

**Bacteriological profile and its antibiotic sensitivity in UTI patients in
hospitals and diagnostic centers in Bangladesh**

Submitted by,

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partial fulfillment of the requirements for the degree of
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Declaration

I hereby declare that the thesis is entitled “**Bacteriological profile and its antibiotic sensitivity in UTI patients in hospitals and diagnostic centers in Bangladesh**” is based on my work and it contains no material previously published or written by another person and not accepted for the award of any other degree of a university or other institute of higher education. This research work was done in Thyroid Care and Diagnostic Center, Mohammadpur. The work was completed under the supervision of Akash Ahmed, lecturer in the microbiology program at the Department of Mathematics and Natural Science, BRAC University.

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Approval

The thesis “**Bacteriological profile and its antibiotic sensitivity in UTI patients in hospitals and diagnostic centers in Bangladesh**” submitted by Noshin Nahar (ID: 16326012) of summer16, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of microbiology on October 9, 2021.

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Abstract

Among the common bacterial infection urinary tract infection is the common one in Bangladesh. Women are predominant to be infected by UTI, most commonly the women of reproductive age (18-40 years). This study was designed to determine the prevalence of UTI microorganisms that causes the disease and the sensitivity pattern among UTI patient. This study was done in the Thyroid Care and Diagnostic center in Dhaka during the time period of February 21- June 21. 500 urine samples were collected from UTI suspected patients of different ages and gender. After the samples were collected they were inoculated and then incubated for 24 hours after that (Kirby Bauer's) disk Diffusion method was used to see antibiotic susceptibility of the isolated pathogens. Most of the samples were from Dhaka and Chittagong, among these 500 samples 186 (37.2%) showed positive growth results. Various pathogens were detected, among them, *Escherichia coli* constituted 26.4%. The organisms were found most resistant against common drugs like Amoxyclave, Ctimoxazole, Nalidixic acid, Tetracycline and sensitive against Amikacin, Gentamycin, Imipenem and Meropnem. The prevention from more complexity of UTI is the early detection of the disease and to be treated with local sensitivity pattern of that specific pathogen

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Declaration	2
Approval	3
Abstract	4
Acknowledgement	5
Chapter	1
Introduction	1
Chapter	2
Methodology	4
Specimen collection:	4
Urine sample analysis:	4
Bacteria identification:	4
Sensitivity test	5
Chapter3	
Result:	7
Chapter	4
Discussion	16
References	22

List of Tables

Table 1: Number of samples collected from different districts

Table 2: Gender distribution of culture-positive and negative cases

Table 3: Distribution of cases UTI affecting in different age group

Table 4: Presumptions of the bacteria by Biochemical test results

Table 5: Prevalence of Different Organisms among Culture Positive Cases

Table 6: Antimicrobial sensitivity pattern of the organisms

List of Acronyms

UTI Urinary Tract Infection

Introduction

Urinary tract infection is a provocative reply of the urethra to the attack of pathogenic microorganisms (Neuhauser et al., 2003; Saber et al., 2021). UTI is acknowledged as the most frequent and exorbitant health-related complication globally. This infection is the maximal concern after respiratory tract infection and occupies the second position among the most frequent in children and adults and the most crucial infection in newborns. Again this disease plays an important role in the morbidity and mortality of Bangladesh (Fatima et al., 2020). This is a result of a lack of proper research, faulty diagnostic procedures, abuse of antibiotics, and little or no knowledge about how to prevent it (Yasmeen et al., 2015). UTI is one of the most frequent diseases that appear at different ages and ill-treatment of it can give birth to severe difficulty for example disarray of urinary tract hypertension, uremia, early delivery, and even abortions in expecting women (Zare et al., 2018). Nevertheless, proper knowledge of UTI and related risk factors help to take the sickness under control fast. (Angoti et al., 2016; Odoki et al., 2019).

