTCG
ACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGG
AGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACG
GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
TGCAACGCGAAGAACCTTACCTGGTCTTGACATCC
ACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGG
AACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGC
TCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGC
CGGGAACTCAAAGGAGACTGCCAGTGATAAACTG

GAGGAAGGTGGGGATGACGTCAAGTCATCATGGCC
CTTACGACCAGGGCTACACACGTGCTACAATGGCG
CATACAAAGAGAAGCGACCTCGCGAGAGCAAGCG
GACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGT
CTGCAACTCGACTCCATGAAGTCGGAATCGCTAGT
AATCGTGGATCAGAATGCCACGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCATGGGA
GTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCG
GGAGGGCGCTTACCACTTGATCAG

>Contig – *Klebsiella* sp. (MW857551): 16S rDNA sequences

GTAGCTAATACCGCATAACGTCGCAAGACCAAAGT
GGGGGACCTTCGGGCCTCATGCCATCAGATGTGCC
CAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTC
ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCAGAC
TCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA
TGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGT
GAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCG
GGGAGGAAGGCGTTAAGGTTAATAACCTTGGCGAT
TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC
GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA
GGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTCGAAACTGGCAGGCTA
GAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAG
CGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTC
AGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGTCGATT
TGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGC
TAACGCGTTAAATCGACCGCCTGGGGAGTACGGCC
GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC
AACGCGAAGAACCTTACCTGGTCTTGACATCCACA
GAACTTTCCAGAGATGGATTGGTGCCTTCGGGAAC
TGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGG
GAACTCAAAGGAGACTGCCAGTGATAAACTGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGGCCCTT
ACGACCAGGGCTACACACGTGCTACAATGGCATAT
ACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAC
CTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTG
CAACTCGACTCCATGAAGTCGGAATCGCTAGTAAT
CGTAGATCAGAATGCTACGGTGAATACGTTCCCGG
GCCTTGTACACACCGCCCGTCACACCATGGGAGTG
GGTTGCAAAAGAAGTAGGTAGCTTAACCTTC

>Contig – *Proteus* sp. (MW857554): 16S rDNA sequences

AAAGGGTGGCTAATACCGCATAATGTCTACGGACC
AAAGCAGGGGCTCTTCGGACCTTGCACTATCGGAT
GAACCCATATGGGATTAGCTAGTAGGTGGGGTAAA
GGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAG
AGGATGATCAGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
CACAATGGGCGCAAGCCTGATGCAGCCATGCCGCG
TGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTT
CAGCGGGGAGGAAGGTGATAAGGTTAATACCCTTA
TCAATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTG
CAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCA
CGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCC
GAGCTTAACTTGGGAATTGCATCTGAAACTGGTTG
GCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGT
GTAGCGGTGAAATGCGTAGAGATGTGGAGGAATA
CCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTG
ACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCTGTAAACGATG
TCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCT
GGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTA
CGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC
GATGCAACGCGAAGAACCTTACCTACTCTTGACAT
CCAGCGAATCCTTTAGAGATAGAGGAGTGCCTTCG
GGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTATCCTTTGTTGCCAGCACGTG
ATGGTGGGAACTCAAAGGAGACTGCCGGTGATAA
ACCGGAGGAAGGTGGGGATGACGTCAAGTCATCAT
GGCCCTTACGAGTAGGGCTACACACGTGCTACAAT
GGCAGATACAAAGAGAAGCGACCTCGCGAGAGCA
AGCGGAACTCATAAAGTCTGTCGTAGTCCGGATTG
GAGTCTGCAACTCGACTCCATGAAGTCGGAATCGC
TAGTAATCGTAGATCAGAATGCTACGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCAT
GGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACC
TTCGGGAGG

Table 2: Two closest species match obtained from FASTA searches between the *Escherichia* sp. (MW857549): 16S rDNA sequence and the sequences downloaded from GenBank/EMBL/DDBJ databases

