

## Isolation and Characterization of Antibiotic-Resistant Bacteria from Urinary Tract Infection on Diabetic Mellitus Patients

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### Abstract

Diabetes mellitus is a chronic disease-which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycemia). Diabetes mellitus is one of the most challenging health problems in India. The present study was investigated for isolation and characterization of antibiotic-resistant bacteria from urinary tract infection on diabetic mellitus patients. The 118 diabetic urine samples were collected and UTI bacteria using HiChrome UTI Agar was isolated. The diabetic UTI isolates were confirmed as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus mirabilis* and *Klebsiella aerogenes* based on morphological and biochemical characteristics. Our study identified that almost all the bacteria were highly sensitive to Cefoperazone/Sulbactam (75/30 mcg), Gentamicin (10 mcg), Meropenem (30 mcg), Piperacillin/Tazobactam (100/10 mcg) and Nitrofurantoin (100 mcg) antibiotics. Interestingly *Klebsiella aerogenes* alone was found to be more resistant to the entire antibiotics used in this study. The antibiotic resistant *Klebsiella aerogenes* is one of the biggest treats to human health, antibiotic resistance occurs naturally, but misuse of antibiotics in humans is accelerating the process.

**Keywords:** Diabetes mellitus; Antibiotic resistant bacteria; Urinary tract infection.

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### 1. Introduction

Diabetes mellitus is one of the most challenging health problems of 21<sup>st</sup> century and is the fifth leading cause of death in developed countries [1]. It is one of the top ten causes of death in the world, due to its complications. One of the common complications of diabetes is its effect on genitourinary system [2]. Some surveillance studies have demonstrated the emergence of highly resistant *Klebsiella spp* [3], *Escherichia coli* [4] and *Pseudomonas aeruginosa* [5] in urinary tract infections in diabetic patients. Asian patients are considered to have a higher risk of developing diabetes and potentially worse

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prognosis. By 2025, the number of individuals with diabetes is expected to be more than double. Nowadays diabetes has become one of the most common problems in clinical field especially in clinical diagnosis. In the developing countries including India, the problem prevails more during the last two decades. The reason for the problem is overuse and misuse of antibiotics. Conjugation, process by which one bacterium transfers genetic material to another through direct contact is a convenient method of transferring drug resistant genetic determinants among intra and inter-generic bacterial populations. Diabetic patients have been tolerating infection poorly. Diabetes mellitus has an altered response to infection as these patients's inherent susceptibility to infection as well as the potential to mount a normal inflammatory response is weak [6]. The aim of this present study was isolation and characterization of antibiotic-resistant bacteria from diabetic mellitus patients with urinary tract infection.

## **2. Experimental**

### **2.1. Materials and methods**

The cross-sectional study was conducted in the Department of Microbiology at Specialty Lab & Research centre, Thanjavur from January 2018 to December 2018. All samples were proven diabetic with fasting glucose >130 mg/dL and postprandial (2 h) glucose >250 mg/dL. Control sample consisted of patients with comparable age and sex with no history of diabetes. All patients were instructed properly to collect clean-catch mid-stream urine in a sterile wide mouthed urine container and the samples were processed within one hour of collection. The collected specimens were inoculated on HiChrome UTI Agar (M1353, HiMedia, India) using a standard quantitative loop and incubated at 35-37 °C for 18-24 h [7]. The test isolates were subjected to morphological and biochemical characterization. Disc diffusion method was used to determine the antimicrobial susceptibility of isolates [8]. The antibiotics against which sensitivity was tested includes Amikacin (30 mcg), Amoxyclav (30 mcg), Ampicillin/Sulbactam (10/10 mcg), Cefixime (5 mcg), Cefoperazone/Sulbactam (75/30 mcg), Ciprofloxacin (5 mcg), Cefalexin (Cephalexin) (30 mcg), Ceftriaxone (30 mcg), Cefotaxime (Cephotaxime) (30 mcg), Cefuroxime (30 mcg), Ceftazidime (30 mcg), Gentamicin (10 mcg), Levofloxacin (5 mcg), Meropenem (30 mcg), Norfloxacin (10 mcg), Ofloxacin (5 mcg), Piperacillin/Tazobactam (100/10 mcg), Sparfloxacin (5 mcg), Azithromycin (30 mcg), Nitrofurantoin (100 mcg), Linezolid (30 mcg), Roxithromycin (30 mcg) and Co-Trimoxazole (Sulpha/Trimethoprim) (23.75/1.25 mcg). The isolated antibiotic resistant strains were further subjected to molecular characterization using 16S rRNA sequencing on an ABI 310 automatic DNA sequencer (Applied Biosystems) using the primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). The 16s rRNA sequence analysis was performed using the BLAST [9] at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) network services. After verifying and adjusting

the alignments manually the phylogenetic tree was constructed using the neighbor-joining method and maximum parsimony algorithms contained in the PHYLIP package [10].

