



## ISOLATION AND IDENTIFICATION OF BACTERIA FROM UTI PATIENT AND ANALYSIS OF MULTI-DRUG RESISTANT BACTERIA

### Microbiology

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### ABSTRACT

In the present study 200 urine samples were collected from UTI patients of various Hospital, Thanjavur, Tamil Nadu. The urine sample was stored on specific aseptic containers. From the samples to isolate bacterial species were isolated using UTI agar and MacConkey agar medium. The bacteria isolated were identified with the help of culture morphological and biochemical characteristics. The bacterial isolates were confirmed as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The sensitivity tests were performed to detect the sensitivity of organisms against bacterial antibiotics. In bacteria, the maximum inhibition was observed in antibiotics Meropenem and Nitrofurantoin. Antibacterial with anticipated effectiveness in patients with chronic renal insufficiency is selected Meropenem and Nitrofurantoin. Other antibiotics should not be used because of low antibacterial activity against all isolated bacteria. The *Staphylococcus aureus* have more resistance to all used antibiotics.

### KEYWORDS:

Antibacterial activity, urinary tract infections and multi-drug resistant bacteria

### INTRODUCTION

Presently, the attitude of people, especially those from urban areas, are suffering from deferent types of infections and towards the treatment they are admitting in the hospitals half of all hospitalized patients received antibiotic are estimated that 25 to 50% of antibiotics are in appropriate as a result incorrect choice in drugs dose or duration. Despite the wide spread availability of antibiotics, UTI remain the most common bacterial infections in human population (Sharmas, 1997). Keeping the above view in mind the present work has been carried out with the following objectives to isolate and identified the different types of bacteria from UTI patient and analysis of multi-drug resistant bacteria.

### MATERIALS AND METHODS

#### Sample Collection

The urine samples were collected from various Hospitals at Thanjavur. The collected specimens were stored on specific aseptic container, for further study.

#### Isolation and Identification of Bacteria

The specimens were inoculated on UTI agar, MacConkey agar and blood agar and incubated at 35-37°C for 18-24 Hrs (Cappuccino and Sherman, 1999). The test isolate were subjected to morphological, biochemical character (Hi25TM identification kit, Himedia Laboratories Pvt. Ltd., Mumbai, India).

#### Antibiotic Disc Used

The commercially available antibiotic disc such as Amikacin (AK), Amoxycylav (AMC), Ampicillin/Sulbactam (A/S), Cefixime (CFM), Cefoperazone (CFS), Ciprofloxacin (CIP), Cefalexin (Cephalexin) (CX), Cetriaxone (CTX), Cefotaxime (CN), Cefuroxime (CXM), Gentamicin (GEN), Levofloxacin (LE), Meropenem (MRP), Norfloxacin (NX), Ofloxacin (OF), Piperacillin (PIT), Sparfloxacin (SPX), Tigecycline (TGC), Azithromycin (AZM), Nitrofurantoin (NIT), Methicillin (MET), Cefazolin (CZ), Cefepime (CPM) and Ticarcillin (TCC) for bacterial culture. The antibiotic disc were purchased from High media chemical Pvt. Ltd, Mumbai.

#### Assay of Antibiotic Sensitivity

##### Antimicrobial Activity

Antimicrobial activity test was carried out following the modification of the method originally described by Bauer et al., (1996). The isolated bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* cultures were swabbed on the Muller-Hinton agar medium separately. After this swabbing different commercial antibiotic discs were placed on the each culture swabbed plate. The plates were incubated at 37°C for 24 hrs. After the incubation period zone of inhibition were noted. The

diameter of the zone of inhibition was measured.

#### Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like Mean ( $\bar{x}$ ) and Standard Deviation (SD) by Zar (1984).

### RESULTS

The urinary tract infections ( $\bar{x}$ ) ent urine specimens were collected aseptically by following protocols the urine total number collected was 200 and UTI positive in 144 samples other 56 samples are Negative (Table -1). With regards to the sex wise categories Male Total No. 45 and positive 226 Female Total No. 155 and positive 236 (63%) here female are more susceptible than the male to the overall age categories the data pertaining to the inference of difference level between the male and female and with different age categories.

From this urine specimen four different bacterial colonies were noted after the incubation. The isolated colonies were named as IUTB1, IUTB2, IUTB3, and IUTB4. The isolates were tentatively identified as *Escherichia Coli* Total No. 71 (36%), *Staphylococcus aureus* 29 (15%), *Klebsiella pneumoniae* 25 (13%) and *Pseudomonas arruginosa* 19 (10 %) on the basis of morphology ad biochemical reactions (Table-2).

Among the study all the bacterial isolates were more sensitive to Meropenem and Nitrofurantoin compared the other antibiotics. At the same time the maximum inhibition (22±2.41; 22±1.72 mm in diameter) was noted Cetriaxone against *Escherichia coli* and *Klebsiella pneumoniae* compared to other organisms. The *Staphylococcus aureus* have more resistance to all used antibiotics (Table-3).

### DISCUSSION

The microbial communities in the rectum and vagina, it is likely that multiple strains of bacteria are simultaneously introduced into the urinary tract of woman during the events that lead to a urinary tract infection, such as sexual intercourse. Intracellular bacteria have been found in human bladder infection (Hooten et al., 1996). In the present study the pathogenic bacteria are isolated such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Similar work was done by Gokal et al., (1982). They isolated Gram positive organism such as *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus faecalis*, *Bacillus*, *Coryne bacterium* sp. and the Gram negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella ozaenae*, *Citrobacter* sp. *Serratia marcescens* and *Acinetabacter calcoaceticus*.