Description	Maximum Score	Total Score	Query Cover	E-Value	Percentage Identity	Accession
Escherichia coli strain JCD2 16S ribosomal RNA gene, partial sequence	2423	2423	100%	0.0	99.92%	MH532520.1
Escherichia coli strain W5 16S ribosomal RNA gene, partial sequence	2422	2422	99%	0.0	99.92%	MN086364.1
Escherichia coli strain JCD1 16S ribosomal RNA gene, partial sequence	2422	2422	99%	0.0	99.92%	MH517447.1
Escherichia coli strain 90-9133 chromosome, complete genome	2418	16841	99%	0.0	100.00%	CP042947.1
Escherichia coli strain CFSAN061772 chromosome, complete genome	2418	16673	99%	0.0	100.00%	CP042833.1
Escherichia coli strain CFSAN061771 chromosome, complete genome	2418	16900	99%	0.0	100.00%	CP042896.1
Escherichia coli strain CFSAN061763 chromosome, complete genome	2418	16840	99%	0.0	100.00%	CP042899.1
Escherichia coli strain CFSAN061761 chromosome, complete genome	2418	16668	99%	0.0	100.00%	CP042903.1
Escherichia coli strain CFSAN061766 chromosome, complete genome	2418	16679	99%	0.0	100.00%	CP042871.1
Escherichia coli strain O15:H7 strain ATCC 43888 chromosome, complete genome	2418	16867	99%	0.0	100.00%	CP041623.1
Escherichia coli strain Ec40743 chromosome, complete genome	2418	16779	99%	0.0	100.00%	CP041919.1
Escherichia coli strain 183-d pink 16S ribosomal RNA gene, partial sequence	2418	2418	99%	0.0	99.92%	MN208122.1
Escherichia coli strain 148-B pink 16S ribosomal RNA gene, partial sequence	2418	2418	100%	0.0	99.85%	MN208218.1
Escherichia coli strain 405 chromosome	2418	16768	99%	0.0	100.00%	CP041563.1

Escherichia coli strain ECOL-18-VL-LA-PA-Ryan-0026 chromosome, complete genome	2418	16646	99%	0.0	100.00%	CP041392.1
Escherichia coli strain U1 chromosome, complete genome	2418	16795	99%	0.0	100.00%	CP041359.1
Escherichia coli strain ATCC 43899 chromosome, complete genome	2418	16867	99%	0.0	100.00%	CP015853.1
Escherichia coli strain MSHS 472 chromosome, complete genome	2418	16696	99%	0.0	100.00%	CP041302.1
Escherichia coli O157:H7 strain COPRO21317 chromosome	2418	16862	99%	0.0	100.00%	CP035706.1
Escherichia coli strain A1_181 chromosome, complete genome	2418	16928	99%	0.0	100.00%	CP040067.1
Escherichia coli strain K71-77 chromosome, complete genome	2418	16884	99%	0.0	100.00%	CP040886.1
Escherichia coli isolate EcMAD1 genome assembly, chromosome: EcMAD	2418	16889	99%	0.0	100.00%	LR595691.1
Escherichia coli strain A1_136 chromosome, complete genome	2418	16696	99%	0.0	100.00%	CP040390.1
Escherichia coli strain A1_180 chromosome, complete genome	2418	16928	99%	0.0	100.00%	CP040381.1
Escherichia coli O157:H7 strain ECP17-1298 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP040570.1
Escherichia coli O157:H7 strain ECP17-46 chromosome, complete genome	2418	16862	99%	0.0	100.00%	CP040572.1
Escherichia coli strain AR24.2b chromosome, complete genome	2418	16928	99%	0.0	100.00%	CP035944.1
Escherichia coli strain 105 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028700.1
Escherichia coli strain 106 chromosome, complete genome	2418	16862	99%	0.0	100.00%	CPO28698.1
Escherichia coli strain 107 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028695.1
Escherichia coli strain 108 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028693.1
Escherichia coli strain 109 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028690.1
Escherichia coli strain 110 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028687.1
Escherichia coli strain 111 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028687.1
Escherichia coli strain 112 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028683.1
Escherichia coli strain 113 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028680.1
Escherichia coli strain 114 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028677.1
Escherichia coli strain 115 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028674.1
Escherichia coli strain 116 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028671.1
Escherichia coli strain 117 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028668.1
Escherichia coli strain 118 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028665.1
Escherichia coli strain 119 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028662.1
Escherichia coli strain 120 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028659.1
Escherichia coli strain 121 chromosome, complete genome	2418	16817	99%	0.0	100.00%	CP028656.1
Escherichia coli strain 122 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028654.1
Escherichia coli strain 123 chromosome, complete genome	2418	16873	99%	0.0	100.00%	CP028652.1

Table 3: Two closest species match obtained from FASTA searches between the *Klebsiella* sp. (MW857551): 16S rDNA sequence and the sequences downloaded from GenBank/EMBL/DDBJ databases

