



**ORIGINAL RESEARCH PAPER**

**Medical Microbiology**

**ISOLATION AND IDENTIFICATION OF BACTERIA FROM SYMPTOMATIC URINARY TRACT INFECTION**

**KEY WORDS:** Asymptomatic, UTI, Children, *E.coli*, antibiotics

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**ABSTRACT** Symptomatic and asymptomatic types of urinary tract infection caused by various microorganisms in different kinds of age groups. This study carry out with symptomatic UTI samples and those samples were collected from 10 years old both male and female. Most of the children shows symptoms like pain or irritation during urination. Collected samples were used to microbiological studies and few bacteria were isolated like *E.coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Streptococcus-B*. Some antibiotics were used to identification of antimicrobial sensitivity test against of isolates.

**INDRODUCTION:**

The urinary tract infection is an infection caused by various microbes. Most of the time urinary tract infections are caused by bacteria sometime fungi and rarely caused by viruses. Kidneys, ureters, bladder, and urethra are linked together and called as urinary tract. The urethra and bladder mostly affected with urinary tract infection (UTI) in the lower tract, ureters and kidneys also affected in the upper tract. The upper tract UTIs are rarely affected than lower tract UTIs. Between 65% and 90% of urinary tract infections in children are caused by *E.coli*. Other pathogens include *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterococcus*.

Types of urinary tract infection: An infection can happen in different parts of your urinary tract. Cystitis (bladder) - need to pee a lot or it may hurt when urination. It is also produce lower belly pain and bloody or cloudy urine. Pyelonephritis (kidneys)-This can cause fever, chills, nausea, vomiting, and pain in your upper back or side. Urethritis (urethra)-This can cause a discharge and burning when urination.

Symptomatic bacteriuria is bacteriuria with the accompanying symptoms of a urinary tract infection such as painful urination, fever, back pain and includes pyelonephritis or cystitis. The most common cause of urinary tract infections is *E.coli*.

In asymptomatic bacteriuria, large numbers of bacteria are present in the urine. The person do not have any symptoms of a urinary tract infection (without any symptoms). There is no clear clarifications why the bacteria don't cause any symptoms in the affected person. The asymptomatic bacteriuria may caused by less virulent bacteria. This condition of infection does not need to be treated.

There are number of conditions are cause painful urination (dysuria). In women, urinary tract infections are a common cause of painful urination. In men, urethritis and certain prostate conditions are frequent causes of painful urination. Pyelonephritis is inflammation that results from a urinary tract infection.

**MATERIALS AND METHODS**

**Sample collection:**

Urine samples were collected from Dharmapuri district surrounding area and it takes nearby three months. Totally 200 samples were collected from symptomatic children. The urine samples were labelled and immediately sent to the laboratory for microbiological analysis (1) The samples are analyze in various aspects like pH, colour and odour and direct microscopic examination (5).

**Culturing:**

Urine samples were inoculated into nutrient agar and incubated. After the proper incubation period the Colonies are selected based on size, shape, margin, texture and inoculated into selective media for the confirmation of bacterial colony morphology. Isolated colonies were examined microscopically for the identification of capsule on bacterial cell wall and motility.

Isolated bacteria were confirmed by biochemical analysis. The biochemical analysis were carried by the application of Peptone water, MR-VP medium, Simmon citrate agar, TSI and Urea broth. The media inoculated with isolated bacteria and incubated at 18-24 hours in 37°C.

**Antimicrobial sensitivity test**

Isolated bacteria are inoculated into peptone water for Antimicrobial sensitivity test. The broth cultures are swabbed into surface of the Muller Hinton Agar plates. After drying the selected antibiotic disc were place on inoculated plates.

The antibiotics used for treatment of urinary tract infections are selected for disc sensitivity method. Antibiotics name as: Ampicillin, Gentamycin, Tetracycline, Chloromphenicol, Piperacillin, Rifampicin, Amoxyclav (AC), Tobramycin, Cefixime and Streptomycin (10).

**RESULTS AND DISCUSSION:**

The collected samples were inoculated into the various media for the macroscopic identification. In direct microscopic observation, crystals and blood cells appear in maximum number of sample. Totally 200 sample were collected from female 108 (54%) and male 92 (46%). The samples are appear in three different colours pale yellow 89 (44.5%), yellow 78 (39%) and deep yellow in 33 (16.5%) samples. The odour divided into two types namely aromatic and ammonical. 127 (63.5) samples are in ammonical and 73 (36.5) samples are in aromatic. 163 (81.5%) samples in acidic condition and 37 (18.5%) in alkaline nature of pH.