### 3. Results and Discussion

Significant bacteriuria is defined as the presence of  $\geq 10^5$  Colony Forming Units (CFU) per unit volume (mL) of urine. A symptomatic urinary tract infection was defined as the presence of bacteriuria in a patient with fever, pelvic pain, increased urge to urinate, pain with urination and blood in the urine. Asymptomatic bacteriuria (ASB) was defined as bacteriuria without any symptoms. A total of 88 bacterial cultures were positive and 30 were negative of the 118 diabetic samples. Out of the 118 samples, male diabetic patients with UTI were on the upper hand compared to females (Fig. 1). The bacterial isolates were identified as *Escherichia coli* (36.4 %), *Klebsiella pneumoniae* (25 %), *Pseudomonas aeruginosa* (14.7 %), *Staphylococcus aureus* (11.4 %), *Staphylococcus saprophyticus* (7.9 %), *Proteus mirabilis* (3.5 %) and *Klebsiella aerogenes* (1.1 %) on the basis of morphology and biochemical reactions (Table 1). In some of the previous studies researchers have isolated intracellular bacteria from human bladder infection [11]. They isolated Gram positive organism such as *Staphylococcus aureus* (11.4 %), *Staphylococcus saprophyticus* (7.9 %), and the Gram-negative organisms such as *Escherichia coli* (36.4 %), *Klebsiella pneumoniae* (25 %), *Pseudomonas aeruginosa* (14.7 %), *Proteus mirabilis* (3.5 %) and *Klebsiella aerogenes* (1.1 %). These findings were similar with study of Savitha and Thangamariappan, [12] such as *Escherichia coli* (48.04 %), *klebsiella* (8.82 %), *P. aeruginosa* (0.98 %), *Proteus. spp* (4.9 %) and Gram positive organisms (37.26 %) (Table 2). Ciragil et al. [13] have noted that 20 to 30 % of UTI urine samples results in significant growth with main infective bacteria like *Escherichia coli* in both community and hospital acquired infections [14]. Urinary tract infections caused by *Klebsiella sp.* appear to be rising and have become a real health problem, especially in hospital settings [15].

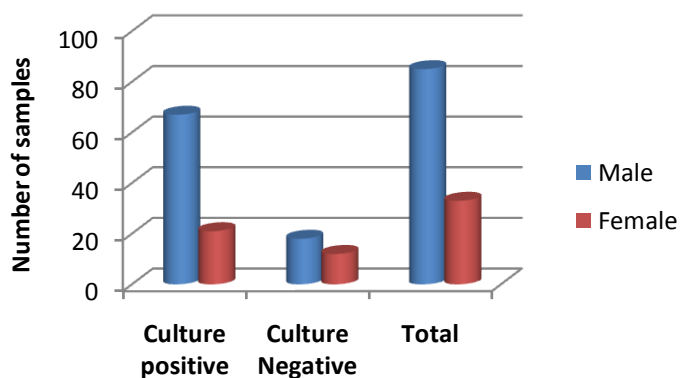


Fig. 1. Bacterial urine culture in urinary tract infection on diabetic mellitus patients.

Table 1. Bacterial isolates causing urinary tract infection on diabetic mellitus patients.

Bacterial isolates	No. of isolates			Percentage (%)
	Male	Female	Total (%)	
<i>Escherichia coli</i>	21	11	32	36.4
<i>Klebsiella pneumonia</i>	17	5	22	25
<i>Pseudomonas aeruginosa</i>	11	2	13	14.7
<i>Staphylococcus aureus</i>	8	2	10	11.4
<i>Staphylococcus saprophyticus</i>	6	1	7	7.9
<i>Proteus mirabilis</i>	3	-	3	3.5
<i>Klebsiella aerogenes</i>	1	-	1	1.1

Table 2. Antibiotic Susceptibility pattern of all the isolates.

Antibiotics	Zone of Inhibition (mm in diameter)							
	EC	KP	PA	SA	SS	PM	KA	
Amikacin (30 mcg)	S	S	S	S	R	S	R	
Ampicillin/Sulbactam (10/10 mcg)	S	I	R	S	S	S	R	
Amoxyclav (30 mcg)	S	R	R	S	S	S	R	
Cefixime (5 mcg)	R	R	S	R	R	R	R	
Cefoperazone/Sulbactam (75/30 mcg)	S	S	S	S	S	S	R	
Ciprofloxacin (5 mcg)	S	I	S	R	S	S	R	
Cefalexin (Cephalexin) (30 mcg)	S	I	R	R	S	S	R	
Ceftriaxone (30 mcg)	S	I	R	R	S	S	R	
Cefotaxime (Cephotaxime) (30 mcg)	R	I	R	R	S	S	R	
Cefuroxime (30 mcg)	S	I	S	R	S	S	R	
Ceftazidime (30 mcg)	S	I	S	R	S	S	R	
Gentamicin (10 mcg)	S	S	S	S	S	S	R	
Levofloxacin (5 mcg)	I	I	S	R	S	S	R	
Meropenem (30 mcg)	S	S	S	S	S	S	I	
Norfloxacin (10 mcg)	R	R	R	R	R	R	R	
Ofloxacin (5 mcg)	S	I	S	R	S	S	R	
Piperacillin/Tazobactam (100/10 mcg)	S	S	S	S	S	S	R	
Sparfloxacin (5 mcg)	S	I	S	R	S	S	R	
Azithromycin (30 mcg)	R	R	R	R	S	R	R	
Nitrofurantoin (100 mcg)	S	S	S	S	S	S	R	
Linezolid (30 mcg)	R	R	R	S	S	R	R	
Roxithromycin (30 mcg)	R	R	R	S	S	R	R	
Co-Trimoxazole (Sulpha/Trimethoprim) (23.75/1.25 mcg)	S	S	R	S	S	R	R	