Description	Maximum Score	Total Score	Query Cover	E-Value	Percentage Identity	Accession
Klebsiella pneumoniae strain DA12090 chromosome, complete genome	2379	18870	100%	0.0	100.00%	CP030072.1
Klebsiella pneumoniae strain KP14003 chromosome, complete genome	2379	18898	100%	0.0	100.00%	CP041934.1
Klebsiella pneumoniae strain R50 chromosome, complete genome	2379	18920	100%	0.0	100.00%	CP040363.1
Klebsiella variicola strain UTI_4 16S ribosomal RNA gene, partial sequence	2379	2379	100%	0.0	100.00%	MK530084.1
Klebsiella pneumoniae strain LB-AMP1KSU 16S ribosomal RNA gene, partial sequence	2379	2379	100%	0.0	100.00%	MH973160.1
Klebsiella pneumoniae strain AR_0135 chromosome, complete genome	2379	18842	100%	0.0	100.00%	CP032178.1
Klebsiella pneumoniae strain QMP B2-170 chromosome, complete genome	2379	18992	100%	0.0	100.00%	CP031798.1
Klebsiella pneumoniae strainMSB1_8A_sc-2280397 chromosome, complete genome	2379	18931	100%	0.0	100.00%	CP031800.1
Klebsiella pneumoniae strainF89-1 chromosome, complete genome	2379	18776	100%	0.0	100.00%	CP026159.1
Klebsiella pneumoniae strain L5-2 chromosome, complete genome	2379	18787	100%	0.0	100.00%	CP025684.1
Klebsiella pneumoniae AJ218 genome assembly, chromosome 1	2379	18820	100%	0.0	100.00%	LR130541.1
Klebsiella pneumoniae strain 08EU827 chromosome, complete genome	2379	18887	100%	0.0	100.00%	CP025576.1
Klebsiella pneumoniae strain SCM96 chromosome, complete genome	2379	18892	100%	0.0	100.00%	CP028716.1
Klebsiella pneumoniae strain NH25 chromosome, complete genome	2379	18926	100%	0.0	100.00%	CP024874.1
Klebsiella variicola strain G5 16S ribosomal RNA gene, partial sequence	2379	2379	100%	0.0	100.00%	MG948566.1
Klebsiella pneumoniae strain KSB1_10J chromosome, complete genome	2379	18920	100%	0.0	100.00%	CP024515.1
Klebsiella pneumoniae strain KSB1_4E chromosome, complete genome	2379	18865	100%	0.0	100.00%	CP024499.1
Klebsiella pneumoniae subsp. pneumoniae strain ST101:960186733 chromosome, complete genome	2379	18807	100%	0.0	100.00%	CP023487.1
Klebsiella pneumoniae subsp. pneumoniae strain ST101:950142398 chromosome	2379	18848	100%	0.0	100.00%	CP023553.1
Klebsiella quasivariicola strain KPN1705 chromosome, complete genome	2379	18865	100%	0.0	100.00%	CP022823.1
Klebsiella pneumoniae strain 19051, complete genome	2379	18920	100%	0.0	100.00%	CP022023.1
Klebsiella pneumoniae strain AR_0107, complete genome	2379	18859	100%	0.0	100.00%	CP021955.1
Klebsiella pneumoniae strain K66-45, complete genome	2379	18765	100%	0.0	100.00%	CP020901.1
Klebsiella pneumoniae strain AR_0117, complete genome	2379	18920	100%	0.0	100.00%	CP020061.1
Klebsiella pneumoniae strain Kp_Goe_121641, complete genome	2379	18842	100%	0.0	100.00%	CP018735.1

