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## ISOLATION AND IDENTIFICATION OF BACTERIA FROM PERSONS WITH ASYMPTOMATIC URINARY TRACT INFECTION AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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### ABSTRACT

Urinary tract infection is one of the most common disease in both male and female of all age groups. Both gram positive and gram negative bacterial pathogens are responsible for UTI. In this study, 25 Urethral samples were collected. Among that, 34 pathogens were isolated. The isolates were identified and confirmed by biochemical characterisation tests and culturing method. Totally seven types of bacterial pathogens were isolated namely *E.coli*, *P.aeuruginosa*, *K.pneumonia*, *P.vulgaris*, *P.mirabilis*, *S.aureus* and *S.saprophyticus*. The most common pathogens affected by the individuals were *Klebsiella* and *E.coli*. These pathogens were treated with different antibiotics and the findings were recorded.

### KEYWORDS

UTI infection, *E.coli*, *P.aeuruginosa*, *K.pneumonia*, *P.vulgaris*, *P.mirabilis*, *S.aureus* and *S.saprophyticus*.

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### INTRODUCTION

Normally urine may contains variety of fluids, salts and waste products but bacteria not present. Urinary tract infection (UTI) is an infection in any part of urinary system-kidneys, uterus, bladder and urethra. The lower urinary tract, bladder and the urethra mostly affected. Urinary tract infection is more common in women. They usually occur in the bladder or urethra but more serious infections involves the kidney<sup>1</sup>. The term Urinary tract infection encompasses a broad range of clinical entities that associated with common findings a  
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positive urine culture<sup>2</sup>. A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and or need to urinate, pain during urination, and cloudy urine<sup>3</sup>.

UTI may caused by bacteria includes cystitis, pyelonephritis, asymptomatic bacteriuria and acute urethral syndrome constitute one of the most causes of illness in humans. *Escherichia coli* mainly causing Urinary tract infection<sup>4</sup>.

*Escherichia coli*, *Staphylococcus sp.*, and *Bacillus sp.*, was the predominant bacterial flora of human urine sample. *E.coli* is the most prevalent pathogen in the sample when compared to *Staphylococcus sp.*, and *Bacillus sp.*<sup>5</sup>.

Asymptomatic bacteriuria (ABU or ASB) is synonymous with asymptomatic UTI and isolates the number of bacteria from the samples of person without symptoms of UTI<sup>6,2</sup>.

Asymptomatic bacteriuria is common in pregnant women with asymptomatic UTI are at an increased risk for adverse outcomes and these can be prevented with antimicrobial treatment of asymptomatic bacteriuria. Asymptomatic bacteriuria is a microbiological diagnosis carried out with urine specimen<sup>6</sup>.

While most microbes are killed by urine, different organisms have distinct bacteruric potentials. Several aspects of urine and antibiotics can stop the growth and progression of bacteriuria<sup>7</sup>. But the patient infected with *E.coli* experience recolonization with the same or similar organisms at high rates<sup>8</sup>.

## MATERIAL AND METHODS

### Sample collection

The patients were asked to clean their external genital part with disinfectant and midstream urine were collected in sterile container.

### Physical examination

Various parameters of collected urine sample were analyzed like pH, color and appearance.

### Microscopic examination

Each urine samples were centrifuged and the sediments were collected. The sediments were observed under microscope by simple wet mount method.

## Cultural characterization

The Urine samples were inoculated into Nutrient Agar and incubated overnight at 37°C. Different colonies were observed. They were further confirmed by inoculating the cultures in Selective media. Different culture media such as Nutrient agar, Mac Conkey agar, EMB, MSA and Blood agar were used for the identification of Colony morphology. Colonies were screened based on color, size, elevation, margins and texture. The morphologically different colonies were observed and sub cultured onto Nutrient Agar.

The isolated colonies were examined microscopically based on their cell wall composition and presence of capsule.

### Biochemical Characterization tests

The biochemical characteristics of the isolated pathogens were identified for the further confirmation of the organism. Media used were Peptone water, MR-VP medium, Simmon citrate agar, TSI and Urea broth. All the above media were autoclaved at 121°C for 15 min. These were inoculated with the isolated pathogens and incubated at 37°C for 24 to 48 hrs.

