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Prevalence, Etiology and Identification of Urinary Tract Infection among Students at Residence Halls in Jashore University of Science and Technology, Bangladesh

M. Hossain , Dr. Selina Akter

Department of Microbiology, Jashore University of Science and Technology, Jashore 7408, Bangladesh

Corresponding author: M. Hossain

Abstract: Urinary tract infection (UTI) is one of the most common domiciliary and nosocomial bacterial infections prevalent in both males and females. UTI is diagnosed on the basis of clinical symptoms, microscopy and quantative culture of urine. In order to assess the prevalence of UTI among students in residence halls of Jashore University of Science and Technology, volunteers were randomly selected and requested to supply freshly void midstream urine samples in sterile container. For the presumptive evaluation of UTI, microscopic observation was performed to count pus cells, red blood cells (RBC) ,white blood cells , platelet cell and epithelial cells of centrifuges urine samples. Presences of bacterial cells were also observed followed by Gram staining. It has been observed that RBC and epithelial cell counts were not sensitive enough to be used for presumptive diagnosis of UTI rather than pus cell counts and Gram stained smear was sufficient to detect bacteriuria. Semi-quantitative culture of urine was performed in addition for confirmation. Escherichia coli was the most predominant microorganism observed. All of the isolates were resistant to vancomycin though Polymixin B as well as ciprofloxacin were found effective antibiotic against the isolates.

Keywords: Urinary tract infection, Escherichia coli, Colony forming unit (CFU) Pus cells, Red blood cells (RBC), White blood cells (WBC), Too Numerous to Count (TNTC), Epithelial cells, Bangladesh

I. Introduction

Urinary tract infection (UTI) is the second commonest bacterial infection after respiratory diseases, prevalent in both males and females. The incidence of urinary tract infection is greatly influenced by age, sex and factors which impair the defense mechanisms that maintain the sterility of the urinary tract. Many predisposing factors have been described for the development of UTI including anatomical, pathological, infective, social and environmental factors (Leigh, 1990). Although UTI differs considerably in pathogenesis, natural history and management, it can be generally stated as a spectrum of diseases involving microbial invasion of any of the urinary tissues extending from the renal cortex to the urethral meatus (Singh, 1991). Urine secreted from kidneys is sterile unless any of the organs are infected. During infection, bacteria undergo multiplication in urine within the urinary tract causing a condition called bacteriuria (Leigh, 1990). Bacteriuria may lead to the infection of the male reproductive system, so the infection of the prostate, epididymes or testes are also included in the definition of UTI (Fowler, 1983). Even though the prevalence of urinary infections may vary in different patient populations, approximately 80% of urine cultures are negative (Kolbeck et al., 1985 and Wu et al., 1985). In an attempt to reduce the cost and time expended in examining these negative cultures, several rapid methods have been developed for characterizing bacteriuria, including microscopic examination, chemical tests, and automated systems (Clarridge et al., 1987).

Among these non-cultural techniques, white blood cell (WBC) , RBC count and Gram stain have been proposed as sensitive and inexpensive methods (Baron and Finegold, 1994; Clarridge et al.,1987; Pezzlo, 1988; Pezzlo, 1990 and

Pollock, 1983). Cultural method, semi-quantitative urine culture with 10000-10^5 colony forming units/ml (CFU/ml) remains the standard diagnostic method to diagnose UTI (Mahon et al., 2007). The main causal agent *is Escherichia coli*. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it (Adult Health Advisor 2005). When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI. The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Although they cause discomfort, urinary tract infections can usually be easily treated with a short course of antibiotics with no significant difference between the classes of antibiotics commonly used (Zalmanovici Trestioreanu A, Green H et al: 2010). The most common organism implicated in UTIs (80–85%) is *E. coli*, (Nicolle LE, 2008) while Staphylococcus saprophyticus is the cause in 5–10%. The bladder wall, in common with most epithelia is coated with a variety of cationic antimicrobial peptides such as the defensins and cathelicidin which disrupt the integrity of bacterial cell walls. (Ali AS, Townes CL et al:, 2009).During cystitis, Uropathogenic *Escherichia coli* (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs) (Justice S, Hunstad D et al., 2006).Theodor Escherich first described *E. coli* in 1885, as Bacterium coli commune, isolated from the feces of newborns. It was later renamed Escherichia coli, and for many years the bacterium was considered as a commensal microorganism of the large intestine, provides benefit to their host by producing vitamin K_2 and giving a barrier against the attachment to community practice (Stamm WE and Hooton TM, 1993; Warren JW et al., 1999). It occurs when a significant number of microorganisms ($\geq 10^5$ cells/ml) present in urine from catheter specimen (Williams DN, 1996). Uropathogenic E. coli (UPEC) is responsible for approximately 90% of urinary tract infections (Todar K, 2007). UPEC utilizes P fimbrine to bind specifically to the P blood group antigen which contains a D-galactose-Dgalactose residue. Binding of this P fimbriae not only specific to red blood cell but to a specific galactose disaccharide that is found on the surface of uroepithelial cells in approximately 99% of the population. UTI may associate both upper and lower tract. Lower tract UTI describes as cystitis. The major symptoms of cystitis are the urgency of urination, dysuria, irritation of urinary tract and tiredness. Most of the cases UTI occur as community acquired infection (Akram M et al. 2007).Antibiotics are the main treatment for all UTIs. A variety of antibiotics are available, and choices depend on many factors, including whether the infection is complicated or uncomplicated or primary or recurrent. For example, if a woman has symptoms, even if bacterial count is low or normal, infection is probably present, and the doctor should consider antibiotic treatment. The following are measures that studies suggest may reduce the incidence of urinary tract infections. A prolonged course (six months to a year) of low-dose antibiotics (usually nitrofurantoin or TMP/SMX) is effective in reducing the frequency of UTIs in those with recurrent UTIs (Nicolle LE ,2008).

Abuse and improper prescribing policy of antibiotics causes remarkable increase of antibiotic resistance pattern among the *E. coli* isolates from UTI (Li Q et al., 2007). These types of resistance associated with genetic mutation and intra or inter species transfer of resistance gene through plasmid (Hughes M and Datta N, 1983). Microorganisms considered multidrug resistance (MDR) when it was resistance to at least three antibiotics (Santo E et al. , 2007). Frequency of UTI cases caused by multidrug resistance *E. coli* required strong concern of medical practitioners and health agencies. Therefore regional studies on pattern of antibiotic sensitivity are very much necessary to overcome this problem. Considering the majority of UTI cases caused by *E. coli* and increasing use of antibiotics followed by growing resistance in bacteria and emerging MDR strains, the present study was conducted to identify the UPEC and also investigate the drug resistance pattern of those *E. coli* strains collected samples from jessore university of science and technology. This will be useful other pathogenic bacteria in the intestine (Bentley R and Meganathan R, 1982; Hudault S, 2001; Reid G et al., 2001).

To assess the usefulness of Gram stain as a urine screening test in the microbiology laboratory to eliminate culture negative specimens, we have analyzed one hundred and twenty three urine samples obtained from male and female persons in Jashore University of science and technology. Between them eighty one are male sample and forty three are female samples. When we compared the results of Gram stain of centrifuged urine to the results of culture, we found that Gram stain is a reliable and sensitive procedure with high sensitivity and specificity for the initial screening of urinary tract infection. In addition, observation of >5 pus cells per high power field added a subsequent value for the preliminary diagnosis. Evaluation of suspected UTI includes history, physical examination and

laboratory investigation. Urine analysis for presence of pus cells, bacteria and culture are important in the adequate management of UTIs.

II. Materials and Methods

Study period: This study was performed from 10th February , 2015 to 30th July ,2015 (within 6 months period) in the Microbiology laboratory at Jashore university of science and technology, Jashore . Routine urine test, microscopy, culture on bacteriological media, isolation and identification of uropathogens and conventional antimicrobial susceptibility testing were carried out during this period.

Sampling site: Urine samples are collected from male and female persons in Shaheed Mashiur Rahman & Sheikh Hasina Hall at Jashore university of science and technology, Jashore.

Sample collection: Total 123 urine samples submitted to the microbiology laboratory of Jashore university of science and technology by suspected persons in Shaheed Mashiur Rahman & Sheikh Hasina Hall were collected by following standard procedure as per the guideline mentioned by Isenberg (1998) and WHO Manuals (1980) and examined microscopically for epithelial cells, leukocytes, erythrocytes and microorganisms in both unstained and Gram-stained centrifuged urine.

Sample processing: Urine samples were processed immediately, but in cases of delay they were refrigerated at 4^0C until processing.

Preparation of urine sediment: This is the first step in microscopic analysis. The importance of standardisation of technique and quality assurance cannot be overstressed to ensure accurate and reproducible analysis. Important steps include centrifugation, resuspension of sediment, slide preparation, and microscopic examination. In brief, 10 ml of urine in centrifuged at approximately 2,000 rpm (1,500-2,500rpm) for 5 minutes. The supernatant 9 ml is discarded and sediment is resuspended in 1 ml. A drop of this is pipetted onto a slide and a coverslip placed. The slide is then examined with or without staining. The urine sediment is routinely examined by bright field microscopy under both low and high power without staining. (Wargotz E.S, J.E Hyde and D.S. Karcher (1987).