Klebsiella pneumoniae strain Kp_Goe_71070, complete genome	2379	18837	100%	0.0	100.00%	CP018450.1
Klebsiella pneumoniae strain Kp_Goe_33208, complete genome	2379	18837	100%	0.0	100.00%	CP018447.1
Klebsiella pneumoniae strain KP36, complete genome	2379	18926	100%	0.0	100.00%	CP017385.1
Klebsiella pneumoniae subsp. pneumoniae strain TGH13, complete genome	2379	18804	100%	0.0	100.00%	CP012745.1
Klebsiella pneumoniae isolate 23, complete genome	2379	18926	100%	0.0	100.00%	CP016926.1
Klebsiella pneumoniae strain BR, complete genome	2379	18931	100%	0.0	100.00%	CP015990.1
Klebsiella pneumoniae subsp. pneumoniae strain TGH10, complete genome	2379	16239	100%	0.0	100.00%	CP012744.1
Klebsiella pneumoniae subsp. pneumoniae strain TGH8, complete genome	2379	16720	100%	0.0	100.00%	CP012743.1
Klebsiella pneumoniae subsp. pneumoniae strain RJF293, complete genome	2379	18854	100%	0.0	100.00%	CP014008.1
Klebsiella pneumoniae subsp. pneumoniae strain NUHL24835, complete genome	2379	18931	100%	0.0	100.00%	CP014004.1
Klebsiella pneumoniae subsp. pneumoniae strain 234-12, complete genome	2379	18881	100%	0.0	100.00%	CP011313.1
Klebsiella pneumoniae subsp. pneumoniae strain MGH 78578, complete genome	2379	18820	100%	0.0	100.00%	CP000647.1
Klebsiella pneumoniae strain 18-2374 chromosome, complete genome	2374	18854	100%	0.0	99.92%	CP041927.1
Klebsiella pneumoniae strain IA565 chromosome, complete genome	2374	18870	100%	0.0	99.92%	CP030070.1
Klebsiella pneumoniae strain NKU_KlebA1 chromosome, complete genome	2374	18765	100%	0.0	99.92%	CP041649.1
Klebsiella pneumoniae subsp. pneumoniae strain KPCTRSRTH07 chromosome, complete genome	2374	18959	100%	0.0	99.92%	CP041099.1
Klebsiella variicola strain PAK/UVAS/PATH/SA21 16S ribosomal RNA gene, partial sequence	2374	2374	100%	0.0	99.92%	MK789289.1
Klebsiella pneumoniae isolate KSH203 chromosome, complete genome	2374	16441	100%	0.0	99.92%	CP034327.1
Klebsiella pneumoniae strain KP18-29 chromosome, complete genome	2374	18721	100%	0.0	99.92%	CP034249.1
Klebsiella pneumoniae strain 4/1-2 chromosome, complete genome	2374	18815	100%	0.0	99.92%	CP023839.1
Klebsiella pneumoniae strain KP_NORM_BLD_2014_104014 chromosome, complete genome	2374	18964	100%	0.0	99.92%	CP034045.1
Klebsiella pneumoniae strain KP_NORM_BLD_2015_112126 chromosome, complete genome	2374	18959	100%	0.0	99.92%	CP034053.1

Table 4: Two closest species match obtained from FASTA searches between the *Proteus* sp. (MW857554): 16S rDNA sequence and the sequences downloaded from GenBank/EMBL/DDBJ databases

Description	Maximum Score	Total Score	Query Cover	E-Value	Percentage Identity	Accession
Proteus mirabilis strain VAC chromosome, complete genome	2396	16729	100%	0.0	99.92%	CP042907.1
Proteus mirabilis strain PmBC1123 chromosome, complete genome	2396	16684	100%	0.0	99.92%	CP034091.1
Proteus mirabilis strain NCTC4199 genome assembly, chromosome: 1	2396	16729	100%	0.0	99.92%	LR134205.1