The prevalence of UTI differs according to gender, age and other factors like poverty, literacy etc. it has proven that females have more chances to be infected by UTI than men. Such sexual intercourse can introduce bacteria in the vagina of women simultaneously which leads to UTI (Prabakar & Rajasekaran, 2017). All most 1 woman out of 3, must have at least 1 experience of UTI which is healed through antibiotics, by age of 24 years and approximately 50% of all women will face at least 1 episode of UTI all along their period (Soiza et al., 2018; Tan & Chlebicki, 2016). It is because of the short urethra and proximity to the anal opening than male (Daoud & Afif, 2011; Mirzarazi et al., 2013; Priyadharsini et al., 2014; Tajbakhsh et al., 2015; Zahera et al., 2011). On the other hand if men get UTI infection, this could turn into a very complicated disease because the prostate is often involved (Geerlings, 2016). Moreover, those

who are at their reproductive age which is 20 to 30 are more susceptible to this disease (Soiza et al., 2018; Tan & Chlebicki, 2016) Previous study shows UTI prevalence ratio differ very much depending on various reasons such as poverty, literacy, sanitation infrastructures that come into place (Wanja et al., 2021). For example, more than 10.8 million patients recorded a prevalence ratio of 16.7% in a study in USA (Sammon et al., 2014). Comparable prevalence ratio (11.2%) were also recorded in Asia, a clear sign that UTI prevalence ratio vary across the world (He et al., 2018).

Usually, UTI is caused by different gram-negative and gram-positive bacteria, which involve *Staphylococcus* sp., *Enterococcus* sp, *Streptococcus* sp. Gram-negative consist a big number of oxygen-loving Bacilli for example *Escherichia coli*, *kebshiella* sp, *Enterobacter* sp, *Citobacter* sp, *Protious* sp. and *Pseudomonas* sp. Among this 80-90% of UTI is caused by *Escherichia coli* (Foxman & Brown, 2003; Kulkarni et al., 2017) and *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus faecalis* are also the maximum regularly isolated. (Millner & Becknell, 2019; Ouno et al., 2013). again Enterococcus isolates cause 2.3% of UTI which is called antibiotic-resistant Opportunistic pathogens (Mollick et al., 2016). Nevertheless, in previous studies, other nonbacterial agents like fungi, viruses, and parasites are also reported as UTI-causing agents. (Wanja et al., 2021; Woldemariam et al., 2019)

Many types of antibiotics are suggested for UTI treatment, they all are similar in their mood of action, for example forming obstacles in cell wall synthesis of the bacteria, inhibition of protein synthesis, interference with nucleic acid synthesis, interference with signaling pathways involved in biosynthetic processes, and disruption of cell membrane integrity. (Al-Naqshbandi et al., 2019) The bacteria isolation in urine in a significant load persistent with the infection. So, to treat the patient with an appropriate antimicrobial agent, the isolation and identification of the pathogen are very crucial. For a urinary tract, infection antibiotics are the specific

treatment, but the resistance to antimicrobial agents is extensive in UTI isolated bacteria in the world. It is very common especially in developing countries like Bangladesh. Doctors need to have knowledge about the local arrangements of microbial sensitivity and financial capability for convenient drug choice to provide appropriate antibiotic treatment (Yasmeen et al., 2015). Therefore, the purpose of this study was to determine the prevalence of UTI and to identify the prevalent microorganisms and the recent trend of their antimicrobial susceptibility patterns of suspected UTI patients all over Bangladesh.

Methodology

Specimen collection:

A fresh discharged 5-10 ml of clean urine samples were collected using sterile plastic containers. Patients were well instructed about how to collect urine. After collection, the urine samples were stored during the transportation using ice packs. All the samples were evaluated soon after reaching the laboratory to secure the pathogenic organism present in the urine and were isolated. This also eliminates the overgrowth of the organisms.

Urine sample analysis:

All the 500 samples went through microscopic observation. First, the samples were centrifuged at 2000 to 3000 rounds for 5 minutes, which separate the urine from other impurities. Then from the supernatant one drop is taken and placed over a glass slide and put coverslip on the drop to observe under a microscope. It was done to determine their there was any pus cell, white blood cell, red blood cell, bacteria, yeast, or crystals.

