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Antibiotic sensitivity Profile of Uropathogenic *Klebsiella pneumoniae*

Sunil B Jadhav, H. J. Bhosale*, Mujahed M Siddiqui, Rania N Ghaleb, S.V. Mamdapure, Pallavi B Jadhav
DST-FIST and UGC-SAP Sponsored
School of Life sciences, Swami Ramanand Teerth Marathwada
University Nanded-431606, India
Corresponding author: bhoslehemlata@gmail.com

ABSTRACT
Klebsiella pneumoniae is an important cause of nosocomial and opportunistic infections associated with urinary tract infections, respiratory and bloodstream infections. The increasing drug resistance and emergence of virulent strains are posing major threat and challenges in clinical field. In the present study, one bacterial strain isolated from urine sample of urinary tract infected patient was characterized for its antimicrobial resistance using disk diffusion assay and 20 commonly used antibacterial agents belonging to different classes of antibiotics. The isolate was identified at molecular level as Klebsiella pneumoniae SJB-1. The varying degrees of resistance were found to different classes of antibiotics. Highest sensitivity (31mm) was observed in presence of Sulfonamide and (25-30mm) quinolone class of antibiotics followed by tetracycline (20-25mm), aminoglycosides (15-25mm) and macrolide (15-20mm), phenicol (20mm).The strain was less sensitive to selected cephalosporin (10-15mm) and polymyxin(10-15mm) antibiotics and completely resistant to tested Beta-lactam antibiotics. The present work suggests the efficacy of Sulfonamide antibiotics in treatment of UTIs caused by Klebsiella pneumoniae.
Key words: *Uropathogens, Drug resistance, Klebsiella pneumoniae, Sulfonamides, Quinolones.*

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INTRODUCTION

The second most frequent microbial illness in humans is a urinary tract infection (UTI), which affects 150 million individuals annually worldwide (1).All age grouped people including young adults, small children's and elderly persons are susceptible to bacterial urinary tract infections. An estimated 40% of women have experienced a UTI at some point in their lives. *Klebsiella pneumoniaeis the second most bacteria that cause UTI followed by E.coli.* The infections are more frequent in diabetic persons and they can happen anywhere along the urinary system. The changes in the kidney's host defense mechanism brought on by microvascular and diabetic illnesses may increase the occurrence of urinary tract infections (2). Several risk factors linked with UTI are cystitis, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (3). Choosing the right antimicrobial treatment to treat bacterial illnesses depends on the identification of the bacteria and testing for their susceptibility. The incorrect use, improper prescription of antibiotics, excessive dosage, and long-term treatment in humans, agro farms, and veterinary, results in recurrent urinary tract infections (40–75%) (4). The problem is made worse by the development of antimicrobial resistance brought on by the over use of empiric broad-spectrum antibiotics for the treatment of simple UTIs. (5,6). For instance, numerous investigations have documented the alarmingly high rates of antibiotic resistance for the *klebsiella pneumoniae* to ampicillin, amoxicillin, co-trimoxazole, and cefoxitin. The bacterial resistance to antibiotics poses a serious danger to the medical sector and a worldwide problem in the treatment of urinary tract infections (UTIs), making it a recurrent topic of conversation everywhere (7).Therefore, choosing the right medication for an effective course of therapy depends on making a correct diagnosis of UTI and identifying an antibiotic sensitivity pattern (8). This study investigates the sensitivity of *Klebsiella pneumoniae* isolated from urinary tract infected patient to different classes of antibiotics.

MATERIAL AND METHODS

Collection of sample

A 35-year-old female patient with an infection of the urinary tract was the source of the sample collection at the Uro care Hospital in Nanded. The urine sample was taken in a single-use, sterile bottle. Samples were taken from the hospital and transferred to the laboratory (9).

Physical examination of urine

Urine specimen was collected, and several physical characteristics including appearance, color, pH, odor, and volume were examined (10).

Isolation of Bacteria

Spread 0.1 ml of urine sample was inoculated onto surface of sterile and solidified Macconkey agar plate, cetrimide agar, mannitol salt agar plates. The plates were kept for incubation at 37^0C for 24 hours (11). After incubation well isolated and morphological distinct isolate were recorded.

Biochemical Examination

Based on morphological and microbiological examinations, the chosen isolate was subjected to biochemical tests (starch hydrolysis, oxidase, catalase, nitrate reductase, citrate utilization, urease test, indole synthesis, VP and MR) to confirm the nature of bacterium as per the criteria used by Geoffrey arasaouno (12)

Molecular Identification of Uropathogenic isolate

NCCS in Pune, the 16S rRNA was identified. Using a Quaigen DNA isolation kit and genomic DNA was extracted from the isolate. Utilizing universal oligonucleotide primers that hybridize at locations 8–27 and 1488–1511 in relation to the E. coli 16S rRNA numbering system, PCR was used to amplification up the 16S rRNA from strain. (Perkin Elmer, USA) (13). The obtained sequence was analyzed for closed homology using BLASTn programme at the NCBI. The related sequences for the strain were obtained from the NCBI database. The Phylogenetic evolutionary history was then determined using the Neighbour Joining Method analysis (14). MEGA 4.0 was used to conduct the phylogenetic analysis. MEGA 4.0 was used to create phylogenetic trees. (15).

