



ISOLATION, CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY TESTING OF ENTERIC BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS

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ABSTRACT

Urinary tract infections (UTIs) are the most common type of infections caused by bacteria. The aim of the present study was to investigate the type of bacterial pathogens present in UTIs and to check their antibiotic sensitivity. In the present study, a total of 60 urine samples were taken, Out of which 8 were of males including 1 of child and 52 were of females. The urine samples were cultured on MacConkey agar and incubated, which resulted into the formation of Lactose Fermenting/Non Lactose Fermenting (LF/NLF) type of colonies. After morphological characterization, different urinary tract pathogens were identified by biochemical tests and inoculated on selective media for final confirmation. Out of 8 samples of male, 6 were positive and out of 52 female samples 25 were positive. It is found that the age group most affected was of 21-40 and the organism most commonly encountered was *E.coli*. The six antibiotics were used to test the antibiotic susceptibility pattern of isolated strains. Maximum isolates were resistant to Ceftazidime & Amikacins.

KEYWORDS: Urinary tract pathogens, Antibiotic susceptibility, Lactose fermenting and non-lactose fermenting.



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INTRODUCTION

Most UTIs result from infection by a single, gram-negative, enteric bacterium, such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas* or *Serratia*. Uropathogenic *E.coli* is responsible for approximately 85% of community acquired infections, besides *Proteus*, *Klebsiella* and *Pseudomonas*¹. On the basis of the work done by Kass, 10⁵ Colony Forming Units (CFU) of a single species per mL in a clean catch midstream sample of urine is considered as significant bacteriuria^{2,3}. Infections of the urinary tract due to microorganisms affect patients of all age groups and both sexes, and they vary in severity from an unsuspected infection to a condition of severe systemic disease. Urine is ordinarily an excellent culture medium for the multiplication of common pathogens of urinary tract. Skin is the major factor responsible for an increased bacterial load in urine. The urine from non-infected or normal persons may be sterile or contain bacteria up to 10³ cfu per ml and when the bacterial count exceeds 10⁵ cfu per ml of urine it indicates significant bacteriuria and is indicative of a urinary tract infection⁴. Urinary tract infections (UTIs) may be referred to as cystitis or pyelonephritis, terms that refer to the lower and upper urinary tract, respectively. The bacteriuria and candiuria describe bacteria or yeast in the urine. UTIs can also lead to urosepsis^{5,6}. Several factors increase the risk of UTI in pregnancy. These factors include relative obstruction of the ureters (secondary to the enlarging uterus), smooth muscle relaxation of the ureter and bladder (secondary to progesterone), and amnoaciduria and glycosuria, which provide a favourable environment for bacteria to grow^{7,8}. Sexual intercourse contributes to increased risk, as does use of a diaphragm and/or spermicide. Women who are elderly, pregnant or have pre-existing urinary tract structural abnormalities or obstruction carry a higher risk of UTI^{9,10}. UTI in women are very common approximately 25-40% of women in the United States aged 20-40 years have had a UTI^{11,12}. A diagnosis of a urinary tract

infection can easily be missed or delayed in the elderly. This is because some symptoms, such as fatigue and weakness, may not be noticed or might be associated with aging¹³. Urinary Tract Infection, commonly known as UTI, affects as many as 50% women at least once during their lifetime and 25% of those who acquire UTI, will have recurrent infection within the following six months.

MATERIALS & METHODS

Collection of samples

Mid stream urine samples were collected aseptically from district Solan and cultured on different media like Nutrient agar, MacConkey agar etc. and incubated for 24 hrs at 37⁰C. Identification of isolated microorganisms was done by Gram staining.

Biochemical Identification

Following biochemical tests namely Catalase, Indole, MR, VP, Citrate, Urease, Nitrate reduction, Motility and carbohydrate reduction tests like Glucose, Sucrose and Lactose were carried out for the identification of bacteria¹⁴.