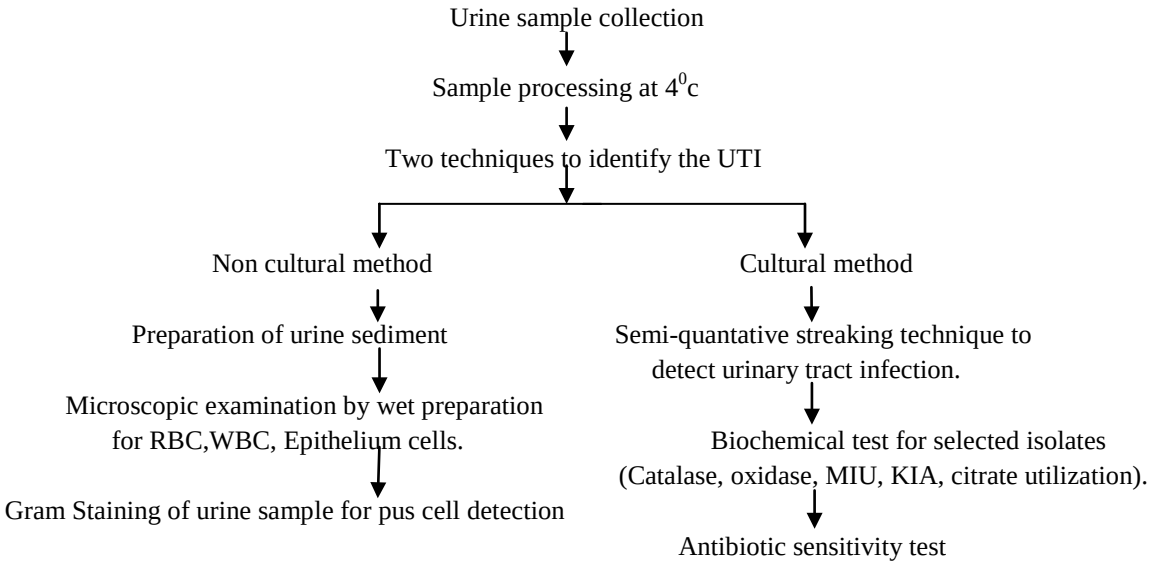

Figure: The design of the experiments of this study

Gram staining of urine sample: Gram staining is of the most common staining procedure developed by Danish Bacteriologist Hans Christian Gram. The procedure is, a drop of urine is applied to glass microscope slide, allowed to air dry. Then it is stained with primary stain gentian violet. After 1 minute it is drained off and washed away. Then the smear is covered with Grams Iodine, a mordant. Then it is washed and treated with acetone, decolorizing agent and rinsed off. Then counter stain usually 5% dilute carbol fuschin is added and washed off after 2-3 minutes. The smear is washed again, air dried and examined microscopically (Collee et al., 1989). A positive Gram stain was defined as the presence of >15 bacteria uniformly distributed per OIF after observation of at least 20 fields, according to the criteria described by Washington et al.(1981) and Cardoso et al. (1998) .

Microscopic examination by wet preparation: Two loopfuls of homogenized urine sediment was placed on a clean dry glass slide which was then covered by a coverslip and observed on 40X dry objective. An average count of WBC, RBC or epithelial cells was taken per high power field (HPF) out of 123 fields examined.
(Washington J.A., C.M. White M., Laganiere and L.H. Smith , 1981).

Culture of urine specimens (Semi-quantitative streaking method): Culture of each uncentrifuged urine specimen was done semi- quantitatively on MacConkey Agar (Oxoid, Unipath Ltd., Basingstoke, England) plates. An inoculating loop of standard dimension was used to take up approximately fixed and a known volume (0.01 ml) of mixed uncentrifuged urine. After incubating the plates aerobically at 37^0C for 24 h, colonies were counted. Cultures were interpreted as positive, negative, non-significant or mixed for UTI according to the standard criteria endorsed by the American Society for Microbiology based on the colony count, the urinalysis findings and patient-specific clinical data as provided on the request slip (Isenberg, 1993). Identification of significant isolates was done by using standard microbiological techniques as described which involves the morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases (Murray et al., 1995; Baron et al., 1994; Collee et al., 1989 and Cheesbrough, 1984).

MUG (beta-Glucuronidase) test for E.coli conformation : At first 2ml mug media are prepaired in the test tube and 500 microlitre urine is added. Incubate the tube at 35-37 °C for 24 hours. The development of a yellow colour in the supernatant indicates a positive test for *E. coli* without uv light. Expected results of MUG Test: Under uv light :- Positive result: Electric blue fluorescence and Negative result: Lack of blue fluorescence. (Tille, P. M., & Forbes, B. A.,2014).

Confirmation of selected strains: Bacterial isolates were identified up to genus level through light microscopic examination and biochemical studies. Biochemical studies included catalase test , Citrate utilization test, Indole test, Methyl red reaction, Oxidase test, KIA test , Motility test ,Urease test , Gram staining (Microbiology laboratory manual, 7^{th} edition, Cappuccino Sherman,2009) .

Antibiotic sensitivity test: Antibiotic sensitivity testing is an in vitro method for estimating the activity of drugs against an infecting microorganism in vivo. The degree of sensitivity or resistivity of the isolated pathogens to an appropriate range of antibiotics was determined by the Kirby-Bauer method i.e. disc diffusion method as described (Collee et al.,1989). The Kirby-Bauer method is based on the observation that the degree of inhibition of bacterial growth on agar medium surrounding an antimicrobial-containing disc correlates with susceptibility to the agent. Paper discs impregnated with standardized amounts of an antimicrobial agent and specifically certified for sensitivity testing were used. Briefly, four to five similar colonies of identified organism from pure culture plates were transferred into 5 ml nutrient broth and incubated at 37^oC for 4 h. With a sterile swab the inoculum was spread on the entire surface of the dried Mueller-Hinton agar plate (Oxoid, Unipath Ltd., Basingstoke, England). The paper discs of selected antibiotics (Ciprofloxacin, Azithromycin, Erythromycin, Streptomycin, Cefaclor, Ceptriaxone, Polymyxin-B, Chloramphenicol, Nalidixic acid, Vancomycin) were gently pressed onto the organism-carpeted plate at a distance of 15 mm away from the edge and 24 mm apart from each other.After incubation at 37^oC for 24 h the diameter of the zone of bacterial growth inhibition around each disc was measured and the susceptibility or resistance to the agent in each disc was determined according to the standardized table provided by the

manufacturer. The test was carried out according to the method described by the National Committee on Clinical Laboratory Standards (NCCLS) (1999) guidelines.

III. Results

All the urine samples analyzed were collected by spontaneous urination (clean catch midstream). The majority of the samples were collected from students of Shaheed Mashiur Rahman & Sheikh Hasina Hall in the Jashore university of science & technology, Jashore . About 65% (81) of the samples were received from males and the remainder 34% (42) from female persons. The ages of the persons ranged from 21 to 25 years with a mean age of 23 years.

I analyzed the culture result and *E.coli* is conformed by the mug test and there was no found the symptomatic persons but they were carried the bacterial populations. 1 to 81 male urine samples were analyzed. But total 7 male and female samples were positive by the semi quantative streaking method . Others were negative culture. The percentage was 6.17% positive culture for male . I considered the rang of colony between 10000-100000 cfu/ml was called the infected person by the semi- quantitative streaking technique. Total 22 isolales were identified by the semi- quantitative urine culture and mug test. Total 5 samples were positive for male by the urine culture method . Only one male urine samples carried more than 10000 colony cfu/ml. So the infection percent was 1.23%.

Forty Two female samples were analyzed in the microbiology laboratory, among which 82-123 were from female students. Two samples were positive by the semi- quantitative urine culture carried more than 10^4 cfu/ml. The 4.76% was positive culture and infection percentage was of 4.76%. And another 3 samples were positive for female person by the mug test method. I also analyzed the physical appearance of the urine and its results are shown in the Table III (a) and III (b).

Table III (a). :- Physical and microscopic examination of urine samples collected from male volunteers.