S- Sensitive; I- Intermediate; R- Resistant

EC - *Escherichia coli*; KP - *Klebsiella pneumonia*; PA - *Pseudomonas aeruginosa*; SA - *Staphylococcus aureus*; SS - *Staphylococcus saprophyticus*; PM - *Proteus mirabilis*; KA -

A total of 88 isolated positive samples were analyzed for sensitivity by disk diffusion method separately. All bacterial isolates were more sensitive to Cefoperazone/sulbactam (75/30 mcg), Gentamicin (10 mcg), Meropenem (30 mcg), Piperacillin/Tazobactam (100/10 mcg) and Nitrofurantoin (100 mcg) antibiotics. However, *Klebsiella aerogenes* was more resistance to all antibiotics used in this study (Table 3). According to Sirot *et al.* [16], the Cephalosporins showed minimum zone of inhibition against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. The diabetic UTI isolated resistant bacterial strains were characterized and results are shown in Table 3.

Table 3. Morphological and Biochemical characterization of antibiotic resistant *Klebsiella aerogenes*.

Characteristics	<i>Klebsiella aerogenes</i>
Grams staining	-
Cell shape	Rod
Cell size (µm)	0.5-0.8
Cell diameter (µm)	1.5-3
Motility	-
Indole test	-
Methyl red test	-
Voges Proskaur test	+
Citrate utilization test	+
Triple Sugar Iron Test	A/G
Urease test	-
Starch	+
Lipase	-
Hydrogen sulphide production test	-
Catalase test	+
Oxidase test	-
Nitrate reduction test	+
Casein	-

+ - Positive; - - Negative; A – Acid; G - Gas

The obtained 16S rRNA sequence was subjected to BLAST. The antibiotic resistant bacteria alignment sequencing showed sequence similarities of about 99 % with *Klebsiella aerogenes*. The sequence has been deposited at GenBank Bethesda, MD, USA (Accession No. MN589745). After the alignment, the tree building option can be activated using Bioedit Software. The tree viewing software NJ plot was used to generate a cladogram for the bacterial isolates as shown in Fig. 2. In the light of this study, antibiotic resistance is a serious public health problem. It can be prevented by minimising unnecessary prescribing and over-prescribing of antibiotics, the correct use of prescribed antibiotics, and good hygiene and infection control.

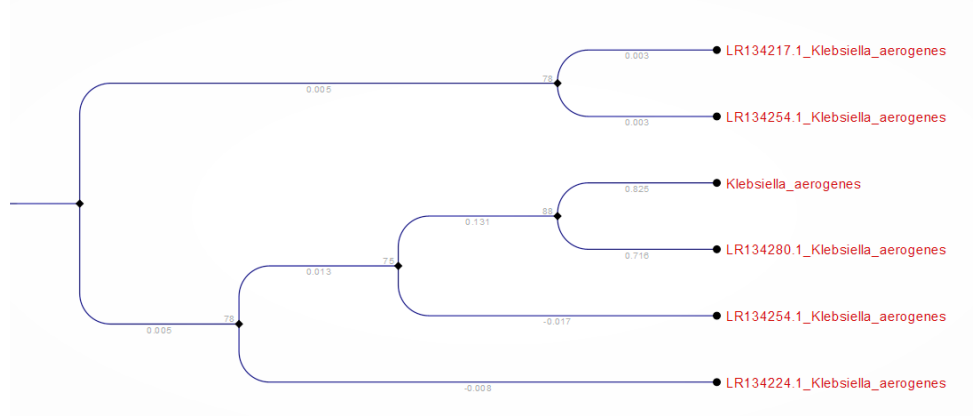


Fig. 2. Phylogenetic tree of isolated antibiotic resistant *Klebsiella aerogenes* strain MRIUTIRB2 based on partial 16S r RNA sequence (Accession No. MN589745).

#### 4. Conclusion

Antibiotic resistance is rising dangerously to high levels in all parts of the world. Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. The present study has isolated *Klebsiella aerogenes* as one of the most resistant species for all the used antibiotics. Several steps should be taken at all levels of the society to reduce the impact and limit the spread of resistance.

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