Proteus mirabilis strain AR_0029 chromosome, complete genome	2396	16734	100%	0.0	99.92%	CP029725.1
Proteus mirabilis strain FDAARGOS_67 chromosome, complete genome	2396	16710	100%	0.0	99.92%	CP026051.1
Proteus mirabilis strain FDAARGOS_80 chromosome, complete genome	2396	16710	100%	0.0	99.92%	CP026059.1
Proteus mirabilis strain T18 chromosome, complete genome	2396	16732	100%	0.0	99.92%	CP017085.1
Proteus mirabilis strain T21, complete genome	2396	16740	100%	0.0	99.92%	CP017082.1
Proteus mirabilis strain 102 16S ribosomal RNA gene, partial sequence	2396	2396	100%	0.0	99.92%	MF399345.1
Proteus mirabilis strain AR_0156, complete genome	2396	16729	100%	0.0	99.92%	CP021852.1
Proteus mirabilis strain AR_0155, complete genome	2396	16734	100%	0.0	99.92%	CP021694.1
Proteus mirabilis strain AR_0159, complete genome	2396	16745	100%	0.0	99.92%	CP021550.1
Proteus mirabilis strain AR_0159, complete genome	2396	16712	100%	0.0	99.92%	CP020052.1
Proteus mirabilis strain PMB31 16S ribosomal RNA gene, partial sequence	2396	2396	100%	0.0	99.92%	KU378106.1
Proteus sp. HW-16 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	KP152656.1
Bacterium COD56 ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	KP195713.1
Bacillus sp. BAB-3447 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	KF917177.1
Proteus sp. BAB-3406 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	KF917137.1
Proteus sp. M1410 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	JF946778.1
Bacterium YCT49 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	JF775434.1
Bacterium YCG20 16S ribosomal RNA gene, partial genome	2396	2396	100%	0.0	99.92%	JF775429.1
Proteus mirabilis strain YCG36 16S ribosomal RNA gene, partial sequence	2396	2396	100%	0.0	99.92%	JF775415.1
Proteus sp. BAB-5300 16S ribosomal RNA gene, partial genome	2392	2392	100%	0.0	99.85%	KT261796.1
Proteus mirabilis strain YCG36 16S ribosomal RNA gene, partial sequence	2392	2392	100%	0.0	99.85%	EF091150.1
Proteus mirabilis strain M17_J16 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MK426652.1
Proteus mirabilis strain CLPM181223 chromosome	2390	2390	100%	0.0	99.85%	CP041391.1
Proteus mirabilis strain Mum2 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MN120786.1
Proteus mirabilis strain PM 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MN022585.1
Proteus mirabilis strain VS-2S 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MK785117.1
Proteus mirabilis strain PmSC1111 chromosome, complete genome	2390	16707	100%	0.0	99.85%	CP034090.1
Proteus mirabilis strain W4 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MK494931.1
Proteus mirabilis strain JN48 tRNA modification GTPase (trmE) gene	2390	2390	100%	0.0	99.85%	MF576130.1
Proteus mirabilis strain UMAGOD06 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MH091057.1
Proteus mirabilis strain TCR46 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MH718833.1
Proteus mirabilis strain TCR20 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MH718824.1