Bacteria identification:

About 5 microliter of the sample was taken and inoculated in the Hichrome UTI agar and MacConkey agar media. As different bacteria give different morphology cases of colors of the colony size, form, elevation, and margins, these were observed to find out the organism primarily. After that, to confirm the bacteria gram staining was performed. Slides had been organized from every exceptional colony determined at the plates and gram staining was done. The results consisting of the gram-positive or gram-negative bacteria biochemical tests such as triple sugar iron, motility indole urease test, catalase were also done to confirm the bacteria, all these processes were done following previously done techniques. (Wanja et al., 2021)

Sensitivity test

The susceptibility of the completely remoted organisms to choose antibiotics which have been typically used to deal with pathogens turned into examined through the Kirby-Bauer Method. Sterile Mueller-Hinton agar plates have been organized and Various antibiotic discs have been selected. Identified pathogens have been inoculated in peptone water tubes one at a time and incubated at 37degree C for 1 hour. Using sterile cotton swabs for every check organism, incubated check organisms have been inoculated at the floor of Mueller-Hinton agar plates 3 times, rotating the plate six hundred after every streaking. Finally, the swab turned into a run around the brink of the agar. The cultures have been allowed to dry at the plate for 5-10 minutes at room temperature. Various antibiotic discs have been positioned at the floor of the agar medium through lightly urgent use of sterile forceps at the pinnacle of the discs (for higher touch and powerful diffusion of the antibiotics into the medium). Make certain that touch is made among the antibiotic disc and the culture. The plates have been incubated in an inverted function for 16-18 hours at 37-degree C. The antibiotics typically used for the remedy of UTIs are decided on for the disc sensitivity method. The antibiotics are as follows: amikacin, ceftazidime, ceftriaxone, ciprofloxacin, cotrimoxazole, cefoxitin, gentamycin, imipenem,

meropenem, netilmicin, nitrofurantoin, levofloxacin, cefuroxime, doxycycline, nalidixic acid, tetracycline, amoxiclav, cefotaxime.

Chapter3

Result:

For this study urine samples from different diagnostic centers and hospitals from all most all the districts of Bangladesh have been collected.

Table 1: Number of samples collected from different districts.

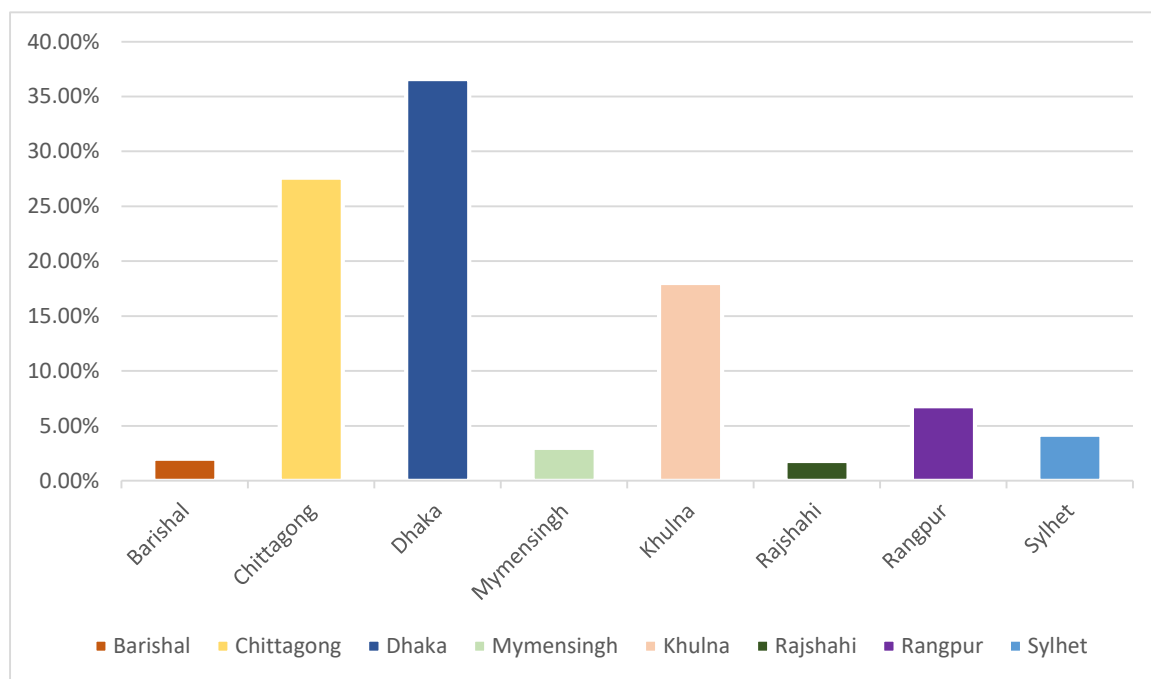


Figure 1: Number of samples collected from different districts

Division	Total Test	Percentage
Barishal	10	2%
Chittagong	138	27.6%
Dhaka	183	36.6%
Mymensingh	15	3%
Khulna	90	18%
Rajshahi	9	1.8%
Rangpur	34	6.8%
Sylhet	21	4.2%
	500	

