In vitro detection of uropathogenic bacteria from UTI infected patients and their suitable drugs selection

Raihanul Islam, Suvamoy Datta, Dayanidhi Sarkar *

Department of Microbiology, Primeasia University, 9 Banani, Dhaka 1213, Bangladesh

ABSTRACT

Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed and management of urinary tract infections is complicated by the increasing prevalence of virulent antibiotic-resistant strains. The present study was designed to isolate the virulent organisms and their drug sensitivity status in a certain community. Total 100 urine samples were collected from patients with clinically diagnosed UTI attending both out-door and in-door patient from the prescription point. The isolates were identified by standard microbiological and biochemical process. From the total collected urine samples of the patients, 80% samples showed culture positive. Moreover, female patients were found more infected than male. *Escherichia coli*, *Klebsiella* spp *& Staphylococcus* spp were prevalent in all urine samples isolated from the diagnostic centers and subjected to characterizations namely cultural and biochemical identification, microscopic observation, identification of ESBL positive strain, antibiogram, and hemolytic assay study for the detection of virulence property. In this study 86% *E. coli* and 40% *Klebsiella* spp shown positive result for the presence of β lactamase. *E. coli*, *Klebsiella* spp *& Staphylococcus spp*. showed multiple resistances of 6 to 8 antibiotics. *E. coli and Klebsiella* spp were 100% sensitive to imipenem and amikacin and compared to the other antibiotics tested and therefore these may be the drugs of choice for the treatment of UTIs.

Keywords: Urinary tract infections, drug resistance, *Escherichia coli*, Extended spectrum β-lactamases (ESBL)

1. Introduction

Urinary tract infections (UTIs) remain a leading cause of morbidity and health-care expenditure for people of all age groups [1]. Urinary tract infection (UTI) is one of the commonest domiciliary and nosocomial bacterial infections [2], comprising of variety of clinical conditions caused by microbial invasion of tissue lining the urinary tract which extends from renal cortex to urethral maetus. Infection of adjacent structures such as prostrate and epididymis is also included in this entity. It also refers to the presence of bacteria undergoing multiplication in urine within the urinary drainage system [3] and presence of more than 10^5 organisms per ml. in the mid stream sample of urine. UTI describes a condition in which there are micro organisms established and multiplying within the urinary tract. It is most often due to bacteria (95%), but may also include fungal and viral infection [3]. It also contributes the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital-acquired infections. UTI cause increased morbidity and economic cost [5]. Bacteria are the primary organism that causes UTI. Gram negatives cause 80-85% and Gram positives cause 15-20%. Among, a Gram negative *Escherichia coli* is the most frequent pathogen [6] but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Serratia, Enterobacter, and Pseudomonas.* Among Gram positives *S. saprophyticus, E. faecalis, S. agalactiae, S. pyogenes, S. aureus* and *B. subtilis* are usually prevalent and are resistant to a variety of different antibiotics [7]. *Enterococcus* isolates cause 2.3% of UTI and best known as antibiotic resistant opportunistic pathogens [8]. In infants the incidence of urinary tract infection is more in male than female due to higher incidence of obstructive anomalies of urinary tract in boys than in girls. After one year age, the urinary tract infection is more frequent in girls than boys because the female urethra is shorter and the chances of vaginal vestibular contamination with fecal flora are more. In school age, the frequency of bacteriuria in boys is 0.5%, where as in girls it is 2%. After this age, the men have rare urinary tract infection until the age of 40. There is regular increase in the rate of urinary tract infection with age in women. At the age of seventy, about 10% women have urinary tract infection. In younger women, the incidence of the disease has some correlation with sexual activity [9]. During pregnancy, the tendency of urinary tract infection increases partly due to the pressure of gravid uterus on the ureters causing stasis of urine flow and is also attributed to the humoral and immunological changes during normal pregnancy [10]. In patients with various diseases, the incidence of urinary tract infection is 20% for diabetes mellitus, 14% for hypertension, 80% for hydronephrosis and nephrolithiasis and greater than 50% for long term indwelling catheters. Twenty five percent of pregnant women with asymptomatic bacteriuria go on to develop acute pyelonephritis [11].

Increasing resistance in bacterial pathogens is the worldwide concern. The prevalence of antimicrobial resistance in both out and hospital patients with UTI is increasing and can vary according to geographical and regional location [12, 13]. In UTI infection, antimicrobial therapy is initiated even before the results of urine culture are available. Hence, there exists a great need for antimicrobial resistance surveillance at local, national and international level [14]. The microbial resistant organisms result in significantly more morbidity, mortality and cost than those due to susceptible bacteria. Because most UTIs are treated empirically, the selection of an antimicrobial agent should be determined not only by the most likely pathogen but also by its expected susceptibility pattern [15]. Early diagnosis and prompt antimicrobial treatment are required to minimize renal scaring and progressive kidney damage [16].

The present study was designed to identify the etiological agents of UTI in male and female at different age group and to investigate their response against twenty one commercially available antibiotics.

2 Materials and methods

2.1 Sample Collection

Freshly voided midstream urine (MSU) sample was collected in a sterile test tube and then send to microbiology laboratory for culture. Total 10-20 ml urine collected from each patient.

2.2 Total viable bacterial count on Nutrient agar

A total of 0.1ml sample was inoculted on nutrient agar (NA) media and spreaded for heterotrophic count of microbes. Then the plates were incubated at 37 ^{o}C for 24 hours.