**Table2: Isolated pathogens in urine sample**

S. NO	Isolates	Patient code
1	<i>Escherichia coli</i>	1,2,3,4,11,16,17,22,30,31,32,33,48,49,50,72,73,74,75,76,77,92,93,103,107,108,109,110,115,116,118,133,134,140,141,143,147,148,154,162,163,172,173,175,176,186,192,193,197,198

2	<i>Klebsiella pneumonia</i>	5,6,12,18,27,36,37,41,42,47,62,71,82,91,94,95,111,116,117,131,132,142,155,168,174,
3	<i>Staphylococcus saprophyticus</i>	7,13,14,15,28,34,35,55,61,63,69,70,81,83,102,118,120,156,157,158,160,161,170,176,177,178,188,191,194,
4	<i>Pseudomonas aeruginosa</i>	8,19,26,29,38,43,51,54,80,85,90,101,112,113,119,121,122,139,144,145,153,171,179,185,187,
5	<i>Streptococcus-B</i>	9,10,20,21,39,46,53,60,78,84,89,98,99,100,114,123,124,125,127,135,137,138,146,150,181,199
6	<i>Enterococcus faecalis</i>	21,24,25,45,56,57,64,65,79,86,96,104,105,126,128,130,149,159,164,167,180,182,189,
7	<i>Staphylococcus aureus</i>	22,23,40,44,46,52,58,59,61,66,67,68,73,87,88,97,103,106,129,136,149,151,152,165,166,169,183,184,190,195,196,200

There are 200 samples were used to isolate the bacteria. Only one bacteria isolated from most of the sample and two different kinds of bacteria were isolate from seven samples (21,22,46,73,103 and etc).

**Table3: Isolated Pathogens In Different Media**

Media	Pathogens	Colony morphology
Nutrient agar	<i>Escherichia coli</i>	Large circular,white,moist colonies
	<i>Klebsiella pneumonia</i>	Circular,small mucoid colonies
	<i>Pseudomonas aeruginosa</i>	Circle,green colour pigment
	<i>Staphylococcus aureus</i>	Round, golden yellow colonies
	<i>Staphylococcus saprophyticus</i>	White to pink circular colonies
	<i>Enterococcus faecalis</i>	Circular,small opaque white colonies
	<i>Streptococcus-B</i>	Circular with entire margin colonies
Eosin methylene blue agar	<i>Escheria coli</i>	Green metallic sheen colonies are formed
	<i>Klebsiella pnemoniae</i>	Pink to purple colonies
	<i>Pseuomonas aerugionosa</i>	Purple colonies
Mannitol salt agar	<i>Staphylococcus aureus</i>	Ferment mannitol,yellow colour colonies
	<i>Staphylococcus saprophyticus</i>	Ferment mannitol,yellow halo around colonies
Macconkey agar	<i>Escheria coli</i>	Colonies appear flat and pink.circular,smooth,entire colonies
	<i>Klebsiella pneumonia</i>	Pink colour and mucoid colonies
	<i>Pseudomonas aeruginosa</i>	Blue-green pigment
	<i>Staphylococcus aureus</i>	Pale pink colonies
	<i>Enterococcus faecalis</i>	Colourless,white colonies
Blood agar	<i>Escherichia coli</i>	Circular,gray and moist,beta(β)haemolytic colonies are formed
	<i>Klebsiella pneumonia</i>	Grey colour,haemolysis

	<i>Pseudomonas aeruginosa</i>	Grey colonies,βhaemolysi
	<i>Staphylococcus aureus</i>	White,creamy,βhaemolysis
	<i>Staphylococcus saprophyticus</i>	White colonies,no haemolysis
	<i>Enterococcus faecalis</i>	Beta(β)haemolysis
	<i>Streptococcus-B</i>	Beta(β)haemolysis,whitish-gray coloniesSS