In this study, there were 500 samples and 186 of them showed positive results. The 500 samples contained 113 urine samples from men and 387 were from women. Among 186 positive samples, 27 male samples showed positive results and 159 were women.

Table 2: Gender distribution of culture-positive and negative cases

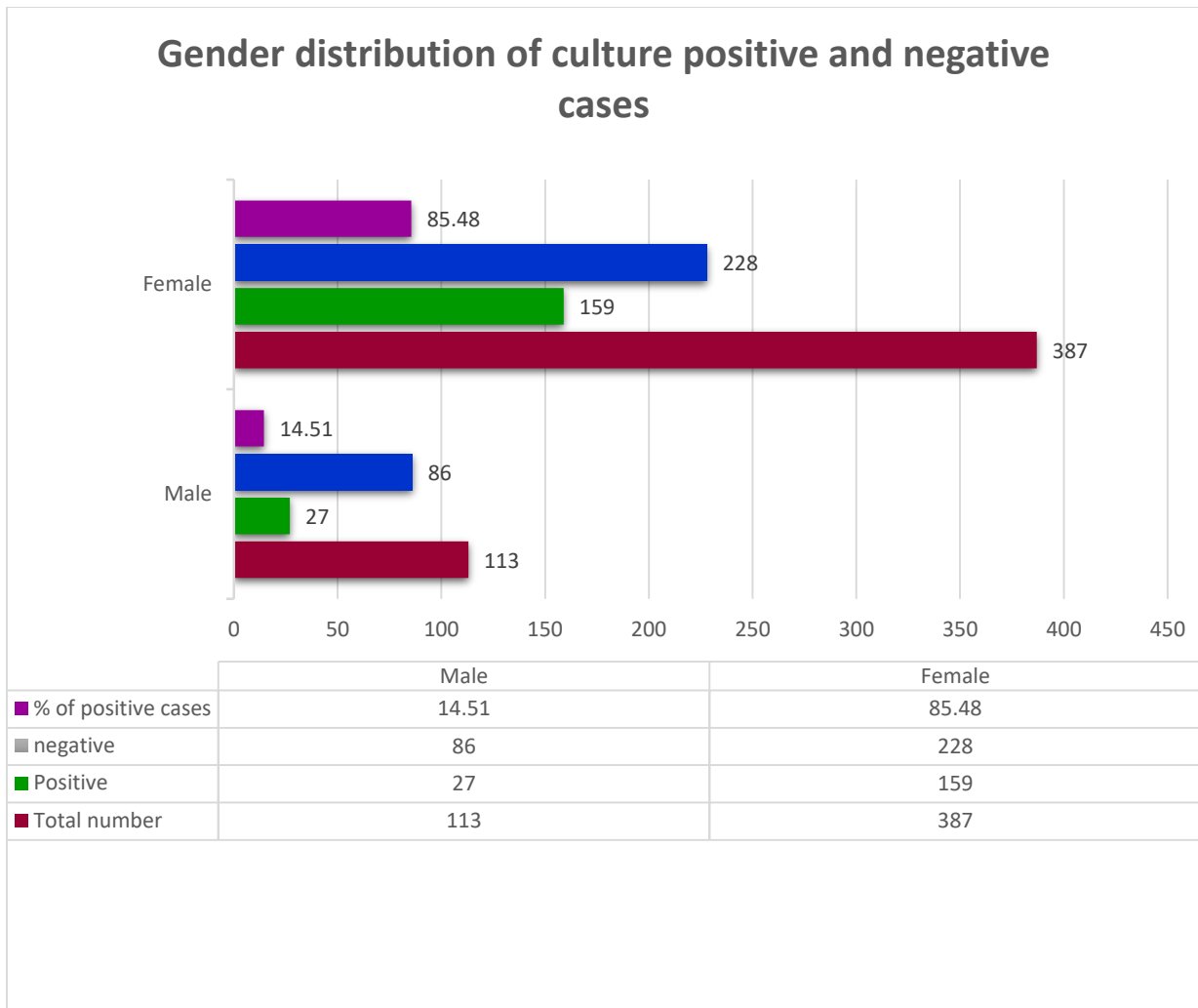


Figure 2: Gender distribution of culture-positive and negative cases

The highest infection rate was found in women. The highest age range is 21-30 years and 31-40 years are the second highest.