2.3 Colifrom count on MacConkey agar

0.1 ml of the diluted sample was inoculated on MacConkey agar media and spreaded by using the glass rod spreader. Then the culture plates were incubated overnigh at 37^{o}C for 24 hours. After 18 hours of incubation, total coliform counts were taken and presumptive *E.coli*, and *Klebsiella* spp colonies were selected.

2.4 Staphylococcus spp count on Manitol Salt agar

0.1 ml of the diluted sample was inoculated on Manitol Salt agar and was spreaded by using the glass rod spreader. Then the culture plates were incubated overnight at 37°C for 24 hours.

2.4.1 Oxidase test

The presence of cytochrom oxidase is detected by Kovac's oxidase lest. The test was performed with 1% solution of N'N'N'N' – tetramethyl-p-phynylenadiamine dihydrochloride which was soaked in a piece of Whatman filter paper. A portion of colony of the test organism was picked tip with a sterile toothpick and touched onto the paper with impregnated reagent. A dark purple color development within 5-10 seconds was considered positive and no change in color was interpreted as negative for the test.

2.4.2 Triple Sugar Iron (TSI) Test

The test was performed to assess the mode of sugar utilization by stabbing the butt and streaking the slant of Triple Sugar Iron (TSI) agar media. After incubation at 37^{o}C for 18 hours, results were recorded for changes in color of the butt and slant, H_2S or other gas production. Formation of acid from sugar in fermentative mode indicated by yellowish of the butt and slant. If gas was formed during the fermentation, it would show in the butt either in bubbles or as creaking of the agar. If no fermentation occurred, the slant and butt would remain red. A blackening the media was indicative of hydrogen sulphide production.

2.4.3 Motility Indole Urease (MIU) Test

Tubes containing Motility Indole Urea (MIU) medium were inoculated with a straight wire by stabbing the medium to a depth not touching the bottom. The tubes were incubated at 37^{o}C for 18 hours. Following incubation, the tubes were observed for the presence of motile organisms which would disperse through the medium leaving the stab line and made the tube turbid. Pink coloration of the MIU medium indicated the positive increase and no change in color would be recorded as negative. The presence of indole would be detectable by adding Kovacs reagent in MIU medium. 3-4 drops of Kovacs reagent was added to observe the production of cherry red reagent layer. The presence of red coloration would demonstrate that the subsrate tryptophan was hydrolysed and indicated an indole positive reaction.

2.4.4 Citrate Utilization Test

Citrate utilization was performed by inoculating each isolates tubes containing Simmons Citrate agar slant by means of streak inoculation. The tubes were incubated at 37^{o}C for 18 hours. Following incubation, citrate positive culture would be identified by the presence of growth on the surface of the slant, which was accompanied by deep Prussian blue coloration. Citrate negative culture would show no growth and the medium would remain green.

2.4.5 Methyl red test

Methyl red test is the test for mixed acid fermentation of glucose by microorganisms. Excreted acid contains large amount of formic, acetic, lactic and succinic acid and causes a major decrease in pH that can be detected by "Methyl Red" indicator. For this test MR broth was inoculated and incubated for 5 days at 37 ^{o}C. After incubation 5-6 drops of methyl red indicator was added to the culture broth. Red color throughout

the broth indicated positive reaction whereas yellow or any yellowish red indicated negative reaction.

2.4.6 Voges- Proskauer (VP) test

Three ml of VP medium containing tubes were inoculated with 3-4 hour fresh culture at 37°C for 24-48 hours. After incubation, 0.6 ml of 6% α-naphthol and 0.2 ml of 40% KOH aqueous solution were added. A pale pink color development within 5-15 minutes after the addition of reagents is interpreted as a positive. After addition of reagent, the tube is shaken vigorously for aeration.

2.5 Microscopic examination of bacterial isolates

A pure colony of each isolates was picked and gram staining was performed. Then the shape arrangement and gram reaction of the isolates were observed in microscopic field [17].

2.6 Extended spectrum β-lactamases (ESBL) detection method by double disc-diffusion (DDD) method

Muller Hilton agar media plate was inoculated with test organism to give a semi confluent growth. An amoxicillin-clavulanate disc (AmC, 20μg + **10** μg) was placed in the centre and cefotaxime (CTX, 30μg), ceftazidime (CAZ, 30μg), cefpodoxime (CPD,10μg) ceftriaxone (CRO,30μg) and aztreonam (ATM, 30μg) discs were placed at a distance of 15 mm from the AmC disc and 90° to each other. The plates were incubated at 37°C for 18 h aerobically before the zone size were recorded. A positive result was indicated by a zone- size difference of ≥5 mm diameter between the combination disc and the corresponding standard antibiotic disc, as recommended an ESBL-producing organism.

2.8 Antibiotic susceptibility assay of bacterial isolates

The standard agar-disc diffusion method known as the Kirby Bauer method [18] was used to determine the bacterial susceptibility to different antibiotics towards Gram-negative bacterial in vitro.