Sample number	Urine color	Opacity	p^H	RBC	WBC	Epitheliu m cell	Platelet	Pus cell
1	straw	low turbid	6	-	-	-	-	-
2	straw	low turbid	6	-	-	-	-	-
3	opaque	transparent	6	-	-	-	-	-
4	straw	low turbid	6	-	-	-	-	-
5	yellow	turbid	6	-	-	-	-	-
6	yellow	turbid	6	-	-	-	-	-
7	opaque	transparent	6	-	-	-	-	-
8	straw	low turbid	5	-	-	-	-	-
9	opaque	transparent	7	-	-	-	-	-
10	straw	low turbid	6	-	-	-	-	-
11	opaque	transparent	7	-	-	-	-	-
12	yellow	turbid	6	-	-	-	-	-
13	opaque	transparent	7	-	-	-	-	-
14	yellow	turbid	6	-	-	-	-	-
15	opaque	transparent	7	-	-	-	-	-
16	opaque	transparent	8	-	-	-	-	-
17	opaque	transparent	8	-	-	-	-	-

Sample number	Urine color	Opacity	pH	RBC	WBC	Epithelium cell	Platelet	Pus cell
18	straw	low turbid	7	-	-	-	-	-
19	straw	low turbid	6	-	-	-	-	-
20	straw	low turbid	6	-	-	-	-	-
21	straw	low turbid	7	-	-	-	-	-
22	straw	low turbid	6	-	-	-	-	-
23	straw	low turbid	6	-	-	-	-	-
24	opaque	transparent	7	-	-	-	-	-
25	straw	low turbid	7	-	-	-	-	-
26	yellow	turbid	5	-	-	-	-	2
27	straw	low turbid	6	-	-	-	-	3
28	straw	low turbid	7	-	-	-	-	-
29	opaque	transparent	7	2	-	-	-	2
30	straw	low turbid	7	3	-	-	-	2
31	yellow	turbid	5	-	-	-	-	TNTC
32	straw	low turbid	7	-	-	-	-	-
33	opaque	transparent	7	2-3	-	-	-	3
34	straw	low turbid	8	-	-	-	-	-
35	opaque	transparent	5	-	-	-	-	1-5
36	yellow	turbid	5	-	-	-	-	TNTC
37	straw	low turbid	7	3	-	-	-	3
38	Yellow	Turbid	6	-	-	-	-	6-7
39	Straw	Low turbid	5	2-3	-	1		1
40	Yellow	Turbid	5	-	-	-	-	-
42	Straw	Low turbid	6	2	-	-	-	-
43	Opaque	Transparent	5	3	-	-	-	TNTC
44	Yellow	Low turbid	7	-	-	-	-	-
45	Opaque	Transparent	6	-	-	-	-	-
46	Yellow	Turbid	7	-	-	-	-	-
47	Opaque	Transparent	7	-	-	-	-	5-7
48	Opaque	Transparent	7	-	-	-	-	-
49	Straw	Low turbid	6	-	-	-	-	1-2
50	Opaque	Transparent	7	-	-	-	-	3
51	Opaque	Transparent	7	-	-	-	-	2-3
52	Opaque	Transparent	7	-	-	-	-	2-3
53	Opaque	Transparent	7	-	-	-	-	2
54	Straw	Low turbid	7	-	-	-	-	-
55	Straw	Low turbid	7	2-3	-	-	-	2-3
56	Opaque	Transparent	7	-	-	-	-	-
57	Yellow	Turbid	5	-	-	-	-	5-6
41,58	Opaque	Transparent	7	-	-	-	-	-
59	Opaque	Transparent	8	-	-	-	-	-
60	Opaque	Low turbid	7	-	-	-	-	-
61	Opaque	Low turbid	7	-	-	-	-	-
62	Opaque	Transparent	7	-	-	-	-	-

Sample number	Urine color	Opacity	pH	RBC	WBC	Epithelium cell	Platelet	Pus cell
63	Opaque	Transparent	7	-	-	-	-	-
64	Opaque	Low turbid	7	-	-	-	-	-
65	Opaque	Transparent	7	-	-	-	-	-
66	Opaque	Transparent	7	-	-	-	-	-
67	Opaque	Transparent	7	-	-	-	-	-
68	Opaque	Transparent	9	-	-	1	-	3-4
69	Opaque	Transparent	8	-	-	-	-	-
70	Opaque	Low turbid	7	-	-	-	-	-
71	Straw	Low turbid	7	-	-	-	-	-
72	Straw	Transparent	7					
73	Opaque	Transparent	7	-	-	-	-	
74	Straw	Low turbid	7	-	-	-	-	-
75	Straw	Low turbid	6	-	-	-	-	-
76	Opaque	Transparent	7	-	-	-	5-6	-
77	Yellow	Turbid	6	2	-	-	-	2-3
78	Straw	Low turbid	6	-	-	-	-	1-2
79	Straw	Low turbid	6	-	-	-	-	-
80-81	Straw	Low turbid	7	2-3	-	-	-	5-7

Table III (b): - Physical and microscopic examination of urine samples collected from female volunteers.

Sample number	Urine color	Opacity	PH	RBC	WBC	Epithelium cell	platelet	Pus cell
82	Straw	Low turbid	7	-	-	-	-	-
83	Straw	Low turbid	7	3	-	-	-	5-6
84	Light straw	Turbid	5	-	-	-	-	-
85-86	Opaque	Transparent	7	-	-	-	-	6-7
87	Opaque	Transparent	7	4	-	-	-	5-7
88	Opaque	Low turbid	6	-	-	-	-	-
89	Straw	Turbid	5	-	-	-	-	-
90	Opaque	Transparent	8	-	-	-	-	-
91	Straw	Turbid	6	-	-	-	-	-
92	Opaque	Transparent	7	-	-	-	-	-
93	Opaque	Transparent	7	-	-	-	-	-
94	Yellow	Turbid	5	-	-	-	-	-
95	Straw	Low turbid	7	-	-	-	-	1-2
96	Opaque	Low turbid	7	-	-	-	-	-
97	Opaque	Transparent	7	-	-	-	-	-
98	Straw	Low turbid	7	-	-	-	-	-
99	Opaque	Transparent	8	-	-	-	-	-

Sample number	Urine color	Opacity	PH	RBC	WBC	Epithelium cell	platelet	Pus cell
100	Opaque	Transparent	7	-	-	-	-	-
101	Yellow	Turbid	5	-	-	-	-	-
102	Straw	Low turbid	7	-	-	-	-	-
103	Straw	Low turbid	7	-	-	-	-	-
104	Opaque	Low turbid	7	-	-	-	-	3-4
105	Opaque	Transparent	7	-	-	-	-	-
106	Opaque	Transparent	8	-	-	-	-	2
107	Straw	Low turbid	7	-	-	-	-	-
108	Light straw	Low turbid	6	-	-	-	-	-
109	Opaque	Transparent	7	-	-	-	-	-
110	Straw	Transparent	7	-	-	-	-	7
111	Opaque	Transparent	9	-	-	-	-	8-9
112	Opaque	Transparent	7	-	-	-	-	2-3
113-114	Opaque	Transparent	9	-	-	1	-	6
115-117	Opaque	Transparent	7	-	-	-	-	-
118	Light straw	Low turbid	6	-	-	-	-	-
119	Straw	Low turbid	7	2-3	-	-	-	3-4
120	Opaque	Transparent	9	-	-	-	-	-
121	Straw	Low turbid	7	-	-	-	-	-
122	Yellow	Turbid	5	-	-	-	-	5-6
123	Opaque	Transparent	7	-	-	-	-	6

Microscopic examination of *Candida sp*.: I analyzed that only two urine samples carried the *Candida sp*.

This results are shown in **Table III (c).** Microscopic examination of *candida sp*:

Sample number	*candida sp*
80	+
118	+

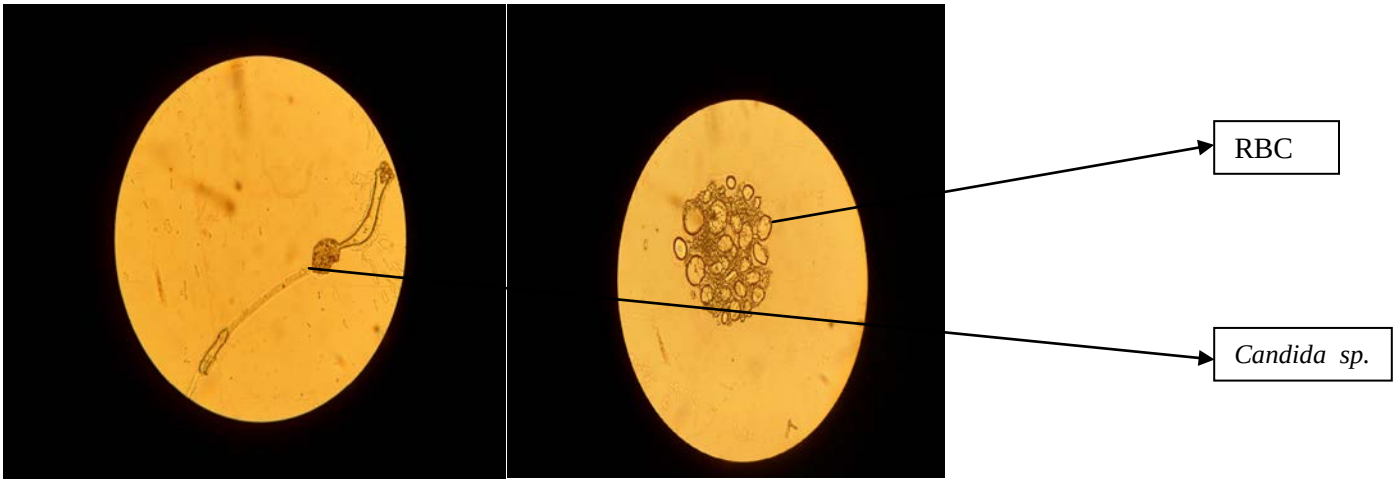

Figure III (A): Microscopic observation of *Candida sp. & RBC* (40X 12.5).