Table 3: Distribution of cases UTI affecting in different age group

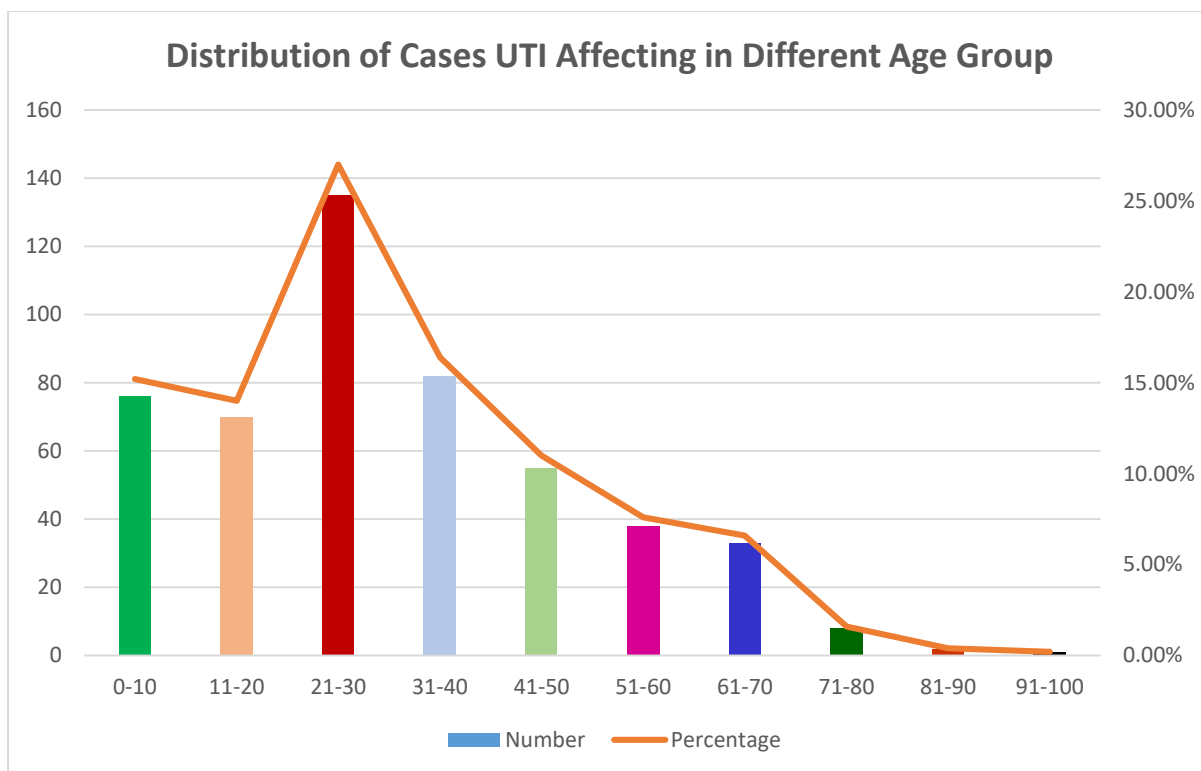


Figure 3: Distribution of cases UTI affecting in different age group

Age group	Number	Percentage
0-10	76	15.2%
11-20	70	14%
21-30	135	27%
31-40	82	16.4%
41-50	55	11%
51-60	38	7.6%
61-70	33	6.6%
71-80	8	1.6%
81-90	2	0.40%
91-100	1	0.20%
Total	500	100%

Among the positive culture cases 124(24.8%) showed growth of *Escherichia coli* and 42(8.4%) showed *klebsiella sp* growth. Other organism's percentages are given bellow