ANTIMICROBIAL SUSCEPTILITY

The susceptibility of the isolated organisms against different antibiotics was checked by disk diffusion method on Mueller Hinton agar. The antibiotics which are commonly prescribed by doctors in UTI's were selected for the test. The antibiotics used were ceftazidime, norfloxacin, ciprofloxacin, nitrofurantoin, amikacin, and gentamycin¹⁵. Inoculum was prepared by using isolated bacterial colonies. The well isolated bacterial colonies were inoculated in the nutrient broth. After that an incubation was given at 37⁰C till turbidity reached equal to 0.5 Mc Farland barium sulphate standard solution^{16&17}. The degree of inhibition shown by different antibiotics was measured by using a transparent plastic ruler and were compared with the ranges published by the manufacturer, Hi-media.

RESULTS

Urinary Tract Infection, commonly known as UTI, affects as many as 50% women, at least once during their lifetime. All individuals are susceptible to urinary Tract Infection (UTI); however the prevalence of infection differs with age, sex and certain predisposing factors. In the present study, a total of 60 samples were taken from people of different age groups. Out of which 29 were sterile, and 31 were pathogenic as shown in table no. 5, from these pathogenic samples *E.coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Pseudomonas*, *Staphylococcus*, *Proteus*, *Micrococcus*, *Streptococcus* and *Bacillus* were isolated. Present study reveals that *E.coli* is the most common uropathogen accounting for 53% of UTIs. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Pseudomonas*, *Staphylococcus*, *Proteus*, *Micrococcus*, *Streptococcus* and *Bacillus* are responsible

for most of the rest. From the present study it can be concluded that nearly 80% of UTIs are caused by gram negative bacilli as shown in table no. 1 which mostly includes the members of Enterobacteriaceae family. A few samples also contain gram positive bacteria. This type of study is very important in the field of Microbiology in order to isolate and identify the root cause of urinary tract infections. Out of 8 samples of male, 6 were found to be positive and out of 52 samples of females 25 were found to be positive. Major causative agents isolated were *E.coli*, Non Lactose Fermenting group & *Enterobacter*, *Klebsiella*, *Staphylococcus*. Maximum isolates were found to be resistant to these antibiotics i.e. Ceftazidime & Amikacins (Table 6). It is found that in females and males the age group affected was of 21-40 as shown in table no. 5 and the organism most commonly encountered was one of the most common uropathogen i.e. *E.coli*.

Table 1
Colony morphology, Gram Staining & Microscopic appearance of positive samples

Sr. No.	Sample No.	Colony Morphology on MacConkey Agar	Colony Morphology on Nutrient Agar	Gram Staining
	A1	LFC	Slimy, white, somewhat translucent, raised growth	Gram-ve bacilli
	A2	LFC	Abundant, thick, white glistening	Gram-ve bacilli
	A3	LFC	Colonies are relatively large	Gram-ve bacilli
	A4	NLFC	Abundant, thin, white medium turns green	Gram-ve bacilli
	A5	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	A6	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	A7	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	A8	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	A9	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	A10	LFC	Colonies are large (2-4 mm	Gram+ve Cocci

		diameter), circular, convex, smooth, shiny, opaque & easily emulsifiable.	
A11	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A12	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A13	LFC	Slimy, white, somewhat translucent, raised growth	Gram -ve bacilli
A16	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A17	LFC	Abundant, thick, white glistening	Gram-ve bacilli
A18	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A20	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A26	LFC	Colonies are 0.5- 1mm in diameter	Gram+ve Cocci
	NLFC	Irregular round colonies, 2- 3 mm in diameter.	Gram+ve bacilli
A29	LFC	Colonies are large (2-4 mm diameter), circular, convex, smooth, shiny, opaque & easily emulsifiable.	Gram+ve Cocci
A31	LFC	Colonies are large (2-4 mm diameter), circular, convex, smooth, shiny, opaque & easily emulsifiable.	Gram+ve Cocci
A33	LFC	Slimy, white, somewhat translucent, raised growth.	Gram-ve bacilli
A37	LFC	Colonies are large (2-4 mm diameter), circular, convex, smooth, shiny, opaque & easily emulsifiable.	Gram+ve Cocci
A40	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	NLFC	Swarming occurs and specific 'fishy' or 'seminal' odour.	Gram-ve bacilli
A42	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
	NLFC	Soft, smooth, yellow growth	Gram+ve Cocci
A46	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli

A49	LFC	Abundant, thick, white glistening	Gram-ve bacilli
A51	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A53	NLFC	Swarming occurs and specific 'fishy' or 'seminal' odour.	Gram-ve bacilli
A56	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A58	LFC	Large, thick, greyish white, smooth opaque or partially translucent disc like colonies.	Gram-ve bacilli
A59	LFC	White, moist, glistening colonies.	Gram-ve bacilli

LFC- Lactose Fermenting Colonies

NLFC- Non Lactose Fermenting Colonies

Table 2
Table showing biochemical results of sample no. A1-A9

Sr.No	Sample No.	Catalase	Indole	MR	VP	Citrate	Glucose	Sucrose	Lactose	Urease	Nitrate	Motility	Results
1	A1	+	-	-	+	+	AG	AG	AG	+	+	-	<i>Klebsiella pneumoniae</i>
2	A2	+	-	-	+	+	AG	AG	AG	-	+	+	<i>Enterobacter aerogenes</i>
3	A3	+	+	+	-	+	AG	-	AG	-	+	+	<i>Citrobacter koseri</i>
4	A4	+	-	-	-	+	-	-	-	-	+	+	<i>Pseudomonas aeruginosa</i>
5	A5	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
6	A6	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
7	A7	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
8	A8	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
9	A9	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>

Table 3
Table showing biochemical results of sample no. A10- A33

Sr.No.	Sample No.	Catalase	Indole	MR	VP	Citrate	Glucose	Sucrose	Lactose	Urease	Nitrate	Motility	Results
10	A10	+	-	+	+	-	A	A	A	-	+	-	<i>Staphylococcus aureus</i>
11	A11	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
12	A12	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
13	A13	+	-	-	-	+	AG	AG	AG	+	+	-	<i>Klebsiella pneumonia</i>
14	A16	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
15	A17	+	-	-	+	+	AG	AG	AG	-	+	+	<i>Enterobacter aerogenes</i>
16	A18	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
17	A20	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
18	A26	-	-	+	-	-	A	A	A	-	-	-	<i>Streptococcus lactis</i>
		-	-	-	+	-	A	A	-	-	+	+	<i>Bacillus cereus</i>
19	A29	+	-	+	+	-	A	A	A	-	+	-	<i>Staphylococcus aureus</i>
20	A31	+	-	+	+	-	A	A	A	-	+	-	<i>Staphylococcus aureus</i>
21	A33	+	-	-	+	+	AG	AG	AG	+	+	-	<i>Klebsiella pneumonia</i>

Table 4
Table showing biochemical results of sample No. A37-A60

Sr.No.	Sample No.	Catalase	Indole	MR	VP	Citrate	Glucose	Sucrose	Lactose	Urease	Nitrate	Motility	Results
22	A37	+	-	+	+	-	A	A	A	-	+	-	<i>Staphylococcus aureus</i>
23	A40	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
		+	+	+	-	-	AG	AG	-	+	+	+	<i>Proteus vulgaris</i>
24	A42	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
		-	-	-	-	+	-	-	-	+	+	+	<i>Micrococcus luteus</i>
25	A46	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
26	A49	+	-	-	+	+	AG	AG	AG	-	+	+	<i>Enterobacter aerogenes</i>
27	A51	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
28	A53	+	+	+	-	-	AG	AG	-	+	+	+	<i>Proteus vulgaris</i>
29	A56	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
30	A58	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>
31	A59	+	+	+	-	-	AG	A	AG	-	+	+	<i>E. coli</i>