A suspension of test organisms was prepared using normal saline by adjusting the turbidity of the borth with the equivalent turbidity standard of Macfarland (0.5 standards). A sterile cotton swab was dipped into suspension and excess fluid was removed by pushing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was then lawned evenly against the inside of the tube above the agar plate to obtain uniform inoculums. Antibiotic discs were applied aseptically to the surface of the inoculated plates at appropriate spatial arrangement by means of sterile needle within a distance of 5 mm. the plates were then inverted and incubated at 37°c for 24 hours. After incubation, the plates were examined and the diameters of the zones of inhibition were measured in mm. The zone diameters for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories according to Clinical Laboratory Standard Institute (CLSI).

Name of the antibiotics are listed in table 3.

Table2: Antibiotics and their potencies used in this study

Antibiotics	**Potencies (μg)**
Amikacin (AK)	30
Amoxycillin (AML)	10
Azithromycin (AZM)	15
Cephradine (CE)	30
Cefoxitin (FOX)	30
Ceftriaxone (CRO)	30
Ceftazidime (CAZ)	30
Chlorfloxacin (CIP)	20
Ciprofloxacin (CIP)	5
Clindamycin (DA)	2
Gentamicin (CN)	10
Imipenem (IPM)	10
Kanamycin (K)	30
Nalidixic Acid(NA)	30
Nitrofurantoin (F)	300
Ofloxacin (OFX)	5
Penicillin G(P)	10
Polymyxin B (PB)	300
Tetracycline (TE)	30
Tobramycin (TOB)	10
Trimethoptim (SXT)	25

2.10 Hemolytic characterization

The hemolytic activity of *E. coli*, *klebsiella* spp, *Staphylococcus* spp were tested on a blood agar plate containing 5% debrinated sheep blood. Initially, the strains were subcultured onto a nutrient agar (NA) plate and incubated at 37°C for overnight. The lower surface of the blood agar plate was marked into several squares of 0.7 cm each and labeled with the test strain number. A portion of the colony from the overnight above culture on the NA plate was transferred into one chamber having a corresponding isolate number. The inoculated plates were incubated aerobically at 37°C for overnight. Following incubation, hemolytic strains showed a prominent zone of hemolysis around each colony, and the hemolytic zones were characterized as α and β depending on the extent of hemolysis around each colony [19]. This experiment was repeated twice.

3. Results

3.1. Demographic result

100 urine samples was received & examined during the study period. 80% of the total sample number showed significant growth. Among the urine sample, 27 sample was taken from male patient (culture positive 19) and 73 from female patient (culture positive 61) that is showed in Table 4 and Figure 3.

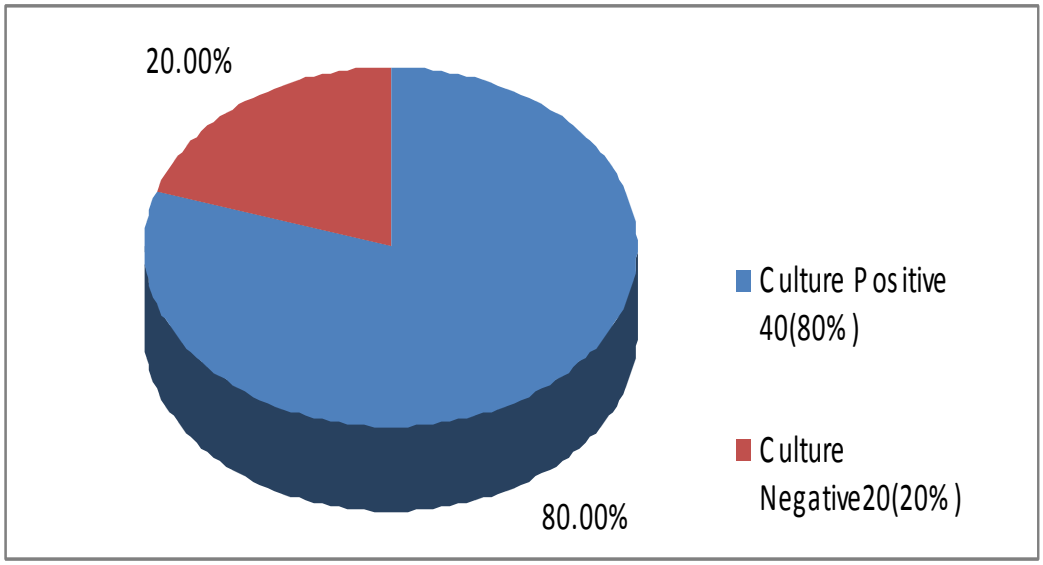


Fig 1 : Rate of culture Positive & Negative urine samples among UTI cases.

Table 3: Distribution of UTI among age and sex groups.