**Table4: Identification of microbes using biochemical tests**

Biochemical tests	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus saprophyticus</i>	<i>Enterococcus faecalis</i>	<i>Streptococcus-B</i>
Gram staining	-	-	-	+	+	+	+
Motility	Rod	Rod	Rod	Cocci	Cocci	Cocci	Cocci
Indole	+	-	-	-	-	-	-
MR	+	-	-	+	+	-	+
VP	-	+	-	+	+	+	±
Citrate	-	+	+	-	-	-	-
TSI	A	A/A,G	K/K	A/A	A/A	A/A	A/K
Nitrate	+	+	+	+	-	+	-
Urease	-	+	-	-	+	-	-
Catalase	+	+	+	+	+	-	-
Oxidase	-	-	+	-	-	-	-
Starch hydrolysis	-	-	+	-	-	-	±
Casein hydrolysis	+	-	+	-	-	+	±
Lactose	+	+	-	+	-	+	+

**Table5: Antimicrobial sensitivity test**

Antibiotics	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>S.aureus</i>	<i>S.saprophyticus</i>	<i>E.faecalis</i>	<i>Streptococcus-B</i>
Ampicillin	+	-	+	+	-	+	+
Gentamycin	+	+	+	+	+	+	-
Chloromphenicol	+	+	+	+	+	+	-
Tetracycline	-	+	+	+	-	+	-
Penicillin	-	-	-	-	-	-	+
Rifambicin	-	-	+	+	-	-	-
Steptomycin	+	-	-	+	-	-	-
Ciprofloxacin	+	+	+	+	-	+	-

Sensitive (+),resistance (-)

**Table6: Zone of inhibition for Kirby-baurer method**

Antibiotics	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>S.aureus</i>	<i>S.saprophyticus</i>	<i>E.faecalis</i>	<i>Streptococcus-B</i>
Ampicillin	20 mm	10 mm	12 mm	22 mm	23 mm	14 mm	18 mm
Gentamycin	18 mm	17 mm	16 mm	18 mm	17 mm	18 mm	17 mm
Chloromphenicol	19 mm	21 mm	17 mm	20 mm	19 mm	16 mm	12 mm
Tetracycline	13 mm	17 mm	19 mm	18 mm	15 mm	17 mm	14 mm
Penicillin	14 mm	15 mm	14 mm	15 mm	16 mm	13 mm	17 mm
Rifambicin	09 mm	11 mm	16 mm	17 mm	12 mm	13 mm	09 mm
Steptomycin	18 mm	13 mm	13 mm	18 mm	10 mm	14 mm	11 mm
Ciprofloxacin	19 mm	15 mm	19 mm	16 mm	10 mm	18 mm	12 mm

Urinary tract infections are among the most common bacterial infections. The most common bacteria found to cause urinary tract infections is *E. coli*. UTI are very common in both male and female children. Some kids have a problem with their bladder or kidneys that make some symptoms like UTI. In this study, 200 samples were collected and processed for isolation and identification of bacteria. Nutrient agar, Macconkey agar, Mannitol salt agar, EMB agar and blood agar were used to identification of culture characters of isolates. The results were interpreted in the table 3.

*E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Streptococcus-B* were isolated from collected sample in various numbers. Initial achievement to identify the isolated bacteria on the basis of colony appearance. *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* are produced  $\beta$ -haemolysis on blood agar. Karzan Mohammed (2017), collected urine samples around 25 to 35 years old and isolated organisms like *E. coli* and *S. aureus*. He reported that *E. coli* was present in the maximum number sample. John Cherulyot (2013) reported that *E. coli* was highest isolated organism from the urine samples and more over it was 80-90%.

Ezeadila (2015) collected sample from 30 to 35 years females and *E. coli* was maximum number of isolate from the whole study. He recorded that Ciprofloxacin was produce maximum level of zone of inhibition against of gram positive and negative bacteria. Nida wasmy shahab (2017) says that, 4 days to 10years age group people are highly affected by *E. coli* and Female children will affected more than male children. According to many researchers report, *E. coli* will cause highest rate of urinary tract infections and followed by *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas*.

Various antibiotics were used to identification of antimicrobial sensitivity against of isolates. reported on table5. Gentamycin, chloramphenicol, ampicillin and ciprofloxacin are more effective against of *E. coli*. Gentamycin, Chloromphenicol and Tetracycline producing zone of inhibition against of maximum number of isolated organisms. The results were interpreted in table 5 and 6.

**CONCLUSION:**

Seven isolates of bacteria were identified from the two hundred samples. This investigation conducted with around ten years old. Mostly gram negative bacteria were isolated from the collected sample. Particularly *E. coli* causing UTI in most of the peoples. The female children highly affected by urinary tract infection than male children. There are so many antibiotics used to treat urinary tract infection and it very necessary to control infection it also support to prevent and reduce of kidney diseases.

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