Table 5
Table showing different types of bacteria isolated from different urine samples

Sr. No.	Sample No.	Age	Gender	Name of the Bacteria isolated
1.	A1	72 yrs	Female	<i>Klebsiella pneumoniae</i>
2.	A2	52yrs	Male	<i>Enterobacter aerogenes</i>
3.	A3	29yrs	Female	<i>Citrobacter koseri</i>
4.	A4	33yrs	Female	<i>Pseudomonas aeruginosa</i>
5.	A5	60yrs	Male	<i>E.coli</i>
6.	A6	45yrs	Female	<i>E.coli</i>
7.	A7	28yrs	Male	<i>E. coli</i>
8.	A8	48yrs	Male	<i>E.coli</i>
9.	A9	47yrs	Female	<i>E. coli</i>
10.	A10	9yrs	Child(Male)	<i>Staphylococcus aureus</i>
11.	A11	25yrs	Female	<i>E.coli</i>
12.	A12	55yrs	Female	<i>E.coli</i>
13.	A13	27yrs	Female	<i>Klebsiella pneumoniae</i>
14.	A14	20yrs	Female	Sterile
15.	A15	42yrs	Female	Sterile
16.	A16	36yrs	Female	<i>E.coli</i>
17.	A17	20yrs	Female	<i>Enterobacter aerogenes</i>
18.	A18	27yrs	Female	<i>E.coli</i>
19.	A19	66yrs	Female	Sterile
20.	A20	65yrs	Male	<i>E.coli</i>
21.	A21	49yrs	Male	Sterile
22.	A22	32yrs	Female	Sterile
23.	A23	24yrs	Female	Sterile

24.	A24	43yrs	Female	Sterile
25.	A25	60yrs	Male	Sterile
26.	A26	22yrs	Female	<i>Streptococcus lactis</i> & <i>Bacillus cereus</i>
27.	A27	22yrs	Female	Sterile
28.	A28	23yrs	Female	Sterile
29.	A29	22yrs	Female	<i>Staphylococcus aureus</i>
30.	A30	22yrs	Female	Sterile
31.	A31	22yrs	Female	<i>Staphylococcus aureus</i>
32.	A32	22yrs	Female	Sterile
33.	A33	22yrs	Female	<i>Klebsiella pneumoniae</i>
34.	A34	23yrs	Female	Sterile
35.	A35	23yrs	Female	Sterile
36.	A36	22yrs	Female	Sterile
37.	A37	21yrs	Female	<i>Staphylococcus aureus</i>
38.	A38	21yrs	Female	Sterile
39.	A39	18yrs	Female	Sterile
40.	A40	22yrs	Female	<i>E. coli</i> & <i>Proteus vulgaris</i>
41.	A41	22yrs	Female	Sterile
42.	A42	21yrs	Female	<i>E. coli</i> & <i>Micrococcus luteus</i>
43.	A43	20yrs	Female	Sterile
44.	A44	24yrs	Female	Sterile
45.	A45	21yrs	Female	Sterile
46.	A46	22yrs	Female	<i>E. coli</i>
47.	A47	22yrs	Female	Sterile
48.	A48	22yrs	Female	Sterile
49.	A49	21yrs	Female	<i>Enterobacter aerogenes</i>
50.	A50	22yrs	Female	Sterile
51.	A51	22yrs	Female	<i>E. coli</i>
52.	A52	22yrs	Female	Sterile
53.	A53	22yrs	Female	<i>Proteus vulgaris</i>
54.	A54	22yrs	Female	Sterile
55.	A55	23yrs	Female	Sterile
56.	A56	23yrs	Female	<i>E. coli</i>
57.	A57	25yrs	Female	Sterile
58.	A58	24yrs	Female	<i>E. coli</i>
59.	A59	20yrs	Female	<i>E. coli</i>
60.	A60	20yrs	Female	Sterile