Submission of Sequence of isolates to NCBI: -

After phylogenetic analyses, the sequences was submitted to the DNA Databank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL), European Bioinformatics Institute (EBI), European Nucleotide Archive (ENA) and the accession numbers was obtained.

Antibiotics used

The selected antibiotics are used for the disc sensitivity method which is used in the treatment of urinary tract infection (6). The antibiotics used are as follows: Gentamycin, Kanamycin, Tobramycin, Erythromycin, Rifamycin, Norfloxacin, Nalidixic acid, Sparfloxacin, Tetracycline, Oxytetracycline, Amoxycillin, Ampicillin, Augmentin, Cefoxitin, Cefotaxime, Cefalexin and Co-trimoxazole.

Antibiotic Sensitivity Testing

The different group of antibiotics commonly used in treatment of UTIs were used to check the antibiotic sensitivity profile of selected isolate using disc diffusion method. The disc diffusion method, an antimicrobial sensitivity test was performed in accordance with CLSI standards (16).Different antibiotic discs were chosen and Mueller-Hinton agar plates were set up. The identified bacterial isolate was inoculated in nutrient broth and incubated at 37 °C to activate the culture and attain the cell density approaching 0.5 McFarland standard 0.1 ml of the active culture was inoculated and evenly spread over the surface of Mueller-Hinton agar plates (11). Sterile forceps were used to gently put the tops of the various antibiotic discs onto the surface of the agar medium. These plates were kept for incubation at 37 °C for 24 hours after 30min diffusion at 4^o C (17), (18). The diameter of each zone of inhibition was measured in mm after an overnight incubation.

RESULTS AND DISCUSSION

Physical Parameters of Urine Sample

The collected urine sample from urinary tract infected patient was examined for physical parameters includes p^H was acidic, pale yellow in color, turbid in appearance and with aromatic odor.

Isolation of Bacterial pathogen

The cetrimide agar and mannitol salt agar plates did not show any growth after 24 hrs incubation and the distinct and well isolated colonies appeared only on MacConkey agar plates. The selected colonies showing visible growth, pink center and pink edges, mucoid in consistency with smooth surface were used for further analysis.

Biochemical Characteristics of Selected Isolate

Table 1. The result of biochemical characterization of selected isolate are shown in table 1.

Biochemical Tests	Bacterial isolate
Grams Nature	-ve
Indole Production	-ve
Methyl Red	+ve
Voges-Proskauer	+ve
Citrate Utilization	+ve

Starch Hydrolysis	-ve
Oxidase Test	-ve
Catalase Test	+ve
Nitrate Reduction	+ve

Molecular Identification

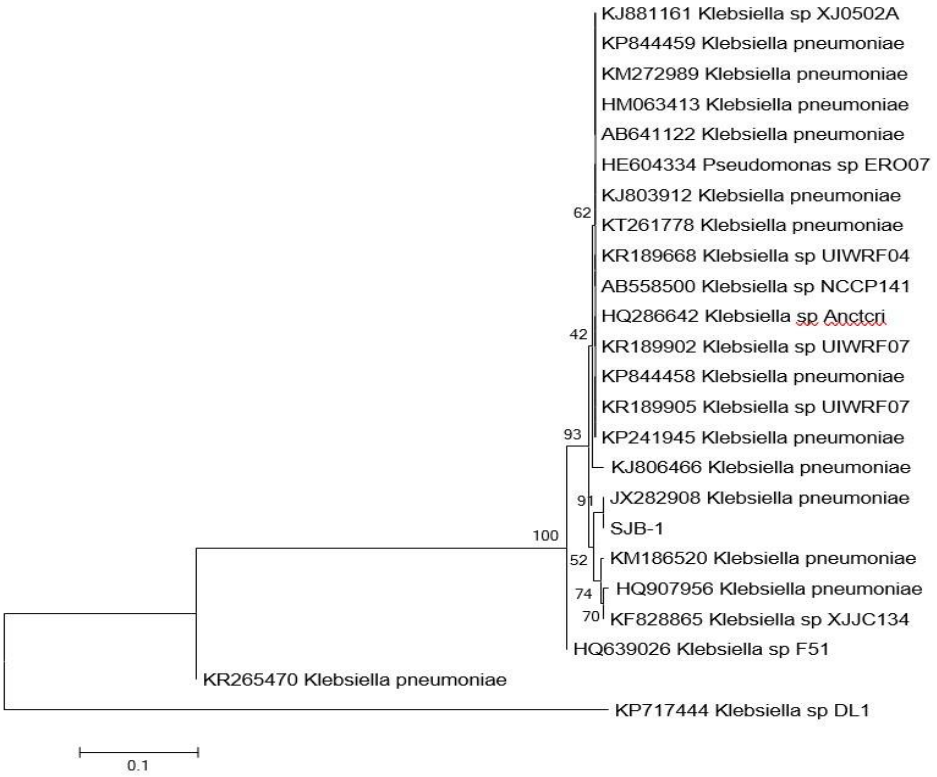
The bacterial strain that was chosen for molecular characterization 16S rRNA sequencing was identified as *klebsiella pneumoniae*. The obtained 16S rRNA sequence of the strain *klebsiella pneumoniae* was deposited to GenBank and obtained the accession number LC720890.