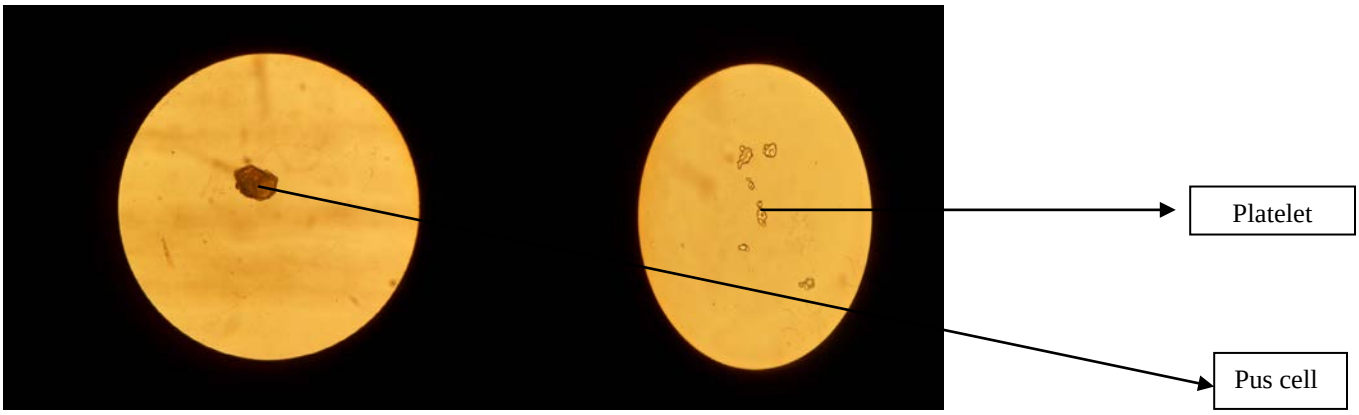

Figure III (B): Microscopic observation of pus cell & platelet (40X12.5).

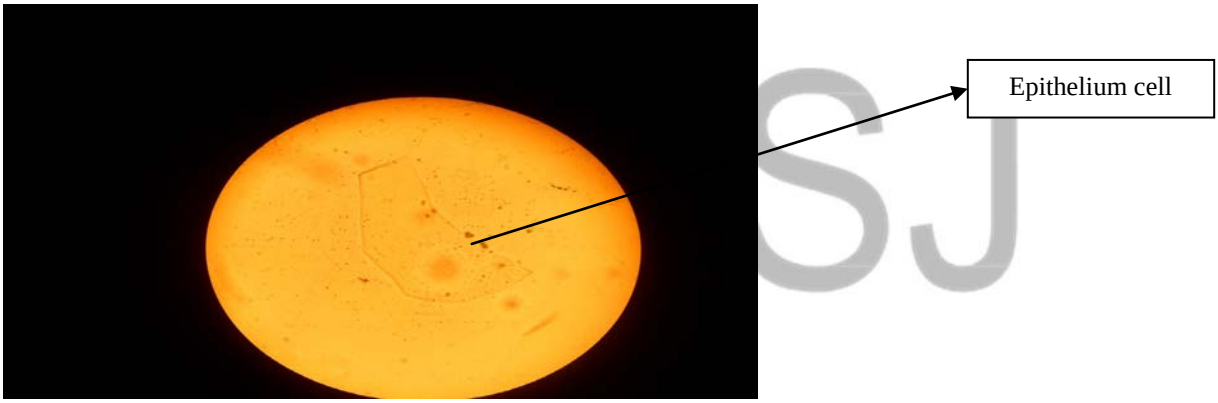

Figure III(C): Microscopic observation of epithelium cell (40X12.5).

Semi- quantitative culture of urine from male volunteers : One to 81 male urine samples were analyzed. But total 5 samples were positive. All others were negative.The percentage of 6.17%% was positive culture. Infection percent was 1.23% because one male persons urine carry 35000 cfu/ml. The colony numbers are shown in the Table III (d).

Table III (d). Urine culture results and colony number for male sample:

Number of sample	Number of isolate	Colony color for isolates	CFU/ml	Gram staining of urine sample	Symptoms of person
1-40	-	-	0	Negative(-)	Asymptomatic
41	1	pink	300	-	Asymptomatic
42-44	-	-	0	-	Asymptomatic
45	1	pink	100	-	Asymptomatic
46	-	-	0	-	Asymptomatic
47	1	pink	1500	-	Asymptomatic

48	1	pink	1100	-	Asymptomatic
49-67	-	-	0	-	Asymptomatic
68	3	a)pink b)cream c)transparent	35000	-	Asymptomatic
69-79	-	-	0	-	Asymptomatic
80	-	-	0	-	Asymptomatic
81	-	-	0	-	Asymptomatic

Semi- quantitative urine culture's result for female volunteers: Forty Two female samples were analyzed.82-123 were female sample .2 sample were positive by the semi- quantitative urine culture. The 4.76% was positive for female. Infection percentage was also 4.76%.The female urine culture are shown in the Table III (e).

Table III (e). Female urine culture results:

Number of sample	Number of isolate	Colony color	CFU/ml	Gram staining results	Symptoms of person
82-84	-	-	0	-	Asymptomatic
85	2	a)pink b)pinkish	25000	-	Asymptomatic
86	-	-	0	-	Asymptomatic
87	2	a)pink b)pinkish	40000	-	Asymptomatic
88-123	-	-	0	-	Asymptomatic

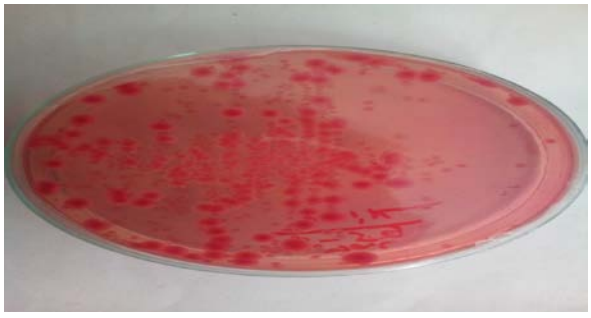

Figure III (D): Semi- quantitative urine culture on MacConkey Agar for infected UTI person.

MUG test results for conformation of *E.coli* for male and female samples: Total 123 urine samples were analyzed but 13 samples were mug positive. And the total percentage were 10.56% positive for *E.coli* by using this method. Between 1 to 81 samples were male samples and 82 to 123 samples were female samples. Between the total 123 sample ,only 8 sample were mug positive for male person and their percentage was 6.50% and total 5 samples were mug positive for female person. Their percentage was 11.9%. The mug results are given in the table III (f).

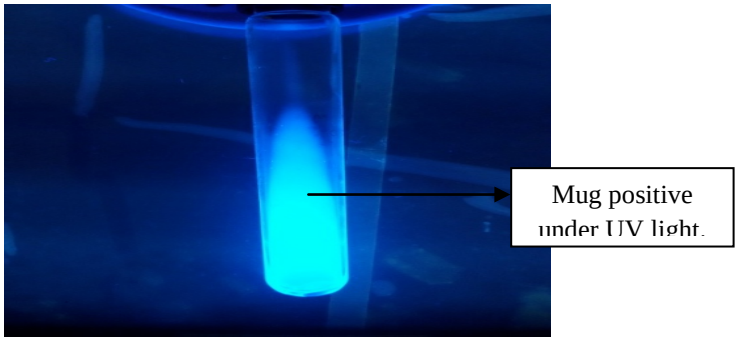

Figure III (E): Mug test for *E.coli*

Table (f). Mug test results for *E.coli*:

Sample number	MUG test result for *E. coli*
1-34	-
35	+
36-40	-
41	+
42-44	-
45	+
46	-
47-48	+
49-57	-
58	+
59-66	-
67-68	+
69-79	-
80	-
81-84	-
85	+
86	-
87	+
88-114	-
115	+
116-117	-
118	+
119-122	-
123	+

Biochemical test results: *E.coli* and all other organisms identified by the short biochemical test such as KIA,MR-VP, MIU, catalase and oxidase test etc. These results are shown in the table Table (g).