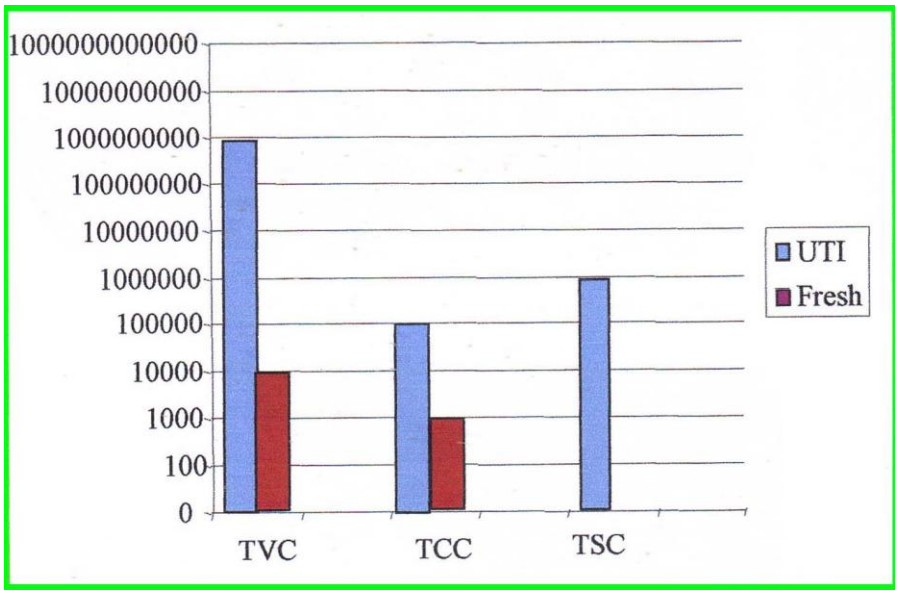
Age	Sample	Sex	Distribution	Culture positive (%)	Culture negative (%)
0-15	1	M	0	0(0/0)	0(0/0)
		F	1	0 (0/0)	100(1/1)
16-50	55	M	15	61.1(9/15)	38.8 (6/15)
		F	40	86.11(33/40)	13(7/40)
51-80	42	M	12	66.66(8/12)	33.33(4/12)
		F	32	93.75(30/32)	6.25(2/32)

3.2 Comparative microbiological analysis of UTI and non UTI patients

After inoculation of samples on Nutrient agar, MacConkey agar and Mannitol salt agar, results were shown in Table 5 and Fig. 4.

Table 4: Comparative microbiological analysis of UTI and non UTI patients

Sample	Average total viable Count (cfu/ml)	Average total Coliform count (cfu/ml)	Total *Staphylococcus* sp.count (cfu/ml)
UTI Patients	$>1 \times 10^8$	1×10^4	$1x10^5$
Non UTI Patients	1×10^3	1×10^2	-



Total Vaible Count (TVC), Total Coliform Count (TCC), Total *Staphylococcus* spp. count (TSC)

Fig 2 : Graphical represent of UTI vs Fresh urine sample.

3.7 Extended spectrum β-lactamases (ESBLs) producer:

From total 100 UTI samples, we randomly selected *E.coli*, *Klebsiella* and *Staphylococcus* spp. from culture plate each sample we randomly selected 2 isolates for each species. Result is shown in table 8. Finally shown that 241 isolates from 600 were found ESBL positive which all are belongs to gram negative bacteria.

Table 7 : ESBL positive strains.

Isolates	**Number tested**	**ESBL Number Positive**	**ESBL Positive Percentage (%)**
E. coli	200	163	86%
Klebsiella spp.	200	78	40%
Staphylococcus spp.	200	-	0%
Total	600	241	63%

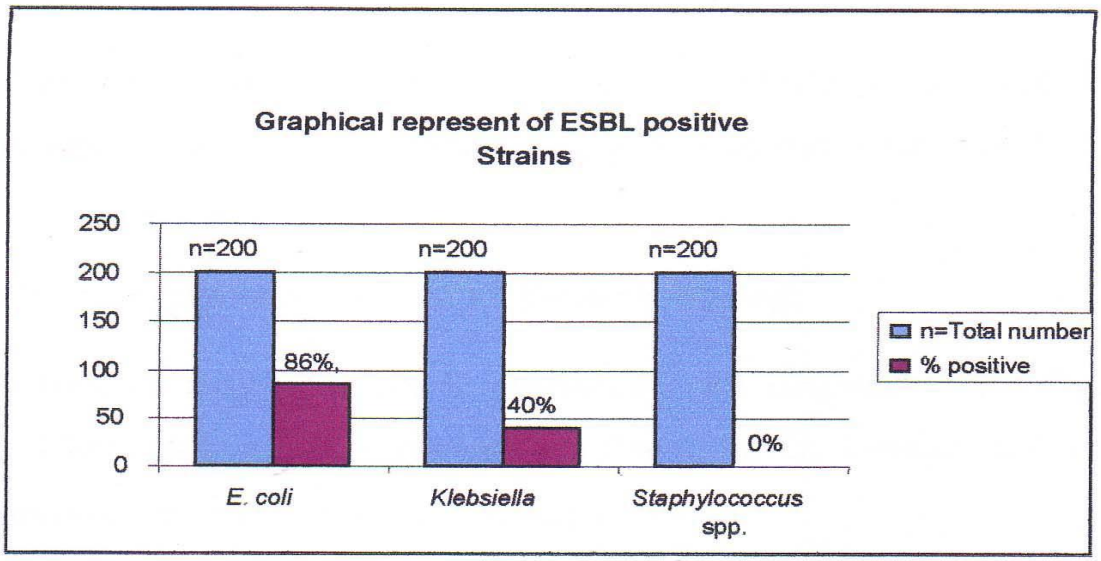


Fig 16: Graphical represent of ESBL positive strains.

Table 8 : ESBL producer isolated from UTI.