>*Klebsiella pneumoniae SJB-1*

AGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAC
GGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGAC
TCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCGGGAACTGCATTCGAAACTGGCAGGCTAGAGTC
TTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC
GGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
CGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGC
CTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCAACCC
TTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGAT
GACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCT
CGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGTAGATCAGAATGCTA

Phylogenetic analysis

The 16S rRNA sequence of bacterial isolate was analyzed for its molecular phylogenetic relationships and determined that it is most closely related to Klebsiella sp. with 100% sequence similarity. Its other closest phylogenetic relatives are *Klebsiella pneumoniae* isolate JX282908.



Antibiotic Sensitivity Tests

In accordance with CLSI recommendations, the isolated bacteria were tested for antibiotic sensitivity against various classes of antibiotics, including macrolide, aminoglycoside, quinolone, Tetracycline, Beta-Lactam, Cephalosporin, Sulfonamide, phenicol, oxazolidine, and polymyxin. All antibiotic classes, with the exception of beta-lactam were shown to be sensitive to *Klebsiella pneumoniae*. The antibiotics with the highest levels of sensitivity were co-trimoxazole, norfloxacin, nalidixic acid, gentamycin, tetracycline,

sparfloxacin, and chloramphenicol, respectively. These were followed by tobramycin, furazolidone, rifamycin, and kanamycin. cefotaxime, cefalexin, and colistin reduced the strain's sensitivity. amoxycillin, ampicillin, cefoxitin, and augmentin were the antibiotic classes having the highest levels of resistance throughout the trial. The ability to produce extended spectrum beta lactamases, which can deconstruct the beta lactam ring, has been linked to the ability to develop resistance to the beta lactam class of antibiotics (19). *Klebsiella pneumonia* are generally resistant to ampicillin and amoxycillin due to constitutively expressed class-A Beta-lactamase (20). Different antibiotic sensitivity patterns are present in the bacterial pathogen (Table 3). The patterns of *Klebsiella pneumonia*e sensitivity to every tested drug are showing in Figure 3.

Table 2. Antimicrobial Sensitivity Test Against *Klebsiella pneumoniae*

Class of Antibiotics	Antibiotics	Disc Concentration(µg)	Zone (mm)
Macrolide	Erythromycin (E) Rifamycin (RIF)	15 05	16 17
Aminoglycoside	Kanamycin (K) Gentamycin (G) Tobramycin (TOB)	 20 10	16 25 19
Quinolone	Norfloxacin (NX) Nalidixic acid (NA) Sparfloxacin (SC)	10 30 05	28 26 20
Tetracycline	Tetracycline (TE) Oxytetracycline (O)	30	21 22
Beta-Lactam	Amoxycillin (AMC) Ampicillin (AMP Augmentin (AM) Cefoxitin (CX)	30 10	00 00 10 00
Cephalosporin	Cefotaxime (CTX) Cefalexin (CN)	30	10 12
Sulfonamide	Co-Trimoxazole (COT)		31
Phenicol	Chloramphenicol (C)	10	20
Oxazolidine	Furazolidone (FR)		18
Polymyxin	Colistin (CL)		13

Fig 1. Antibiotic Sensitivity by Disc diffusion Method

CONCLUSION

The goal of the current study was to identify the patterns of antibiotic sensitivity among the isolated bacterial strains from urinary tract infection patients. This study looked at how isolated bacteria from urinary tract infections responded to 20 different common antibiotics. The result indicated the sensitivity profile of uropathogenic K*lebsiella pneumoniae* such as highest sensitivity was observed in sulfonamide and quinolone class of antibiotics followed by tetracycline group and aminoglycosides and macrolide. The selected strain was less sensitive to selected cephalosporin group of antibiotics and completely resistant to beta-lactam antibiotics. The present study suggests the efficacy of *Sulfonamide* and Quinolone antibiotics in treatment of urinary tract infection caused by *klebsiella pneumoniae.*

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