Table (g). Biochemical test results:

Isolate number	Colony color	Lactose	Glucose	H_2S	MR	Citrate	oxidase	catalase	motility	urease	indole	Organisism
35	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
41	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
45	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
47	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
48	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
58	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
67(a)	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
67(b)	cream	+	+	-	-	+	-	+	-	-	-	*E.aerogens*
67(c)	transparant	+	+	-	+	+	-	+	-	+	-	*K .pneumoniae*
68(a)	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
68(b)	cream	+	+	-	-	+	-	+	-	-	-	*E. aerogens*
68(c)	transparant	+	+	-	+	+	-	+	-	+	-	*K. pneumoniae*
85(a)	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
85(b)	pinkish	+	+	-	-	+	-	+	+	-	+	*E.aerogens*
87(a)	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
87(b)	pinkish	+	+	-	-	+	-	+	+	-	+	*E.aerogens*
112(a)	cream	+	+	-	-	+	-	+	-	-	-	*E.aerogens*
112(b)	cream	+	+	-	+	+	-	+	-	+	-	*K .pneumoniae.*
115	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
118(a)	pink	+	+	-	+	-	-	+	-	-	+	*E. coli*
118(b)	colorless	+	+	-	+	-	-	-	-	-	-	*L. lactis*
123	Pink	+	+	-	+	-	-	+	-	-	+	*E. coli*

Note: MR= Methyl red, KIA= killer iron agar, MIU= motility, indole, urease. *E.aerogens =Enterobacter aerogens , K .pneumoniae.=Klebsiella pneumonia, L. lactis=Lactococcus lactis.*

A total of 22 isolates from 14 urine sample were identified. From this isolates *E. coli* 59.07%, 22.7% *E. aerogens*, 13.63% *K. pneumonia* and 1% *Lactococcus lactis* were identified. Their distribution percentage and frequency are shown in the table Table (h) .

Table (h). Distribution and percentage of microorganisms:

Microorganisms	Frequency	Percentage
E. coli	13	59.07%
E.aerogens	5	22.7%
K. pneumoniae	3	13.63%
Lactococcus lactis	1	1%

From biochemical test, i observed that total 13 persons carried *E. coli*, 5 persons carried *E. aerogens* , 3 person carried *K. pneumoniae* and only one person carried *Lactococcus lactis*.

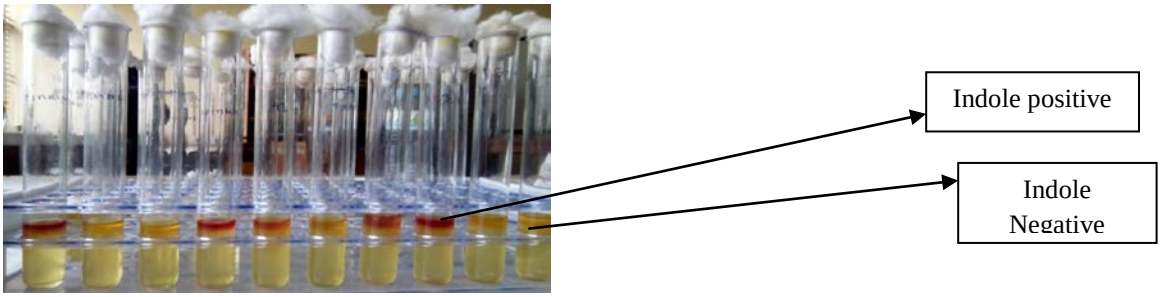

Figure III (F): Indole test

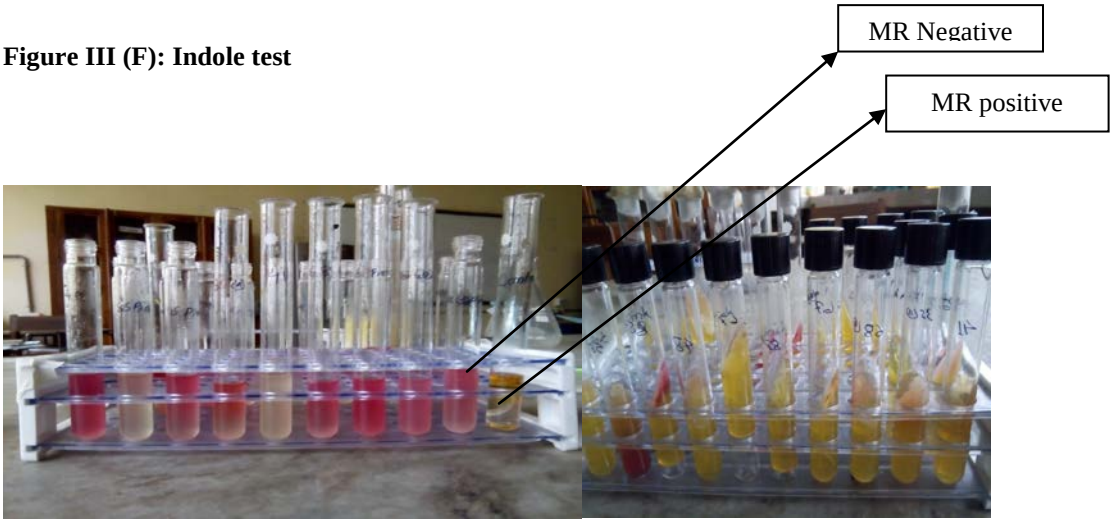

Figure III (G): Methyl Red (MR) test

Figure III (H): KIA test

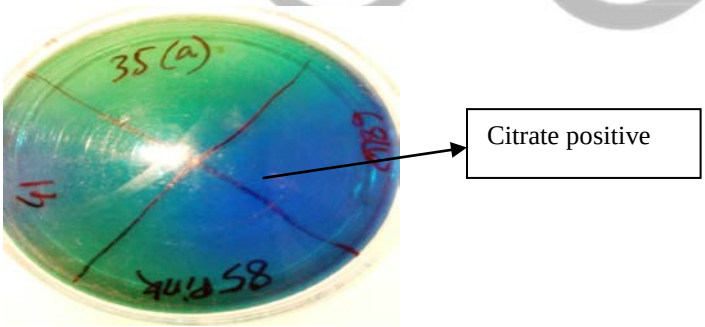

Figure III (I): Cimon citrate test.

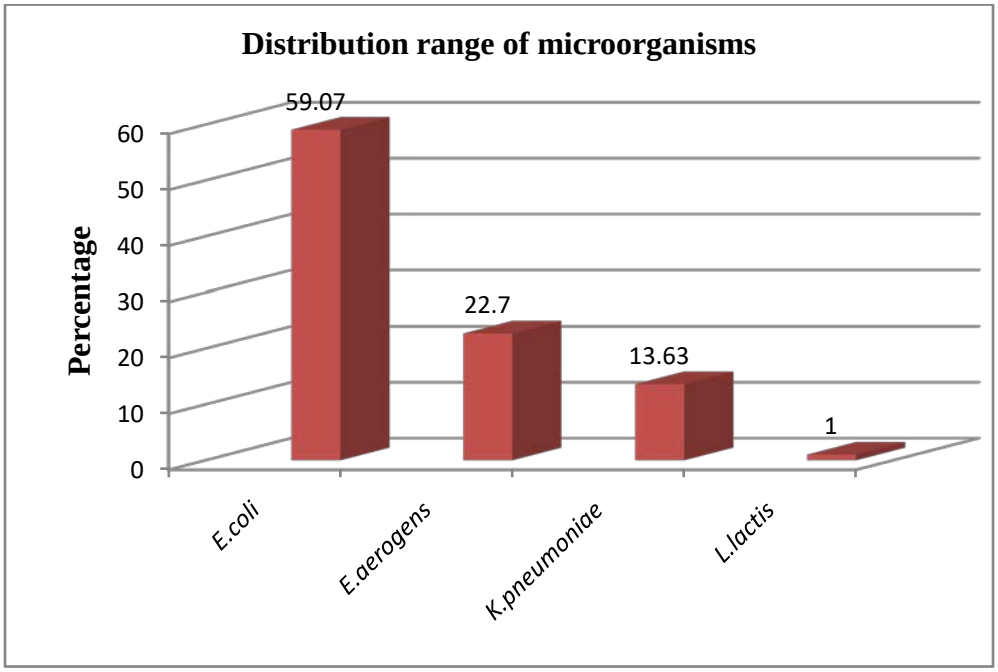

Figure III (J): Distribution range of microorganisms.

Correlation between pus cell count and culture result: I also compared between pus cell count and culture results, as shown in the Table (I).

Table (I). Relationship between pus cell count and culture result

Pus cells/HPF	Frequency of sample	Frequency of growth positive	Frequency of growth negative
<5	22(17.88%)	2	20
5-10	12(9.75%)	5	7
TNTC	3(2.43%)	0	3
0 /hpf	86(69.91%)	7	79

Correlation between WBC cell count and culture result:

I also compared between WBC cell count and culture results.These results are shown in the Table (J).

Table(j). Relationship between WBC cell count and urine culture result :

WBC/HPF	Frequency of sample	Frequency of growth positive
0	123	7

Correlation between RBC cell count and culture result: I also observed correlation between RBC cell count and culture result. These results are given in the Table (k).