Proteus mirabilis strain CCUG 70746	2390	16731	100%	0.0	99.85%	CP023273.1
Proteus mirabilis strain WWi280 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MH396761.1
Proteus mirabilis strain WTPii140 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MH396753.1
*Proteus mirabilis*ES14 gene for 16S ribosomal RNA, partial sequence	2390	2390	100%	0.0	99.85%	LC385633.1
Proteus mirabilis strain AR379 chromosome, complete genome	2390	16718	100%	0.0	99.85%	CP029133.1
*Proteus mirabilis*strain TSPPTL-17/col5 16S ribosomal RNA gene, partial sequence	2390	2390	100%	0.0	99.85%	MF143079.1
Proteus sp. JCM 2150 gene for 16S ribosomal RNA, partial sequence	2390	2390	100%	0.0	99.85%	LC382139.1
*Proteus mirabilis*strain K 1609 chromosome	2390	2390	100%	0.0	99.85%	CP028522.1
*Proteus mirabilis*strain K 1609 chromosome	2390	2390	100%	0.0	99.85%	CP028522.1

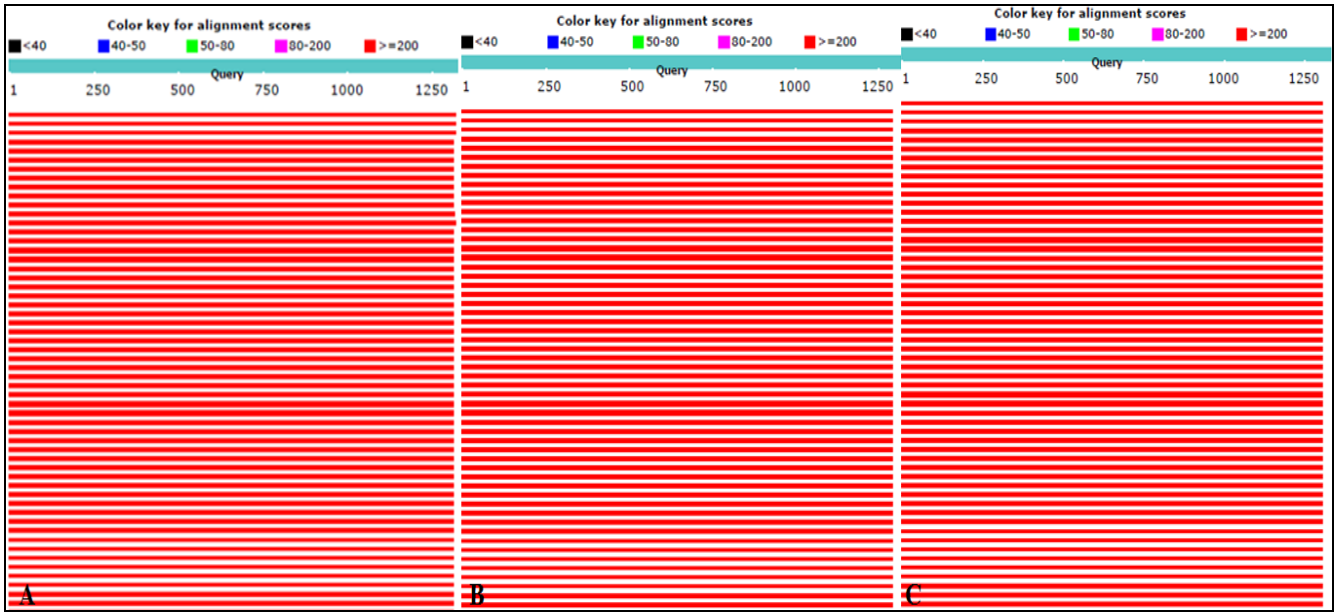

Fig 2: Sequence alignment between the 16S rDNA of the experimental organisms with the downloaded sequences from NCBI-GenBank; **A:** *Escherichia* sp. (MW857549), **B:** *Klebsiella* sp. (MW857551) and **C:** *Proteus* sp. (MW857554)

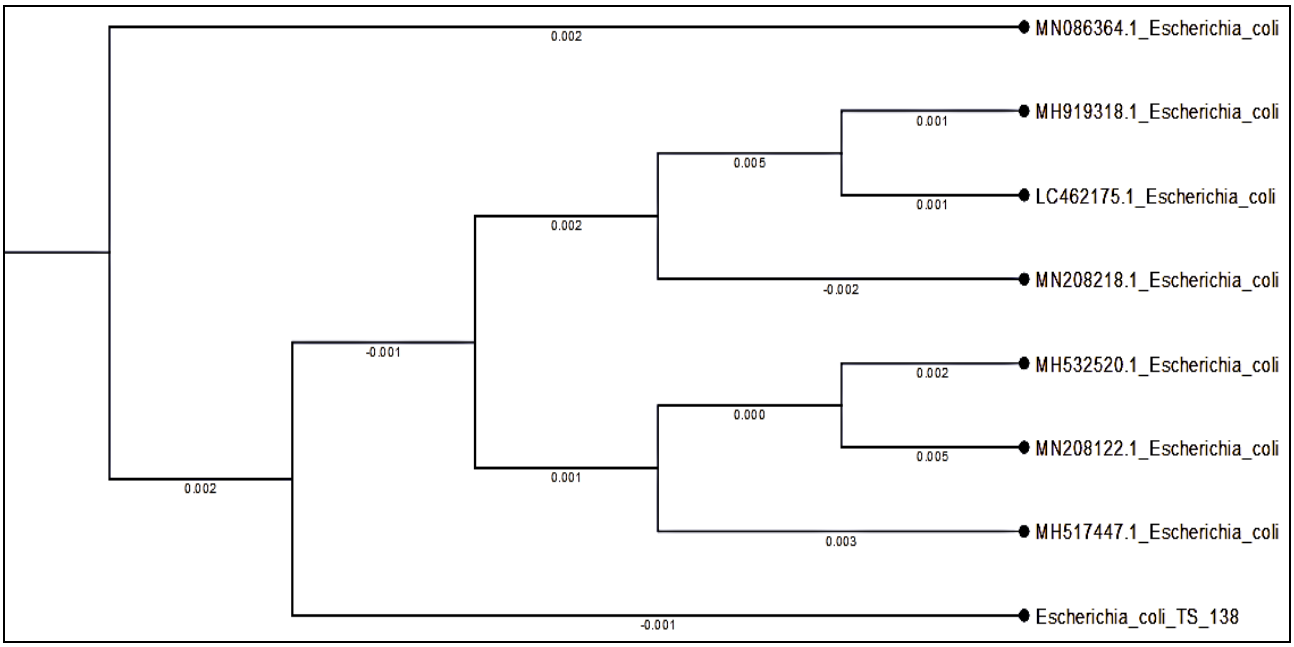

Fig 3: The phylogenetic tree of *Escherichia* sp. (MW857549) based on maximum-likelihood method using PHYLIP package