### Antimicrobial sensitivity testing (Kirby-Bauer method)

The susceptibility of isolates to antibiotics were demonstrated by using five specific antibiotics (Gentamycin, Amikacin, Ampicillin, Chloramphenicol and Streptomycin). Isolates were inoculated in peptone water and incubated in 37°C, 18-24 h. The broth cultures were swabbed onto the Mueller-Hinton agar plate. After drying for about 5-10 min, selected antibiotic discs were placed on the cultured plates using sterile forceps and incubated for 24 hours at 37°C.

## RESULTS AND DISCUSSION

Totally 25 samples were collected from the persons without the symptoms of urinary tract infection in Nallampalli Taluk, Dharmapuri District. The age groups of them were between 25 and 35. The physical properties of the collected samples were examined and the morphology of the cells were identified by microscopy. The abnormal RBC cells and epithelial cells and crystals were detected and the results were shown in Table No.1.

**Microscopic identification**

Gram staining, capsule staining and motility tests were performed for the identification of microscopic morphology of the isolates. Among the isolates, two gram positive cocci and five gram negative bacilli were identified. *K.pneumonia* produces capsule and all other isolates were non-capsulated.

**Culture in various media**

Later, the samples were cultured and 34 cultures were isolated. All the colony characteristics were recorded. The colonies were further identified by plating on various selective media<sup>9</sup>. The various types of media were prepared for the cultivation and characterization of specific isolates and the results were given in the Table No.2.

The isolates were inoculated in various selective media like Mac Conkey agar, Blood agar, EMB agar and Mannitol Salt Agar for the identification of cultural characteristics. The results were shown in Table No.2.

EMB agar and Mannitol Salt Agar were respectively used for the confirmation of *E.coli* and *S.aureus* present in the urine sample. Mac Conkey agar were used for the confirmation of lactose fermented and non lactose fermented bacteria and Blood agar used to find out the ability of hemolytic activity of bacteria.

Several biochemical characterization tests were carried out for the further confirmation of bacteria, such as IMVIC test, TSI test, catalase and oxidase tests. In catalase test, the isolates were positive and in oxidase test all are negative except *P.aeruginosa*. The biochemical analysis and the results were reported in the Table No.3.

Among 25 samples, 34 cultures were isolated. Those are enlisted in the Table No.4. Priyadharsini *et al*, 2014 reported that, *Escherichia coli*, *Klebsiella pneumonia* are common causative agents of urinary tract infection<sup>10</sup>.

**Antimicrobial sensitivity testing (Kirby-Bauer method)**

After the proper incubation period the ABST plates were observed to find out antimicrobial activity of selected antibiotics. Based on the zone of inhibition (mm), it has been identified that both streptomycin and ampicillin do not control *Pseudomonas sp.*, *Klebsiella sp.*, *Proteus sp.*, and all other antibiotic have the ability to control the pathogens well. The results were listed in the Table No.5.

**Table No.1: Physical properties of the samples**

S.No	Gender	Age	pH	Color	Appearance
1	MALE	30	Acidic	Yellow	Clear
2	FEMALE	28	Acidic	Red	Turbid
3	FEMALE	30	Acidic	Yellow	Clear
4	MALE	29	Acidic	Pale Yellow	Clear
5	MALE	25	Acidic	Yellow	Clear
6	MALE	26	Acidic	Yellow	Clear
7	MALE	26	Acidic	Yellow	Clear
8	FEMALE	31	Acidic	Red	Turbid
9	FEMALE	30	Acidic	Pale Yellow	Clear
10	MALE	33	Acidic	Yellow	Clear
11	FEMALE	28	Acidic	Yellow	Clear
12	MALE	35	Acidic	Red	Turbid
13	FEMALE	25	Acidic	Yellow	Clear
14	MALE	26	Acidic	Yellow	Clear
15	FEMALE	33	Acidic	Yellow	Clear

16	FEMALE	34	Acidic	Yellow	Clear
17	MALE	25	Acidic	Yellow	Clear
18	MALE	26	Acidic	Yellow	Clear
19	MALE	25	Acidic	Pale Yellow	Turbid
20	MALE	26	Acidic	Pale Yellow	Clear
21	FEMALE	30	Acidic	Pale Yellow	Clear
22	FEMALE	26	Acidic	Red	Clear
23	MALE	26	Acidic	Yellow	Turbid
24	MALE	28	Acidic	Pale Yellow	Clear
25	FEMALE	29	Acidic	Yellow	Clear