Table (k). Relationship between RBC cell count and urine culture result :

RBC/hpf	Frequency of sample	Frequency of growth positive
1-4	13(10.56%)	1
>5	0	0

Correlation between urine P^H and culture result: I also observed correlation between PH and culture result. These results are given in the table (l).

Table (l). Correlation between urine P^H and urine culture results :

PH range	**Pus cell range**	**Frequency of sample for P^H**	**Frequency of growth positive**
5	TNTC (2.43%)	14(14.38%)	0
6	0	24(19.51%)	2
7	1-9	65(52.84%)	11
8-9	2-9	20(16.26%)	0

Correlation between opacity of urine and culture result: I also observed the opacity of urine. These results are given in table (m).

Table (m). Correlation between opacity of urine and urine culture result :

Opacity of urine	**Frequency of sample**	**Frequency of growth positive**
Transparent	52(42.27%)	13(100%)
Turbid	14(11.38%)	0
Low turbid	57(46.34%)	0

Correlation between epithelium cell of urine and culture result: Correlation between epithelium cell of urine and culture results are shown in the table (n).

Table (n). Correlation between epithelium cell of urine and urine culture results :

Epithelium cells of urine	Frequency of sample	Frequency of growth positive
1	4(3.25%)	1(0.81%)

Antibiotic sensitivity test: Total 10 antibiotics were used for all isolates. The antibiotic sensitivity test results are shown in the table (o) and (p).

Table (o). Antibiotic sensitivity test for *E.coli* :

Antibiotics	***E. coli***

name	Number of isolates												
	35	41	45	47	48	58	67a	68a	85a	87a	115	118a	123
CIP(5mg)	+	+	_	+	+	_	+	+	+	+	+	_	+
AZM(15mg)	+	+	+	+	+	+	_	_	+	_	+	+	+
ERY(300mg)	+	+	_	+	+	_	_	_	+	_	+	_	+
STR(300mg)	+	+	+	+	+	_	+	_	+	_	+	+	+
CEC(30mg)	+	+	_	_	+	+	_	+	+	+	+	_	+
CPX(30mg)	+	+	+	_	+	_	+	_	+	+	+	_	+
PXB(300mg)	+	+	+	+	+	+	_	+	+	+	+	+	+
CHP(30mg)	+	+	+	+	_	+	+	+	+	+	+	_	+
NXA(30mg)	_	+	_	_	_	_	_	_	+	+	+	_	+
VNC(30mg)	_	_	_	_	_	_	_	_	_	_	+	_	_

Note: + =Sensitive, - =Resistance, CIP=Ciprofloxacin, AZM=Azithromycin, ERY= Erythromycin, STR= Streptomycin, CEC= Cefaclor, CPX= Ceptriaxone, PXB=Polymixim-B, CHP=Chloramphenicol, NXA=Nalidixic acid, VNC=Vancomycin.

I analyzed the antibiotic sensitivity test for four types of bacteria by using ten types of antibiotics.From this analysis, i observed that Ciprofloxacin 76.9% sensitive and 23.07% resistance for total thirteen types isolate of *E.coli*. I also observed that Azithromycin 76.9% sensitive and 23.07% resistance , Erythromycin 53.84% sensitive and 46.15% resistance, Streptomycin 76.9% sensitive and 23.07% resistance, Cefaclor 69.23% sensitive and 30.76% resistance , Ceptriaxone 69.23% sensitive and 30.76% resistance, Polymixim-B 92.30% sensitive and 7.69% resistance, Chloramphenicol 84.61% sensitive and 15.38% resistance, Nalidixic acid 38.46 % sensitive and 61.53% resistance and Vancomycin 26% sensitive,74% resistance for *E. coli* .

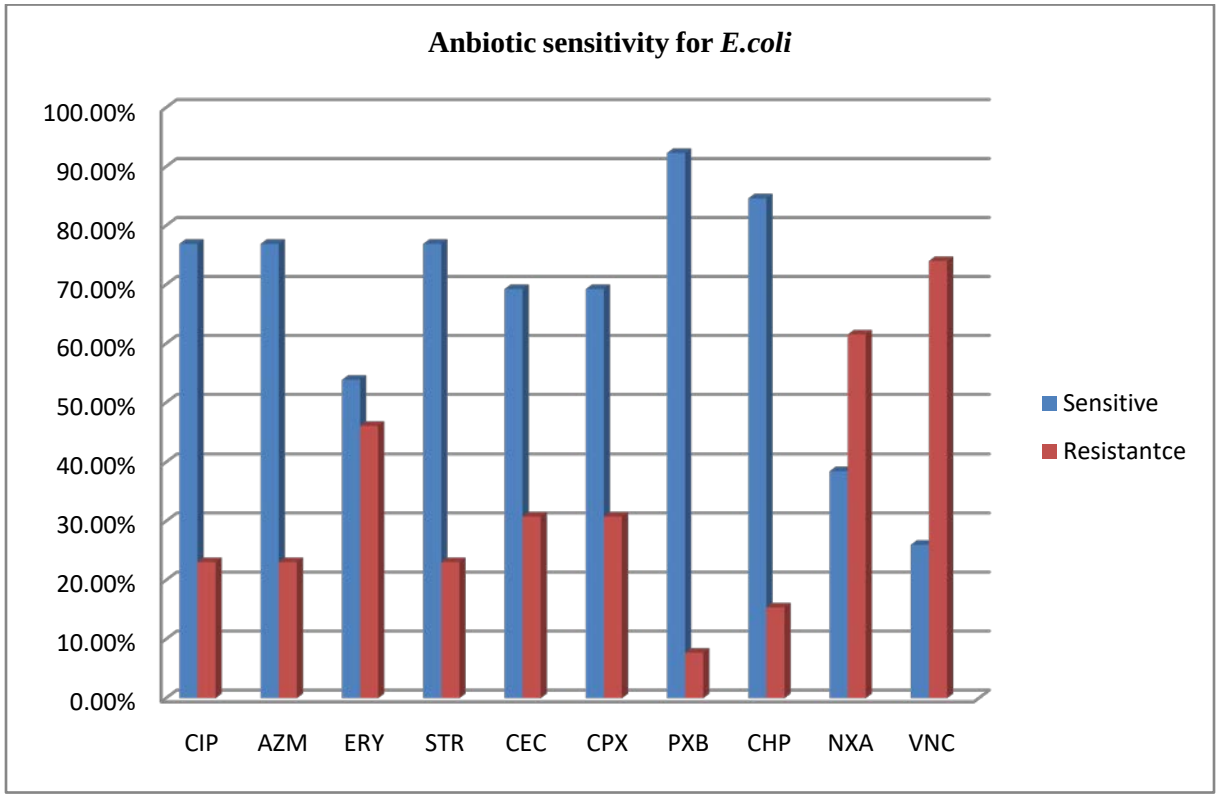

Figure III (K): Antibiotic sensitivity for *E.coli*.

Table (p). Antibiotic sensitivity test for *Enterobacter aerogens*, *Klebsiella pneumoniae* and *Lactococcus lactis*:

Antibiotics name	*Enterobacter aerogens*				*Klebsiella pneumoniae*				*Lactococcus sp*
	Number of isolates								
	67b	68b	85b	87b	112a	67c	68c	112.b	118
CIP(5mg)	+	_	+	+	_	_	_	+	_
AZM(15mg)	_	_	+	_	+	_	_	+	+
ERY(300mg)	_	_	+	_	_	_	_	_	_
STR(300mg)	+	+	+	_	_	+	+	+	+
CEC(30mg)	_	_	+	+	+	_	_	+	_
CPX(30mg)	+	_	+	+	_	+	+	_	_
PXB(300mg)	+	+	+	+	+	+	+	+	+
CHP(30mg)	+	+	+	+	+	+	+	+	_
NXA(30mg)	_	_	+	_	+	_	_	_	_
VNC(30mg)	_	_	_	_	_	_	_	_	_

Note:+= Sensitive, - = Resistance. CIP=Ciprofloxacin, AZM=Azithromycin, ERY= Erythromycin, STR= Streptomycin, CEC=Cefaclor, CPX=Ceptriaxone, PXB=Polymixim-B, CHP=Chloramphenicol, NXA=Nalidixic acid, VNC=Vancomycin.