Strain ID.	Individual					Combined Disc (Amoxicillin/Clavunic acid) 20/10µg against Cefpodoxime Ceftazidime Aztreonam Cefotaxime Amoxicillin
	Cefpodoxime 10 µg	Ceftazidime 30 µg	Aztreonam 30 µg	Cefotaxime 30 µg	Amoxicillin 20 µg	
E1, E5, E8, E18, E25-45, E50-80, E90-115	R	R	R	R	R	S
E10, E15, E17, E25, E28, E30-40, E54-65, E75-90	R	R	R	R	R	R
K1, K5,K15, K17, K25, K30, K45-55, K60-85	R	R	R	R	R	S
K10, K15, K17, K20-25, K35-45, K65-80	R	R	R	R	R	R
S1, S5, S7, S10, S12, S15, S18, S20, S25, S35-45, S55-80	S	S	S	S	S	-

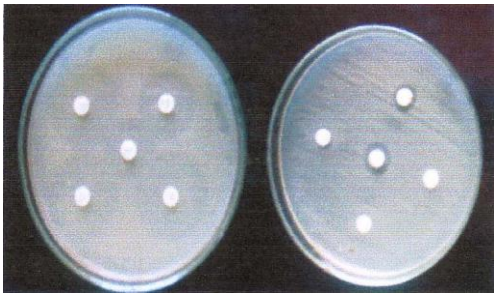


Fig 17 : ESBL detection by disk diffusion method.

3.2 Antibiotic suscepility pattern of most frequent isolates

The isolated organisms from urinary tract infections were tested for antimicrobial suscepti-bility against twenty one commercially available antibiotics Fig-23.

3.2.1 Antibiotic Susceptibility Pattern of *Escherichia coli*

100% E.coli isolates were sensitive to Amikacin, and Imipenem. The figure 20 also indicates that 100% isolates were resistant to Penicillin G, Gentamicin, isolates were resistant to Amoxycillin and Nalidixic acid respectively.

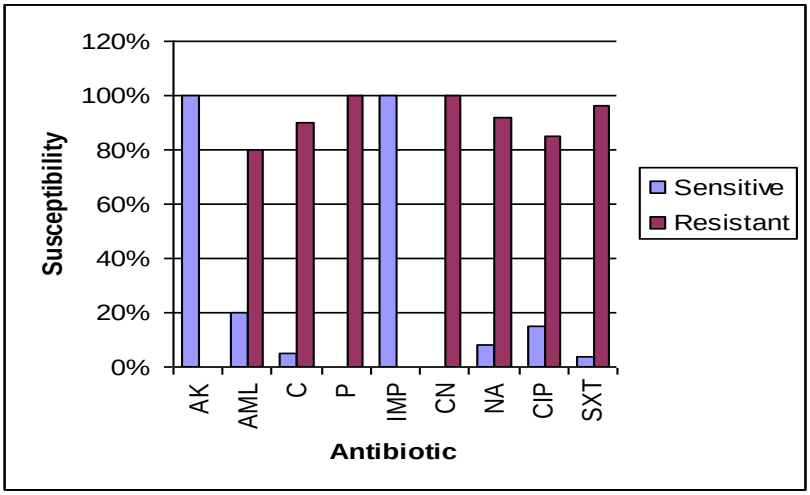


Fig 18: Antibiotic susceptibility pattern of *Escherichia coli*.

3.2.2 Antibiotic susceptibility Pattern of *Klebsiella spp.*

100% *Klebsiella* spp. isolates were sensitive to Amikacin and Imipinem; and showed 100% resistance to Gentamicin and Penicillin which is shown in figure 21.

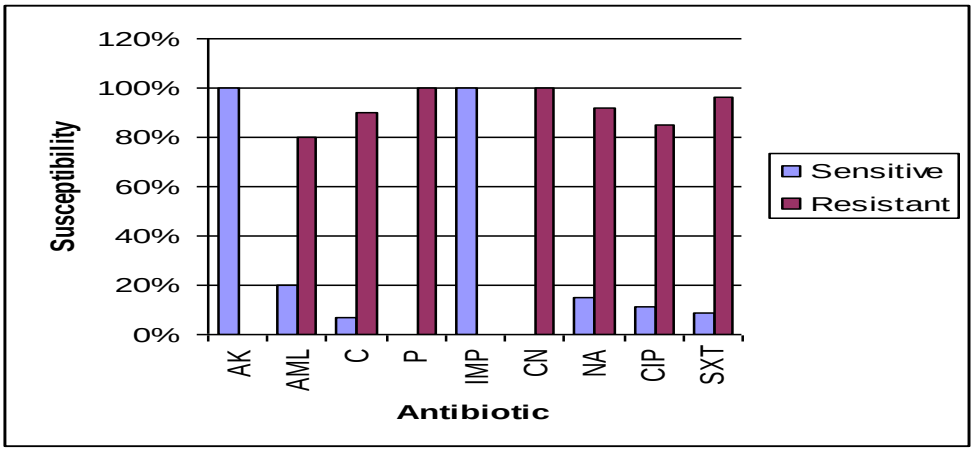


Fig 19: Antibiotic susceptibility pattern of *Klebsiella* spp.

3.2.3 Antibiotic susceptibility Pattern of *Staphylococcus* spp.

100% *Staphylococcus* spp. were sensitive to Amoxicillin, Azithromycin, Cephradine, And resistant to Nafcillin or Cloxacillin and 100% resistant to Vancomycin and Penicillin.