Table 4: Presumptions of the bacteria by Biochemical test results

Test	E.co li	Klebshiel la sp.	Pseudomo nas sp	Enterococc us sp.	Acina to bactor sp.	Proteo us sp.	Staphylococ cus aureus
Gram stain	-	-	-	+	+	-	+
catala se	-	+	+	-	+	-	+
Oxida se	+	+	+	-	-	+	-
indole	+	-	-	-	-	+	-
urease	-	+	+	-	-	+	-
motilit y	+	-	+	-	-	+	-
TSI	+	+	-	-	-	+	-
MR	+	-	-	-	-	+	+
VP	-	+	-	+	-	-	+
Citrat e	-	+	+	-	+	-	-

Table 5: Prevalence of Different Organisms among Culture Positive Cases

Organism	Positive cases	Percentage
<i>Acinetobacter spp.</i>	1	0.53
<i>Klebsiella spp.</i>	42	22.58
<i>Escherichia coli</i>	132	70.96
<i>Enterococcus spp.</i>	1	0.53

<i>Proteous spp</i>	2	1.075
<i>Pseudomonas spp</i>	6	3.22
<i>Staphylococcus aureus.</i>	2	1.075
<i>Proteous spp</i>	2	1.075
<i>Pseudomonas spp</i>	6	3.22
<i>Staphylococcus aureus.</i>	2	1.075

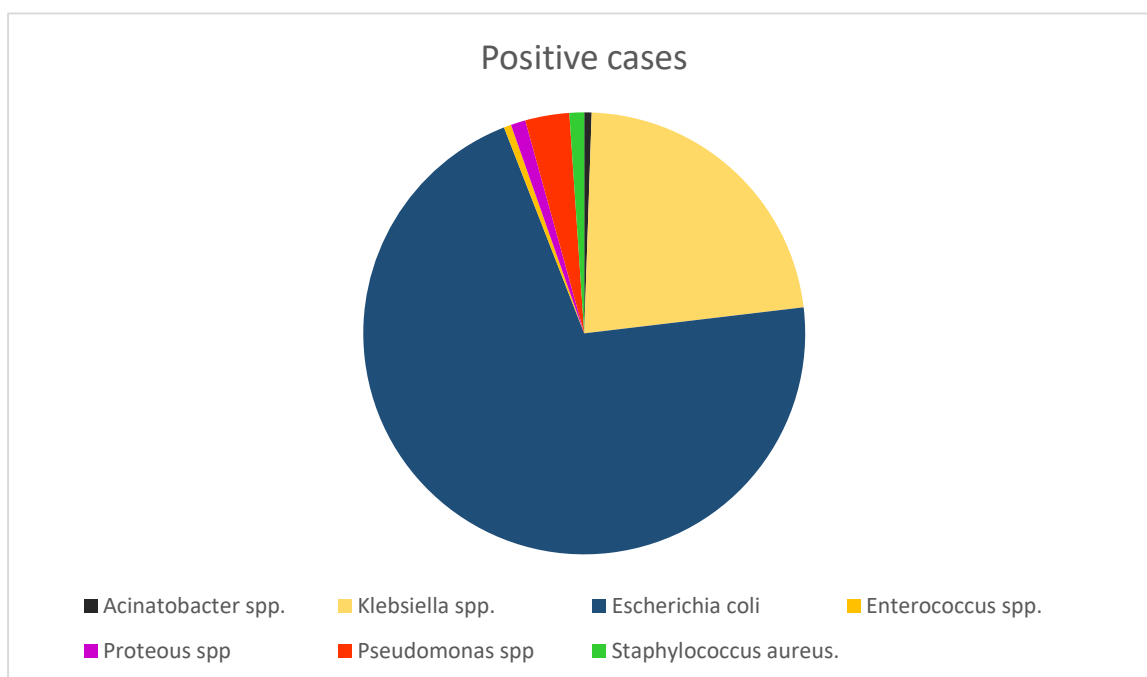


Figure 4: Prevalence of Different Organisms among Culture Positive Cases

For the sensitivity test Amikacin, Tetracycline, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Cotrimoxazole, Cefoxitin, Gentamycin, Imipenem, Meropenem, Nalidixic Acid, Netilmicin, Nitrofurantion, Levofloxacin, Amoxyclave, Doxycycline, Cefotaxime were used and the sensitive rates are given in table 4