I also examined the antibiotic sensitivity test for other three types of bacteria by using the same ten antibiotics. I observed that Ciprofloxacin 60% sensitive and 40% resistance for *Enterobacter aerogens.* I also analyzed that Azithromycin 40% sensitive and 60% resistance, Erythromycin 80% sensitive and 20% resistance, Streptomycin 60% sensitive and 40% resistance, Cefaclor 60% sensitive and 40% resistance , Ceptriaxone 60% sensitive and 40% resistance, Polymixim-B 100% sensitive, Chloramphenicol 100% sensitive , Nalidixic acid 40 % sensitive and 60% resistance and Vancomycin 100% resistance for *Enterobacter aerogens.*

For *Klebsiella pneumoniae,* I observed tha Ciprofloxacin 33.33% sensitive and 66.67% resistance, Azithromycin 33.3% sensitive and 66.67% resistance , Erythromycin 100% resistance, Streptomycin 100% sensitive, Cefaclor 33.33% sensitive and 66.67% resistance , Ceptriaxone 66.67% sensitive and 33.33% resistance, Polymixim-B 100% sensitive, Chloramphenicol 100% sensitive, Nalidixic acid 100% resistance and Vancomycin 100% resistance for *Klebsiella pneumoniae.*

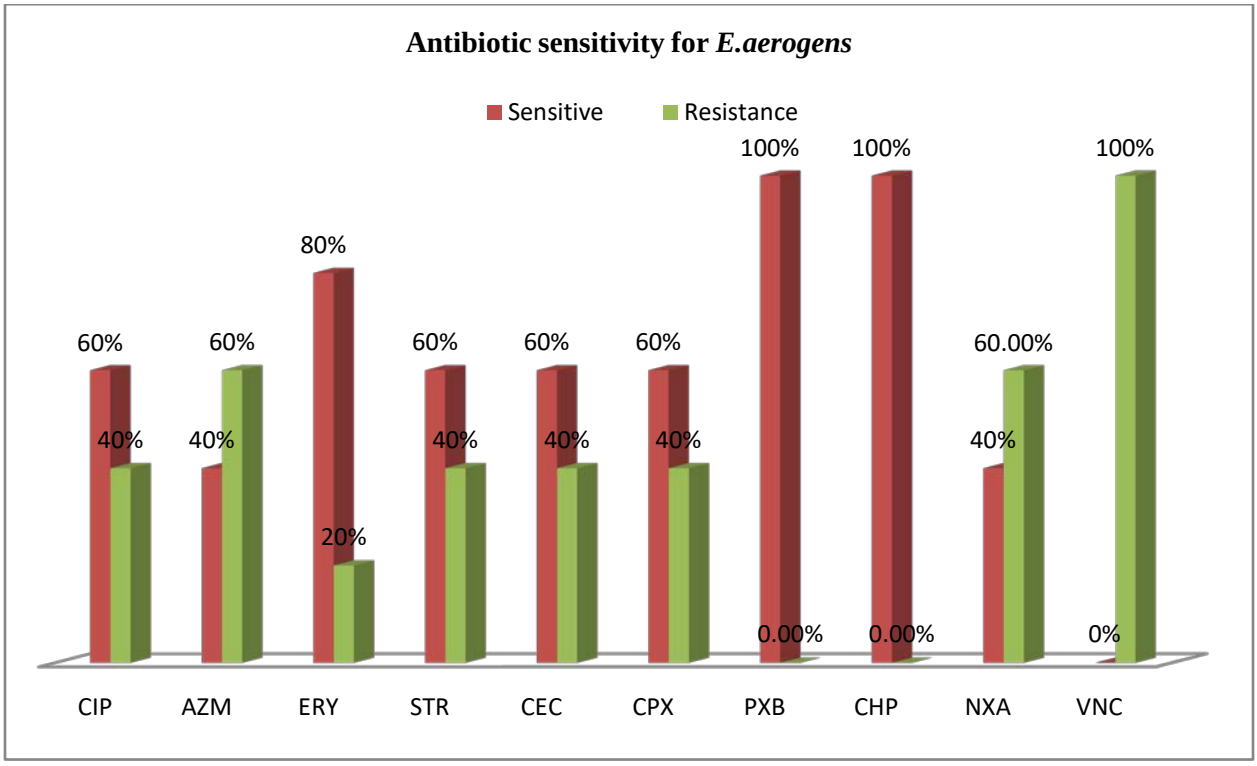

Figure III (L): Antibiotic sensitivity for *Enterobacter aerogens.*

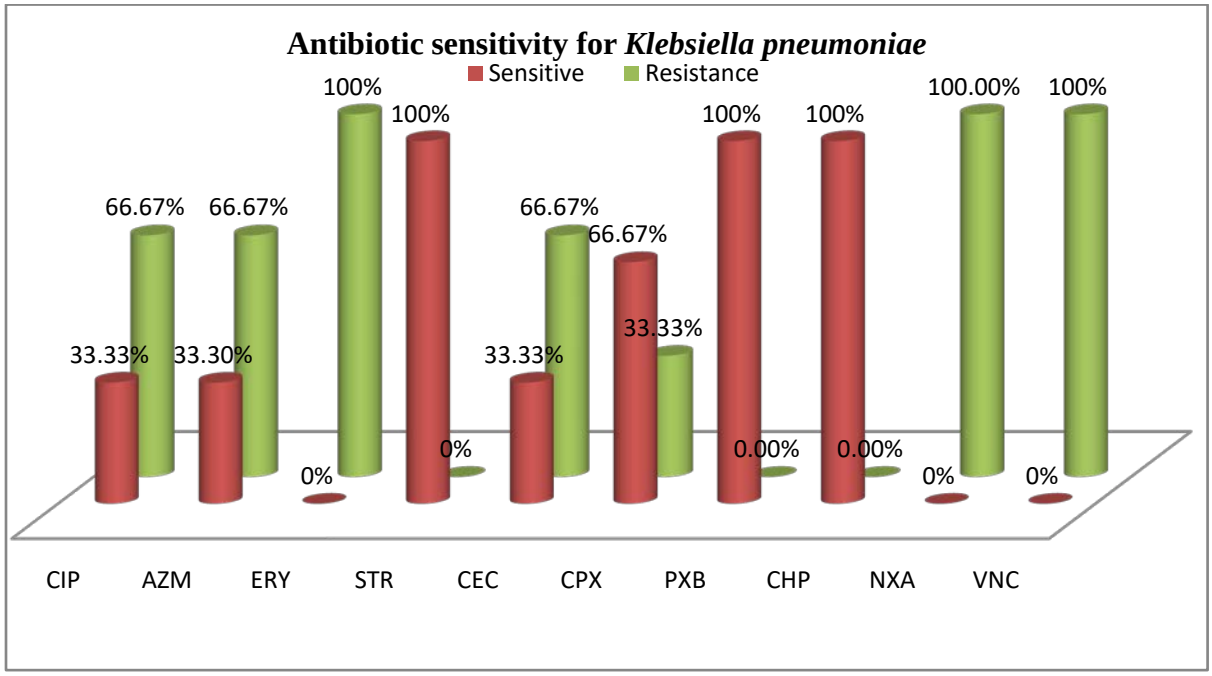

Figure III (M): Antibiotic sensitivity for *Klebsiella pneumoniae*

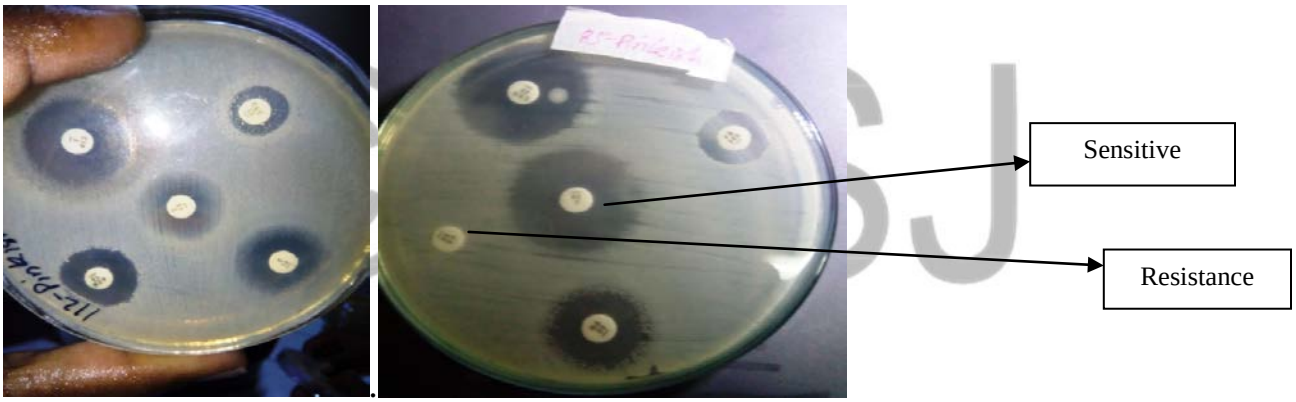

Figure III (N): Antibiotic sensitivity *test*

IV. Discussion

All the urine samples analyzed were collected by spontaneous urination (clean catch midstream). The majority of the samples were collected from students of Shaheed Mashiur Rahman and Sheikh Hasina Hall in the Jashore university of science & technology, Jashore. About 65% (81) of the samples were received from males and the remainder were 34% (42) from female persons. The ages of the persons ranged from 21 to 25 years with a mean age of 23 years. I analyzed the culture result and *E. coli* was confirmed by the mug test and there was no found the symptomatic persons but they were carried the bacterial populations. 1 to 81 male urine samples were analyzed. But, total 7 male and female samples were positive by the semi-quantative streaking method. Others were negative culture. The percentage was 6.17% positive culture for male. Total 5 samples were positive for male by the urine culture method. Only one male person carried more than10000 colony cfu/ml. So, the total infection percent was 1.23%, but this

person was asymptomatic. 42 female samples were analyzed in the microbiology laboratory.82-123 were female samples .2 sample were positive by the semi- quantitative urine culture. Two female persons carried more than10000 colony cfu/ml. The 4.76% was positive culture and infection percentage was of 4.76% but they were asymptomatic.