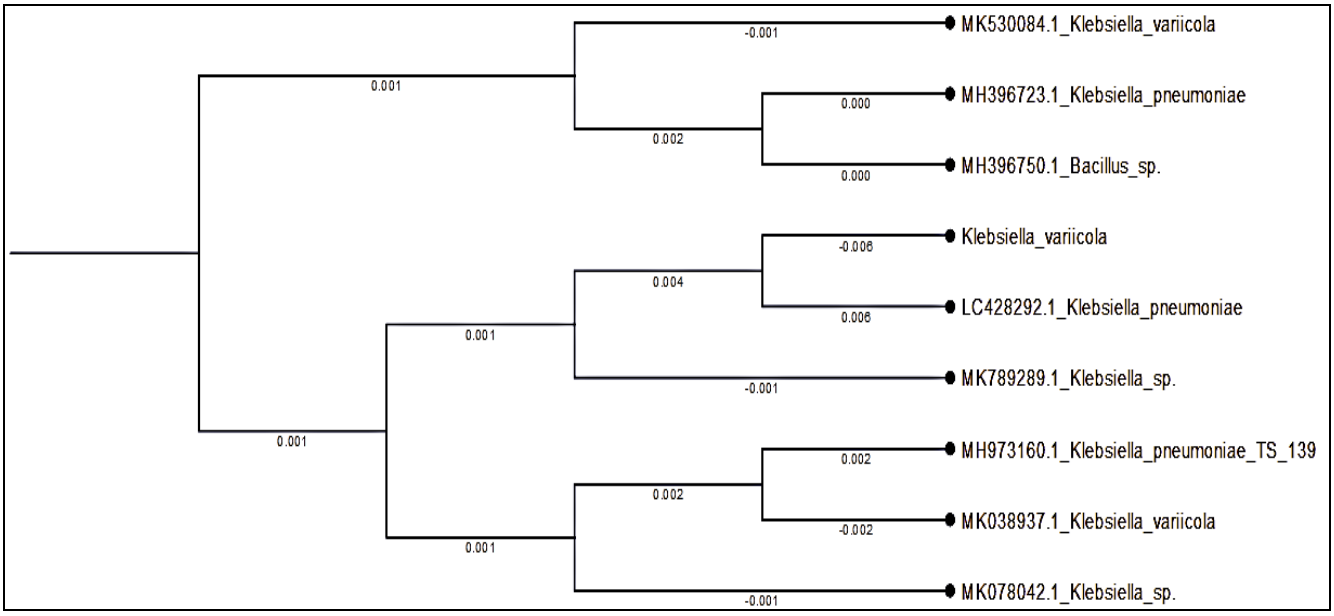

Fig 4: The phylogenetic tree of *Klebsiella* sp. (MW857551) based on maximum-likelihood method using PHYLIP package

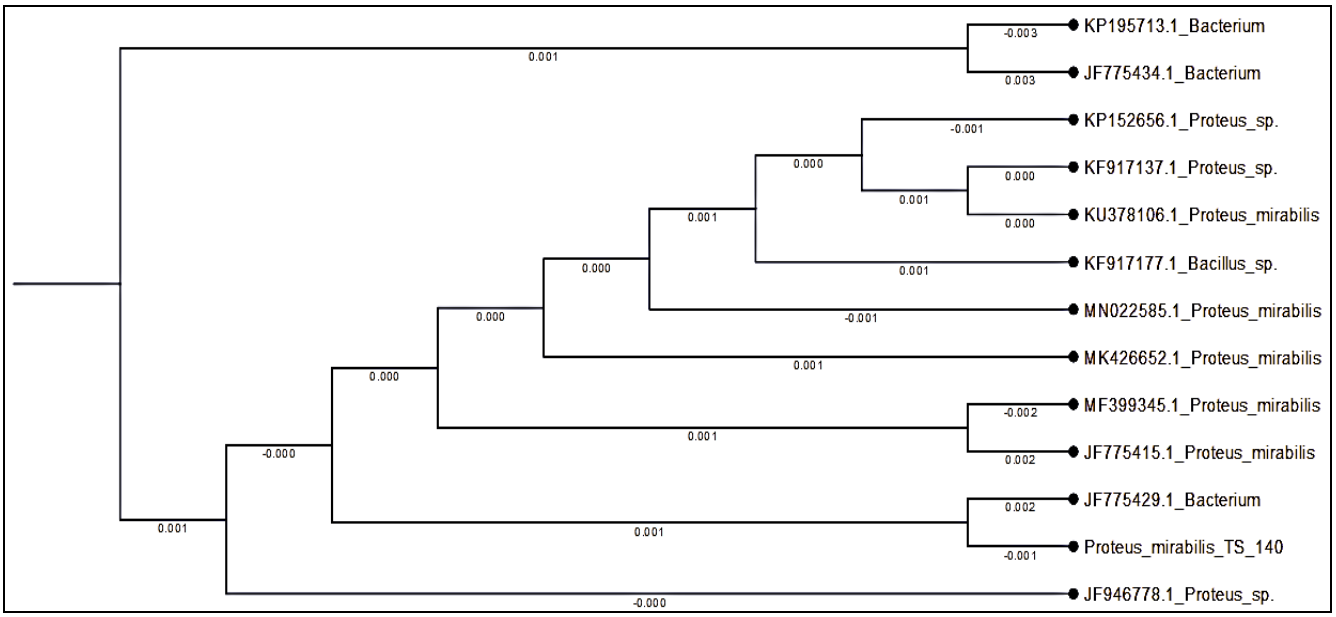

Fig 5: The phylogenetic tree of *Proteus* sp. (MW857554)based on maximum-likelihood method using PHYLIP package