Table 6: Antimicrobial sensitivity pattern of the organisms

	<i>Escheri chia coli</i>	<i>Klebsi ella spp.</i>	<i>Pseudom onas spp.</i>	<i>Staphyloc occus aureus.</i>	<i>Acinato bacter sp.</i>	<i>Prote ous Sp.</i>	<i>Enteroc occus sp.</i>
Amikacin	1.9%	0%	1.07%	7.2%	0	0	0
Tetracycli ne	85.71%	90.1%	100%	98.36%	100%	100%	100%
Ceftazidim e	20.78%	23.81 %	33%	50%	100%	50%	100%
Ceftriaxon e = CRO	10.39%	19.05 %	100%	95%	100%	100%	100%
Cefuroxim e = CXM	79.23%	95.46 %	100%	99%	100%	50%	100%
Ciprofloxacin = CIP	33.77%	52.39 %	50%	0%	100%	100%	100%
Cotrimoxazole	94.81%	97.73 %	100%	0%	100%	100%	100%
Gentamycin	0%	0%	2%	1.22%	0%	0%	0%
Imipenem	0%	0%	17%	5.5%	0%	0%	0%

Meropenem	0%	0%	16.67%	1.7%	0%	0%	0%
Nalidixic Acid	100%	95.46%	95%	100%	100%	100%	100%
Netilmicin	9.10%	0%	33.34%	96%	100%	100%	100%
Nitrofurantion	7.8%	81.82%	100%	98.9%	100%	100%	100%
Levofloxacin	35.07%	50%	50%	99%	100%	100%	100%
Amoxiclavate	100%	95.46%	98.9%	50%	100%	100%	100%
Doxycycline	51.95%	56.82%	100%	100%	100%	100%	100%
Cefotaxime	19.49%	20.46%	100%	0%	100%	100%	100%

Discussion

In medical institution settings UTI has taken into consideration the maximum frequent contamination. Around the globe, each 1/3 of girls suffer from UTI at some factor in their lifetime. Untreated and partly handled UTI can also additionally change into a whole lot of headaches and might create a great chance to turn into a complicated disease. It implies the most important significance of early detection and suitable remedy of UTI amongst sufferers at any age. In our observation, we attempted to discover the incidence of UTI amongst males and females from every age group.

It is said that UTI is mainly a disease of the female because compared to adult males, females have a short period of the urethra, absence of prostatic secretion, pregnancy, and easy infection of the tract with fecal flora (Singh 2016). At present, this study shows UTI infection rate in women is higher (85.48%) compared to men (14.51%) which is similar to the report by (Akter et al., 2013; R Parveen et al., 1970; Yasmeen et al., 2015) where they reported that the prevalence of UTI was predominant in female than male. Moreover, the paper by (He et al., 2018) mentioned that UTI infection was higher in women than men, the percentage was 24.13% v/s 3.67%. Again the high peak of females of having the UTI was also mentioned in (Nerurkar et al., 2012; Sammon et al., 2014). Furthermore, adult women have a higher infection (12.6%) than men (3%)(Foxman & Brown, 2003). Additionally, 0% prevalence was found in 1234 men who were under 50 years in a Japanese study. Another report by Wilson et al shows 1..5% prevalence in 405 men whose age was between 20 to 70(Nicolle, 2003).

In this study, among 500 samples 186 (37.20%) samples showed positive growth results. As it shows only 37.2 % of positive results, this indicates that treatment of UTI based on clinical symptoms can provide us with the false result.(Al-Naqshbandi et al., 2019; Medina-Bombardó & Jover-Palmer, 2011) This rate is closer to the report by (Ju et al., 2020) where they worked with 300 samples and 126 (42%) showed a positive results. However, this study differs from the study done by(R Parveen et al., 1970; Wanja et al., 2021; Yasmeen et al., 2015) where they showed 27.96%, 20.73%, and 27.6% positive growth respectively which is lower than this study. A higher frequency of UTI is reported by(Akter et al., 2013; Al-Naqshbandi et al., 2019; Nerurkar et al., 2012; Saber et al., 2021) which was 57.30%, 41.20%, 60%, 79.9% respectively.