**Table No.2: Cultural characteristics of the isolates**

S.No	NA	MA	MSA	BA	EMB
<i>E.coli</i>	Small white colonies	Pink color lactose fermented colonies	Small colorless colonies	$\beta$ -hemolytic colonies	Malachite green colored colonies
<i>P.aeruginosa</i>	Pigment produced in plate	Media color changed by growth of bacteria	-	Non-hemolytic colonies	Dark color formed in media
<i>K.pneumonia</i>	Mucoid colonies	Pink color lactose fermented colonies	-	$\beta$ -hemolytic colonies	Dark pink colored colonies
<i>P.vulgaris</i>	Creamy color colonies with odor	Non-lactose fermented colonies	Very small colonies	Non-hemolytic colonies	Dark color colonies
<i>P.mirabilis</i>	Creamy color colonies with odor and swarming motility appear	Non-lactose fermented colonies	Very small colonies	Non-hemolytic colonies	Dark color colonies
<i>S.aureus</i>	Yellow color colonies	Lactose fermented pink color colonies	Golden Yellow color colonies	$\beta$ -hemolytic colonies	Dark color colonies
<i>S.saprophyticus</i>	Yellow color colonies	Lactose fermented pink color colonies	Yellow color colonies	$\beta$ -hemolytic colonies	Dark color colonies

**Table No.3: Biochemical characteristics of the isolated bacteria**

S.No	Isolates	Gram stain	Capsule stain	Indole	MR	VP	Citrate	TSI	Motility	Catalase	Oxidase
1	<i>E.coli</i>	-/Rod	-	+	+	-	-	AG/AG	+	+	-
2	<i>P.aeuruginosa</i>	-/Rod	-	-	-	-	+	AK/A,	+	+	+
3	<i>K.pneumonia</i>	-/Rod	+	-	-	-	+	A/A,G	-	+	-
4	<i>P.vulgaris</i>	-/Rod	-	+	+	-	+	AK/A,G, H <sub>2</sub> S	+	+	-
5	<i>P.mirabilis</i>	-/Rod	-	+	+	-	-	AK/A, G, H <sub>2</sub> S	+	+	-
6	<i>S.aureus</i>	+/Cocci	-	-	-	+	+	A/A,	-	+	-
7	<i>S.saprophyticus</i>	+/Cocci	-	-	-	+	-	A/A, H <sub>2</sub> S	-	+	-

**Table No.4: Number of isolates**

S.No	Bacteria	Isolates
1	<i>E.coli</i>	9
2	<i>P.aeuruginosa</i>	3
3	<i>K.pneumonia</i>	9
4	<i>P.vulgaris</i>	4
5	<i>P.mirabilis</i>	2
6	<i>S.aureus</i>	5
7	<i>S.saprophyticus</i>	2

**Table No.5: Antibiotic sensitivity test**

S.No	Bacteria	Streptomycin	Amikacin	Gentamycin	Chloramphenicol	Ampicillin
1	<i>E.coli</i>	12	16	23	24	17
2	<i>P.aeuruginosa</i>	-	12	11	12	-
3	<i>K.pneumonia</i>	-	12	23	13	-
4	<i>P.vulgaris</i>	-	17	09	20	-
5	<i>P.mirabilis</i>	-	12	21	15	09
6	<i>S.aureus</i>	14	14	19	24	22
7	<i>S.saprophyticus</i>	14	11	16	26	24

## CONCLUSION

A total of 25 samples were collected for the analysis of pathogens in urine. On physical analysis, all samples were in acidic pH level, clear in appearance except few samples. On the other hand, samples few samples were yellow and pale yellow in color, four samples were red in color. Totally 34 bacteria were isolated from the sample. The isolates were inoculated in the various media for the identification and biochemical analysis were undergone for the identification of bacteria. The results shows that most of the people were affected by *E.coli* (26%) and *Klebsiella sp.*, (26%). Amikacin, Gentamycin and Chloramphenicol controls the pathogens isolated from Urine compared to other antibiotics.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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