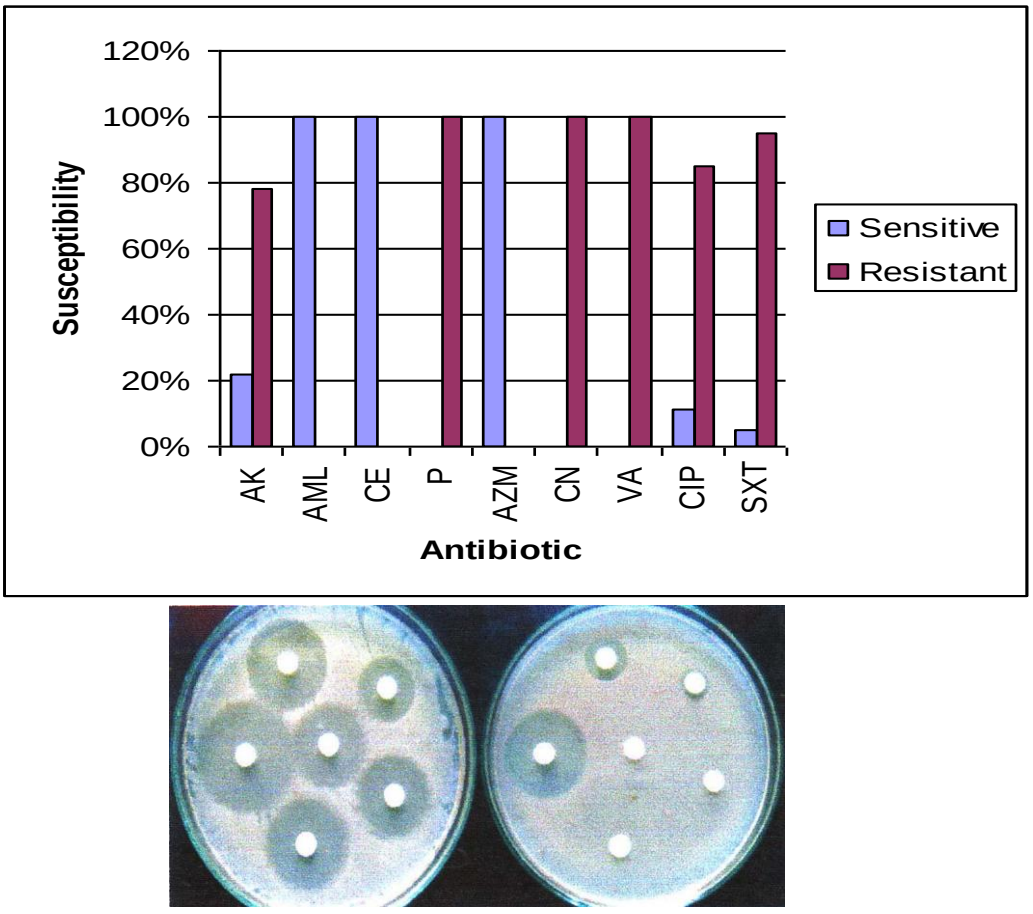


Fig 21: Antibiogram on Muller Hinton agar**.**

3.3 Hemolytic activity

A total 200 strains of each species (*E.coli*, *Klebsiella & Staphylococcus* spp.) were tested for hemolytic activity and variation was found on their hemolytic property that is shown in table 10.

Table 9 : Hemolytic Pattern *E.coli, Klebsiella & Staphylococcus* spp. isolates.

Organism	α-hemolysis (%)	β- hemolysis (%)	γ-hemolysis (%)
E.coli	70(140/200)	5(10/200)	35(70/200)
Klebsiella	40(80/200)	3(6/200)	57(114/200)
Staphylococcus	50 (100/200)	7(14/200)	43(86/200)

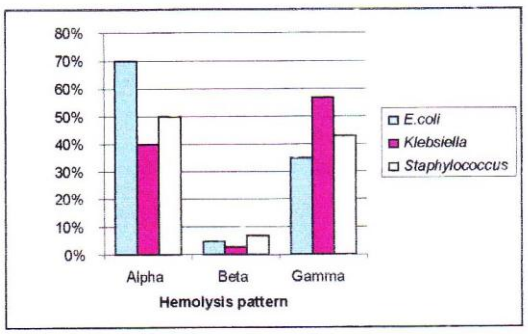


Fig 22: Ratio of the hemolysis pattern of *E.coli* *Klebsiella* and *Staphylococcus* spp.

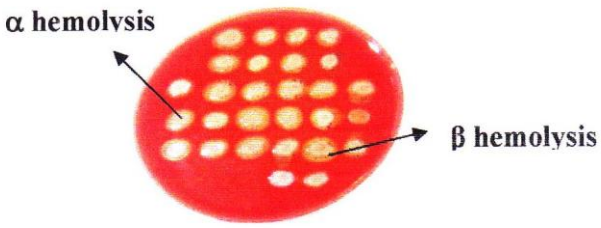


Fig 23: Hemolysis on blood agar.