In the present study *Escherichia coli* isolated was about 36 % of UTI patients and it's followed the *Klebsiella pneumoniae* about 15 % of patients. *Pseudomonas aeruginosa* was isolated 13 % from UTI and *Staphylococcus aureus* was isolated from 10 % of patients. Verbrugh et al., (1984) demonstrated the coagulase negative *Staphylococci* was the most frequent isolated organism from UTI patients. In this present study the second major organisms was *Klebsiella pneumoniae* is about 15% of the urine samples.

The antibiotic susceptibility pattern of microorganisms isolated from infected urine sample was studied which showed that the most effective antibiotics against bacteria was Meropenem and Nitrofurantoin. According to Sirot et al. (1987), the Cephalosporins showed minimum zone of inhibition against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*.

Meropenem and Nitrofurantoin was the most effective agent tested in these studies of UTI and demonstrated the greatest reduction in mean bacterial numbers. In the light of this study, I will be able to know that which organisms are most frequently responsible for chronic kidney infection and to which antibiotic they are usually sensitive. This will help the clinicians to select the proper antibiotic for the management of chronic kidney infection. Similar type of sensitive pattern has been observed by Sharma (1997).

### CONCLUSION

In bacteria, the maximum inhibition was observed in antibiotics Meropenem and Nitrofurantoin. Antibacterial with anticipated effectiveness in patients with chronic renal insufficiency is selected Meropenem and Nitrofurantoin. Other antibiotics should not be used because of low antibacterial activity against all isolated bacteria. The *Staphylococcus aureus* have more resistance to all used antibiotics.

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**Table – 1 Isolation of Bacteria**

S. No.	Bacterial Isolates	Observation	Percentage (%)	Identified Bacteria
1	IUTB1	71	36	<i>Escherichia coli</i>
2	IUTB2	29	15	<i>Staphylococcus aureus</i>
3	IUTB3	25	13	<i>Klebsiella pneumoniae</i>
4	IUTB4	19	10	<i>Pseudomonas aeruginosa</i>
5	No growth	56	28	-

**Table – 2 Identification of Isolated Bacteria**

S. No.	Morphological and Biochemical Characterization	Isolated Bacterial Colony			
		IUTB1	IUTB2	IUTB3	IUTB4
1.	Gram staining	-	-	-	+
2.	Shape	Rod	Rod	Rod	Coccus
3.	Motility Test	+	-	+	-
4.	Indole Test	+	-	-	-
5.	Methyl Red Test	+	±	-	+
6.	Voges Proskauer Test	-	+	-	+
7.	Citrate Utilization Test	-	+	+	±
8.	Catalase Test	-	-	+	-
9.	Coagulase Test	+	+	-	+
10.	Oxidase Test	+	+	+	+
11.	Ureas Hydrolysis Test	+	-	-	+
12.	Triple Sugar Iron Test	AG	AG	-	AG

“+” – Positive,

“-” – Negative,

“AG” – Acid/Gas

“A” – Acid

**IUTB1 – *Escherichia coli***

**IUTB2 – *Staphylococcus aureus***

**IUTB3 – *Klebsiella pneumoniae***

**IUTB4 – *Pseudomonas aeruginosa***

**Table – 3 Assay of Antibacterial Activity**

S. No.	Antibiotics	Zone of Inhibition (mm in diameter)			
		IUTB1	IUTB2	IUTB3	IUTB4
1.	Amikacin	-	-	18±1.30	-
2.	Amoxycylav	-	-	-	-
3.	Ampicillin/Sulbactam	10±1.20	13±1.24	-	-
4.	Cefixime	10±1.42	17±1.30	-	-
5.	Cefoperazone	15±1.23	18±1.74	14±1.31	-
6.	Ciprofloxacin	-	-	-	-
7.	Cefalexin (Cephalexin)	-	-	-	-
8.	Cetrixone	22±2.41	22±1.72	-	-
9.	Cefotaxime	18±1.55	20±0.98	-	-
10.	Cefuroxime	10±1.27	13±1.79	-	-
11.	Gentamicin	-	10±1.26	13±0.62	-
12.	Levofloxacin	-	-	12±1.20	08±1.30
13.	Meropenem	10±1.43	16±1.40	16±1.41	10±1.21
14.	Norfloxacin	-	-	-	-
15.	Ofloxacin	-	-	14±1.07	10±1.47
16.	Piperacillin	17±1.54	19±1.76	08±1.40	-
17.	Sparfloxacin	-	-	14±1.21	-
18.	Tigecycline	-	18±1.40	18±1.30	16±1.34
19.	Azithromycin	-	13±1.67	-	-
20.	Nitrofurantoin	13±0.75	13±1.38	17±1.25	16±1.66
21.	Methicillin	-	-	-	-
22.	Cefazolin	-	-	-	-
23.	Cefepime	19±1.20	21±1.24	10±0.29	-
24.	Ticarcillin	16±1.32	-	-	-

Values are expressed in Mean ± Standard deviation, n=3

**IUTB1 – *Escherichia coli***

**IUTB2 – *Staphylococcus aureus***

**IUTB3 – *Klebsiella pneumoniae***

**IUTB4 – *Pseudomonas aeruginosa***

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