Based on 16S rRNA sequence analysis and phylogeny tree constructed with the downloaded homologous sequences from NCBI-GenBank, the experimental three bacterial strains were identified up to species level as *Escherichia coli* (MW857549; https://www.ncbi.nlm.nih.gov/nuccore/MW857549), *Klebsiella pnemonae* (MW857551; https://www.ncbi.nlm.nih.gov/nuccore/MW857551) and *Proteus mirabilis* (MW857554; https://www.ncbi.nlm.nih.gov/nuccore/MW857554). These three UTI bacterial species have proved to be prevalently occurred in the in- and out-door patients of the Deoghar district, Jharkhand, India.

Proper identification of UTI bacteria and their authentication is significantly important for public health surveillance and clinical care to understand theirbiology of pathogenicity of infectious clinical syndromes for better use of specific antibiotic along with the control strategies for patients and populations. The standard culture-based approaches can correctly distinguish between isolates up to genus level that many diverse species share the same morphological, physiological and biochemical phenotypes. However, to authenticate and identify up to species level based on routine culture-based methods arenot enough that may lead to be misidentified. Therefore, in this study, we used partial sequences generated from 16S rRNA gene amplicons to compare and to construct the phylogeny based molecular identification of clinical isolates UTI and to ascertain accuracy of routine microbiological identification of a wide range of clinically applicable bacterial species. Here, we used a model-based approach for species level classification of clinical organisms of UTI bacteria. Our finding is in concordance with the findings of Bruce *et al* 1992; Cedergren *et al* 1993; Pace *et al* 1986 where theydemonstrated very high confidence genus level identification and good species level identification with exceptions in certain genera such as *Stenotrophomonas*, *Enterobacter*, *Citrobacter*, and *Escherichia* [35, 36, 37].

One of the most attractive potential uses of 16S rRNA gene sequence informatics is to provide genus and species

identification for isolates that do not fit any recognized biochemical profiles, for strains generating only a "low likelihood" or "acceptable" identification according to commercial systems, or for taxa that are rarely associated with human infectious diseases (Woese 1985; 1987) [38, 39]. Some of the previous workers depicted that the cumulative results from a limited number of studies to date suggest that 16S rRNA gene sequencing provides genus identification in most cases (99%) but less so with regard to species (65 to 83%) (Chen *et al* 2013; Tringe and Hugenholtz 2008; Klemetsen *et al* 2019; Bhattacharyya *et al* 2014) [40, 41, 42, 43]. Difficulties encountered in obtaining a genus and species identification include the recognition of novel taxa, too few sequences deposited in nucleotide databases, species sharing similar and/or identical 16S rRNA sequences, or nomenclature problems arising from multiple genomovars assigned to single species or complexes (Rahman *et al* 2012; Barman *et al* 2014; Chowdhury *et al* 2014; Tanti *et al* 2012; Borgohain and Tanti 2020; Jacobsen *et al* 2008) [44, 45, 46, 47, 48, 49].

Conclusion

Gene amplification and sequencing have contributed to the identification of new pathogens as disease agents and improved our ability to identify microorganisms from culture. The emergence of more unusual microorganisms from culture could represent new human pathogens or indicate our growing understanding of microbial taxonomy of species, which was previously overlooked when using traditional methods. Direct application of broad-range bacterial PCR to clinical samples is an effective adjunctive technique to culture that has proven useful in some clinical situations. Moreover, broad-range PCR can be very helpful, particularly when histological stains are positive, patients have received antimicrobial therapy prior to sample collection, or the likely pathogens are fastidious or uncultivable pathogens. Gene amplification and sequencing from culture and clinical samples can help us better understand microbial pathogenesis and predict treatment responses and outcomes.

The present studies revealed that 79 UTI bacterial species from in- and out-door patients of the Duta Deepti Satsang Charitable Hospital, Deoghar, Jharkhand, India based on morphological, physiological and biochemical characteristics followed by molecular characterization based on 16S rDNA sequences.

Author contributions

PKB was involved conception and design of the study. RD carried out all the experiments and analyzed the data. PKB critically analyzed the findings. Both the authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest

Acknowledgements

Infrastructural facilities provided by Prof. Bhaben Tanti, Department of Botany, Gauhati University, Assam (India) is greatly acknowledged.

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