4. Discussion

UTIs are the commonest infections seen in hospital settings, and the second commonest infections seen in the general population [20] which infection is an important cause of morbidity and mortality throughout the world. Bangladesh is a developing country, and the rate of mortality due to infections diseases is also very high. Moreover the tropical weather of Bangladesh also facilities the occurrence and spread of infection disease more rapidly. Among the different types of infections diseases, urine infection accounts for a large percentage of morbidity and mortality in our country. Quantitative bacteriology is necessary and hence a colony count is necessary to distinguish contamination from true infection particularly where mixed organisms are cultured. Indiscriminate use of antibiotics, lack of proper knowledge and negligence toward diseases increase anti-microbial resistance of common pathogens of urine infection [21]. This infection encompasses a wide variety of clinical entities, the common denomination of which is the presence of microbial invasion of the urinary tract [22]. In almost all cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTI and their resistance patterns may help the clinician to choose the right empirical treatment [23]. Intensive investigations of these infections has been carried out during the past three decades in an attempt to define more accurately the epidemiology, pathogenesis, natural history, treatment and prevention of these infection. These infections result in significant financial and personal costs for both individual patients and the healthcare system [24].The significance of this study is that it provides very crucial information about recent UTIs and their causative agents. It helps to understand the antimicrobial susceptibility pattern of major bacteria that cause urinary tract infection by providing essential information regarding the selection of antibiotic therapy. In addition, the age group distributions o the patients suffering from UTI and the percentage of male and female patients in those groups provide essential information regarding who have a higher prevalence of UTI. Total 100 urine samples collected from community – acquired patient. In this study, urinary samples of children and adult both were included and 80% samples were found to be culture positive. It has been extensively observed in our study that, 88% of female were found UTI culture positive compare to 12% of male culture positive that supported previous results by another researcher that females are more vulnerable to Urinary tract infection then male due to genetic predisposition, physical factors and behavioral factors [25].

Female UTI increased at 16-50 age groups as compared with the male (Table 3). At 51-80 age group male UTI decreased and female UTI increased. These may be due to the different sexual orientation and immunity. The quantitative analysis was carried out by average total vaible count (TVC), total coliform count (TCC) and total *Staphylococcus* sp count (TSC) that is showed in Table 4. This table showed number of *E. coli* is higher in UTIs than *Klebsiella* and *Staophylococcus* spp. Since the isolation and correct identification of *E.coli* & Klebsiela is very

crucial for the cultural based detection purpose that was aimed at this study and for this reason, MacConkey agar media was used in this study.

Extended-spectrum β-lacamases (ESBLs) have emerged as an important mechanism of resistance in Gram-negative bacteria. Increasing resistance to third-generation cephalosporins amongst *E. coli* and *Klebsiella* spp. is predominantly due to the production of wxtended-spectrum b-lactamases (ESBLs). Accurate laboratory detection is important to avoid clinical failure due to inappropriate antimicrobial therapy Organisms that produce ESBL have important therapeutic implication as they show resistant to a variety of antimicrobial agent, including third generation's cephalosporin, broad spectrum penicillin & monobactams. Using the Double disk diffusion method, the combination disc containing Amoxicillin/Clavlanic acid detected the presence of ESBL positive in 241 strains out of 600 (200 isolates for each sperices) tested for *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. We didn't find any β-lactamase producer by *Staphylococcus* but *E. coli* (86%) and *Klebsiella* spp (40%). So this above result give a clear outline that most frequent β-lactamase producer are *E. coli.*

In this study, *E.coli* was sensitive to Amikacin (100%), and Imipenem (100%) showed in figure 20.

In this present study, *Klebsiella* spp. were shown high sensitivity to Imipenem (100%), and Amikacin (100%) showed in figure 21. Similar rates were found in other countries such as Pakistan, Karachi (94.9%) [26, 27] 89% isolates were resistant to Gentamicin (99) which was similar to East Africa (87%) [28]. In this study, 100% *Staphylococcus* spp. were sensitive to Amoxicillin, Azithromycin, Cephradine, Cotrimoxazole. And resistant to Nafcillin or Cloxacillin and 100% resistant to Vancomycin and Penicillin Showed in figure 20.

It was accounted that majority of the organisms isolated were belong to Enterobacteriaceae family. This is consistent with the findings of studies in which *E. coli* was the predominant pathogen isolated from patients with community acquired UTIs [29]. However, *Klebsiella pneuemoniae* are rarely encountered in case of community acquired UTI [25, 29, 30]. In the present study, 19.15% of *Klebsiella* app. Isolates were found to be present among all uropathogens studied.

In the present study, Imipenem was the most potential antibiotic (100% sensitivity) followed by Amikacin (90% sensitivity). Imipenem exerts a bactericidal action by inhibiting cell wall sybthesis in aerobic and anaerobic gram negative bacteria including most strains which are beta- lactamase producer. According to this study, 100% isolates were found to be resistant to Penicillin. All the isolates show differences in resistance between Cephradin, Cefoxitine, Ceftriaxone, Nalidixic Acid, Nitrofurantoin, Trimethoprim-Sulfamethoxazole. Hemolytic property of selected isolates were analyzed on sheep blood agar and our study revealed that 70%, 40% and 50% of *E.coli, Klebsiealla* and *S.aureus* shown alpha hemoysin respectively which is a clear indication of *Uropathogenic E.coli.*

Drug resistance is the arising problem among same strains that can attributed in different diseases. The proper selection of antibiotics for treatment of disease depends on the results of antibiotic sensitivity test. Therefore, the correct detection of drug resistant bacteria is important. Judicious use of antibiotics and good antibiotic policy are needed to limit the emergence and spread of antibiotic resistance in bacteria.