According to the semi-quantitative urine culture with 10000-10^5 colony-forming units/ml (CFU/ml) remains the standard diagnostic method to diagnose UTI. (Mahon, C.R., Lehman, D.C. & Manuselis, G,2007). Based on this criterion, a total of 22 bacteria belonging to 4 different strains were isolated from 123 urine samples (table (g)) and total 3 persons were infected by UTI, but they were asymptomatic. This is the major findings of my study that young male and female persons carry infection but do not show the symptoms. Among the isolated bacteria, Gram-negative bacteria were the most predominant microorganisms over Gram-positive bacteria. *Escherichia coli* was the most frequent isolate, followed by *Klebsiella pneumoniae, Enterobacter aerogens* , *Lactococcus lactis.* Leigh (1990) reported that infecting organisms are most commonly derived from the patient's own faecal flora. In women, they are usually present on the perineal skin before infection occurs. The majority of the organisms, i isolated also belong to normal faecal flora suggesting that they might have entered the tract through the ascending route. Antibiotic sensitivity tests of all 22 isolates were performed to determine the degree of sensitivity or resistivity to an appropriate range of commonly prescribed antimicrobial drugs. Total 10 antibiotics were used for antibiotic sensitivity test. Antibiotic sensitivity profile (Figure III (K) , Figure III (L), Figure III (M)) showed that Streptomycin and Polymixim-B was the only effective antibiotic against both gram-positive and gram-negative isolates. Increased resistance was observed against other commonly used antibiotics, such as Nalidixic acid and Vancomycin in this study and also in others (Obi et al., 1996; Oh et al., 2002).

However, these antibiotics were reported as effective by Miano et al. (1990) and Schaeffer (1990). The increased resistance suggested that bacteria are acquiring resistance to commonly used antibiotics and also demonstrates a need for reevaluation of common antibiotics used to treat UTI. Culturing urine is the standard diagnostic method but on the basis of clinical symptoms, macroscopic and microscopic observation, initial presumptive diagnosis can be made. In this study, i tried to evaluate these parameters for rapid diagnosis of UTI.

First, macroscopically focused on turbidity of urine and clinical symptoms (Table III (a) and III (b)). I observed that only about 11.38% of the samples that were turbid and 46.34% of the samples were low turbid but all were culture negative. I also examined that 100% culture positive for transparent opacity of urine. My result was consistent with the report of Leigh (1990). Next, i microscopically examined four different cell types; epithelial cells, red blood cells, white blood cell and pus cells to correlate with the positive culture.

Epithelial cells were examined under the microscope. Epithelial cells appear in urine as a result of normal exfoliation along the urinary tract (Schumann and Schweitzer, 1991). It is also reported that the finding of a large number of squamous epithelial cells or approximately 1-2/HPF, in the voided specimen is not uncommon if proper techniques for the collection of an uncontaminated specimen are not followed (Cheesbrough, 1984). As shown in Table III (a) and Table III (b), a very low percentage (0.81%) of samples were culture positive in which i detected a significant number of epithelial cells (1/HPF). From this finding i suggest that microscopy of epithelial cells has very poor significance for UTI prediction.

Red blood cells were examined under the microscope. The mechanism through which RBC enters urine is not known yet, but it is believed that increased numbers of erythrocytes are seen in renal disease, lower urinary tract disease, extrarenal disease, toxic reactions due to drugs and sometimes in physiologic causes including exercise. Schumann and Schweitzer (1991) suggested that the observation of 0 to 2 RBC per HPF on microscopic examination of the sediment is normal both in males and females. In another report, the finding of a red blood count greater than or equal to three per high power field was considered as abnormal (Wargotz et al., 1987; Froom et al., 1986 and Steward et at1985). Based on this criterion i tried to investigate whether observation of greater than 3 RBC/HPF in urine deposits can be established as a potential predictor of significant bacteriuria. Among samples which had more than 3 erythrocytes per HPF, the majority of them (100%) were culture negative (Table III (a) and Table III (b)).My results revealed that microscopic observation of RBC is not sufficiently sensitive to be used as a screening test for the detection of UTI. This is the one of the findings of my study. The observation of leucocytes is suggestive of bacteriuria, but a substantial number of persons may excrete leucocytes in inflammatory disorders of the urinary tract. Increased numbers of leucocytes, principally neutrophils, are seen in almost all renal diseases and diseases of the urinary tract. Pyuria, the presence of pus cell and leucocytes in urine, is considered significant if more than or equal to 5 white blood cells or pus cells are seen per high power field in the sediment (Steward et al.,1985; Wargotz et al.,1987; Pallares et al.1988; Wenz and Lampasso, 1989; Ziloski and Smucker, 1989; Abyad 1991; Ouslander et al.,1996 and Eisinger et al.,1997). I observed that 5 samples out of 12 (9.75%) having 5-10 puscell/HPF in microscopy were culture positive (Table III (a) and Table III (b)). But two persons carried their colony number was between the UTI range. Thus, the presence of 5-10 pus cells per HPF can be a good marker of UTI. I also observed 3 samples carried huge amount of pus cells/HPF but they were culture negative. So I can tell that microscopic observation of pus cell is not sufficiently sensitive to be used as a screening test for the detection of UTI.

I observed that 123 samples carried 0% WBC, among them 7 samples were culture positive but they had no symptoms. (Table III (a) and Table III (b)). Thus, the presence of 5-7WBC per HPF can be a good marker of UTI. Finally urine smears were Gram stained and observed under microscope. The chief advantage of performing microscopic examination of Gram-stained urine is the presumptive rapid diagnosis of urinary infection and guidance for initial persons treatment based on the form and staining properties of the probable etiological infective agent; these can be made available while the clinic awaits the results of the urine culture and antibiotic sensitivity tests, which are generally available within 24 to 48 h (Clarridge et al., 1987 and Jenkins and Matsen, 1986). Although microscopic examination of an uncentrifuged gram-stained urine drop is recognized as the conventional microscopic method for diagnosing urine specimens with counts of 10^5 CFU/ml (Baron and Finegold, 1994; Clarridge et al., 1987; Hoeprich, 1960; Jenkins and Matsen,1986; Pollock, 1983 and Washington et al.,1981), it is a time consuming and tedious process looking for very few microorganisms (2 or less than 2) per field. To overcome this problem, i studied the centrifuged gram stain smear and tried to set off a criterion that distinguished between positive and negative cultures. The correlation of gram stain of urine sediment with culture was examined and the results are summarized in Table III (d) and Table III (e).

During this study, bacteria within the microscopic field of centrifuges urine was very easy and less time-consuming compared to uncentrifuged urine, having no bacteria per field (Weinberg and Gan, 1991),(Table III (d) and Table III (e)). However, urine from asymptomatic persons, with acute pyelonephritis or persons with acute cystitis which

can produce a cfu count of 10^5 per ml, gram stain smear may be used as an accurate and cost effective screening method (Baron and Finegold,1994; Clarridge et al.,1987; Pezzlo, 1988; Pezzlo, 1990 and Pollock, 1983) for UTI.

V. Conclusion

In this study, it have been observed that *E. coli* was found as the most prominent bacteria in urine (59.07%) and it's presence is insignificant varies sex of the volunteers. According to the semi-quantitative urine culture with 10^4-10^5 colony-forming units/ml (CFU/ml) remains the standard diagnostic method to diagnose UTI. Only three cases among 123 samples carried bacterial colony between this ranges, however, they were aymptomatic. Physical conditions of urine such as p^H, turbidity as well as presence of epithelium cell, RBC, WBC, pus cell were also examined. It was found that microscopic observation of pus cell, is not sufficiently sensitive, to be used as a screening test for the detection of UTI. Finally, in the antibiotic sensitivity assay of the isolates, polymixin-B and ciprofloxacin were most effective against them according to antibiotic assay results. In this regard, making the people aware about hygienic life maintaining is too crucial. Besides, it is necessary to monitor UTI asymptomatic persons and will be given better treatment according to their symptom's or severity by the national health regulatory agencies before being worsen of their health condition. Therefore, proper medication will be great concern and it can be stopped to spread microorganisms within human body and, reduce UTI among people.

Author contributions: M. Hossain and Dr. Selina Akter designed the study framework; M. Hossain conducted the experiment, analyzed the data, and wrote the manuscript. Author read and approved the final manuscript by the assistance of Dr. Selina Akter, associate professor, Department of Microbiology, Jashore University of Science and Technology, Bangladesh.

Conflicts of interest: Author declared that there was no conflict of interest.

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