Table 6

Table showing the results of antibiotic sensitivity tests of isolated microorganisms

Sr.No.	Sample no.	Ca Ceftazidime	NF Norfloxacin	CF Ciprofloxacin	Nx Nitrofurantoin	Ak Amikacin	G Gentamycin
1	A1	R	S	S	S	R	MS
2	A2	S	S	S	S		
3	A3	R	S	S	S	S	MS
4	A4	S	S	S	S		
5	A5	MS	S	R	S	R	MS
6	A6	R	S	S	S	S	S
7	A7	R	S	S	S	S	S
8	A8	R	S	S	S	S	S
9	A9	R	S	S	S	S	S
10	A10	R	S	S	S		
11	A11	MS	S	S	S	R	MS
12	A12	MS	S	S	S	R	MS
13	A13	MS	S	S	S	R	MS
14	A14	MS	S	S	S	R	MS
15	A15	MS	S	S	S	R	MS
16	A16	MS	S	S	S	S	S
17	A17	S	S	S	S		
18	A18	R	S	S	S	S	S
19	A19	S	S	S	S		
20	A20	R	S	S	S	S	S
21	A21	R	S	S	S	S	S
		MS	S	S	S		
22	A22	MS	S	S	S	S	S

23	A23	S	S	S	S	S	S
		MS	S	S	S		
		MS	S	S	S		
24	A25	S	S	S	S		
25	A31	MS	S	S	S		
26	A36	S	S	S	S		
27	A37	S	S	MS	S	S	S
28	A40	MS	S	R	S	S	MS
		S	S	MS	S	S	
29	A42	S	R	MS	MS	S	S
		MS	S	S	S		S
30	A46	MS	S	S	MS	S	
31	A49	S	MS	S	S	S	S
32	A51	MS	S	MS	S	S	
33	A53	S	MS	S	S	S	
34	A55	S	S	S	S	S	S
35	A56	MS	R	S	S	S	S
36	A58	MS	S	S	S	S	S
37	A59	MS	S	R	S	S	S
38	A60	S	S	MS	S	S	S

S: sensitive, MS: Moderately sensitive, R: Resistance.

DISCUSSION

According to this study, *E.coli* is responsible for 53% of UTIs and *Staphylococcus aureus* is responsible for 12% of UTIs, this data also resembles with the study done by Nicolle *et al* in 2008, according to their study *E.coli* is the cause of 80-85% of UTIs with *Staphylococcus aureus* being the cause of 5-10% UTIs¹⁷. *E. coli* is found most commonly in UTIs, it is also the most prevalent type in the normal gut flora, and this can be one of the reasons of its presence in urinary samples. The bacteria causing urinary tract infections enter the bladder via the urethra. Infection may also occur via the blood or lymph. The bacteria are usually transmitted to the urethra from the bowel; females are at greater risk due to their anatomy. After gaining entry to the bladder, *E. coli* get attached to the bladder wall and form a biofilm that resists the body's immune response.¹⁸ Except *E.coli* and *Staphylococcus aureus* other bacterial causes of UTI are *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter*, this data is also supported by Salvatore *et al* in 2011. According to

Salvatore *et al* in 2011 most frequently affected age group with UTI is 16-35 years and in this study also the age group most affected is 21-40¹⁸. According to the study of Shaikh *et al* in 2005, the resistance pattern of *E. coli* towards ciprofloxacin and norfloxacin is 17% and 15.30% respectively & in this study also the resistance pattern of *E. coli* towards ciprofloxacin and norfloxacin is 17.64% and 11.76% which is approximately similar.¹⁹

CONCLUSION

On the basis of present study undertaken on UTI it has been found that *E. coli* is the major group of microorganisms found in females and males after which comes *Enterococcus*, *Klebsiella*, *Proteus*, *Pseudomonas* etc. From the present study it can be concluded that nearly 80% of UTIs are caused by gram negative bacilli which mostly includes the members of enterobacteriaceae family.

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