References

[1] Orenstein R, Edward SW. "Urinary tract infections in adults." *American family physician* 1999:59.5: 1225-34.

[2] CDC (Centre for Disease Control). National Nosocomial Infection Study Report. Atlanta, Centre for Diseased Control 1979: pp. 2-14.

[3] Leigh D. Urinary Tract infections. In: Smith GR, Easma Charles SF, eds. Topley and Wilson's principles of bacteriology, virology and immunity. Bacterial disease. 8th edition. Frome and London: Butler and Tanler Ltd. **1990:3:** 197-214.

[4] Cattell WR. Infections of the kidney and Urinary Tract. Oxford University Press **1996:** pp: 1-26.

[5] Foxman B. Epidemiology of urinary tract infection: Incidence, morbidity and ecomic costs. *Dis. Mon*. **2003:** 49: 53-70.

[6] Gales AC, Sader HS, Jones RN. Urinary tract infection trends in Latin American hospitals. *Diagn. Microbial. Infect. Dis.* **2002**: 44: 289-299.

[7] Thomas JG. Urinary Tract Infections. In: *Diagnostic Microbiology* **1995:** pp: 950-969.

[8] Mudur G. Drug resistant cholera in India attributed to antibiotic misuse. *BMJ*. **2000:**321:1368-9

[9] Steven AS. "Genitourinary tract" In: *Current Medical Diagnosis and Treatment*
ELSEVIER New Youk. Amsterdam. Oxford. **1989:** pp 595

[10] Jabbar HA, Moumena RA, Mosli HA, Khan AS, Warda A. Urinary tract Infection in pregnancy. *Annal of Saudi Medicine*. **1991:**11:322-24

[11] Acharya VN. Urinary tract infection – a dangerous and unrecognized forerunner of systemic sepsis. *j Postgrad Med.* 1992: 38: 52-4.

[12] Mathai D, Jones RN, Pfaller MA. Epidemiology and frequency of resistance among pathogens causing urinary tract infection in 1,510 hospitalized patients: a report from the SENTY Antimicrobial Surveillance Program (North America) *Diag Microbiol Infect Dis.* **2001:**40: 129-136.

[13] Karolwsky JA, Jones ME, Thornsberry C, Critchley I, Kelly L, Sahm D. Prevalence of antimicrobial resistance among

pathogens isolated from female outpatients across the US in 1999. *Int J Antimicrob Agents.* **2001:***18:*121-127.

[14] Khan AU, Zaman MS. Multiple drug resistance pattern in Urinary Tract Infection patients in Aligarh. *Biomedical Research.* **2006:** *17 (3): 179-181.*

[15] Vasquez Y, Hand W L. Antibiotic Susceptibility Patterns of Community- Acquired Urinary Tract Infection Isolates from Female Patients on the US (Texas)- Mexico Border. *The Journal of Applied Research.* **2004:** 4: No.2

[16] Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. *Arch Dis Child*. 2003:88(3) : 215-218.

[17] Pelczar, Brian T, et al. "Identifying high-risk patients before head and neck oncologic surgery." *Archives of Otolaryngology–Head & Neck Surgery* 1993:119.8: 861-864.

[18] Bauer A W, Kirby W M M, Sherris J C T, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology* 1966: *45*(4): 493.

[19] Rahim ZS, Khan I, Chopra AK. "Biological characterization of Aeromonas spp. isolated from the environment." *Epidemiology and infection* 2004:132.04: 627-636.

[20] Valiquette L. Urinary tract infections in women. *Canadian Journal of urology* **2001:** 8(1):6-12.

[21] **Nahar SJ, Khanum H, Shimasaki K.** Occurrence of *Escherichia coli* infection among the womwn of Dhaka city. ARPN Journal of Agricultural and Biological Science **2010:** 5(6): 68-73.

[22] Leong CH. Urinary Tract Infection. Published by The Hong Kong College of General Practitioners **1978:** 1(10):1-6.

[23] **Hryniewicz K, Szezypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W.** Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *Journal of Antimicrobial Chemotherapy* **2001:**47: 773-780.

[24] Griebling TL. "Urinary tract infection in women." *Urologic diseases in America* 2007:7: 587-619.

[25] Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta-lactamases among Enterobacteriacea spp. isolated at a tertiary care institute. Indian J. Med. Microbial 2006: 24: 208-11.

[26] **Rizvi MF, Hasan Y, Memon AR, Abdullah M, Saleem S.** Pattern of nosocomial infection in two intensive care units of a tertiary care hospital in Karachi. *j Coll physicians Surg pak.* **2007:**17(3): 136-9.

[27] Sonavane A, Mathur M, Turbadkar D, Baradkar V. Antimicrobial Susceptibility Pattern in Urinary Bacterial Isolates. *Bombay Hospital Journal*, 2008:50:No. 2.

[28] Isaack H, Mbise RL, Hirji KF. Nosocomial bacterial infections among children with servere protein energy malnutrition. *East Afr Med j.* 1992:69(8): 433-6.

[29] Kahlmeter G. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECO. SENS study. *Int.J.Antimicrob. Agents.* **2003:**22:49-52.

[30] Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, Pfaller MA, Doern GV. The SENTRY Study Group (Latin America). Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: Report from the second year of the SENTRY antimicrobial surveillance program (1998*). J. Antimicrob. Chemother* 2000: 45: 295-303.