Most of the positive cases from whom the urine samples were collected were in the age range of 21-30 years which is the highest of the all other 10 age ranges, This got here in settlement with preceding research counseled widespread correlation among misdiagnosed and symptom handiest primarily based totally method for UTI detection which is similar to the report by (Akter et al., 2013; A. Hossain et al., 2020; R Parveen et al., 1970; Yasmeen et al., 2015), where they have also found a higher rate of positive cases in this age range, probably due to their initial exposure to sex because most of the women of this age range get married in Bangladesh or they lack the knowledge of the personal hygienic practice.

Moreover, this report shows that *Escherichia coli* are the predominant organism that has been isolated from most of the positive growth samples. Among the 37.2% of positive growth, 24.8% are *E. coli* isolated from the urine specimen followed by *Klebsiella* sp (8.4%), *Pseudomonas* sp. (1.2%) *Staphylococcus aureus* (0.40%), *Enterococcus* sp (0.20%), *Proteous* sp (0.40%). Similarities can be seen in the report by (Angoti et al., 2016; Arasa-Ouno et al., 2013; Lutter et al., 2005; Nerurkar et al., 2012; R Parveen et al., 1970; Yasmeen et al., 2015; Zahera et al., 2011) they have also found the predominance of *Escherichia coli* among the positive cases. Moreover, in this study *Klebsiella* sp is holding second place among the isolated organisms which is similar to the report by (Setu et al., 2017) and unlikely to the report by (Akter et al., 2013) the second highest was *Staphylococcus aureus*, in (Yasmeen et al., 2015) *Pseudomonas* sp. It was also said that *Escherichia coli* which is uropathogenic do have some special characteristics that helps them to take advantages of the bladder environment. (Foxman, 2014)

UTI is a common urethral disease affecting women than men and the antibiotic sensitivity pattern of Bangladesh is quite concerning. In this study *Escherichia coli* was found 100% sensitive to amikacin, gentamycin, imipenem, meropenem. Then nitrofurantoin (92.20%), netilmicin (90.90%), ceftriaxone (89.61%), cefotaxime (80.51%), ceftazidime (79.22%), and *E. coli* was most resistant to commonly used oral drugs like nalidixic acid 100%, amoxyclave 100%, cotrimoxazole (94.81%), tetracycline (85.71%) levofloxacin (35.07%), cefuroxime (79.23%), doxycycline (51.95%). The similarity was found with the report by (Parveen & Rahim, 2018), *E.coli* was most sensitive to imipenem (100%). Amikacin (87.5%) and nitrofurantoin (83.33%) and most resistant to nalidixic acid (79.16%), cefuroxime (75.0%) and cotrimoxazole (54.16%). This also correlates with (Akter et al., 2013; Setu et al., 2017) of Bangladesh where the susceptibility rate to amikacin and imipenem remained between 93.0 to 100%.

In different region of the world resistance pattern of antimicrobial agents which are used to treat UTI are different. UTI is common in Bangladesh and it has been reported that all age groups, both hospitalized patients and outpatients do suffer from UTI(M. D. Hossain et al., 2014).From this study we can see raising microbial resistance against common oral drugs such as nalidixic acid, cotrimoxazole, tetracycline, levofloxacin, cefuroxime, ciprofloxacin which are very useful for the treatment of many grams positive and gram negative bacterial infection as well as UTI in Bangladesh. if we compare the previous reports with this report we can see these organisms have become more resistance to regular use drugs, such as *E. coli* was 63.31% resistant in 2017 (Setu et al., 2017), 79.16% in 2018(Rezina Parveen & Rahim, 2018) and 100% at present against nalidixic acid. If we see cotrimozole, *Escherichia coli* was resistant 54.54% in 2015 (Yasmeen et al., 2015), 53.33% in 2016, 57.94% in 2017(Mollick et al., 2016; Setu et al., 2017), 94.81% in 2021. This comparison between the present antibiotic resistance result with previous ones can support the above mentioned statement.

UTI causing pathogens are showing an raised resistance to regularly used drugs in studies from India, Bangladesh and Nepal (Shilpi et al., 2013).These findings are alarming with regard to the selection of powerful healing alternatives to treat UTI. Such as nalidixic acid showed 63.31% resistance in (Setu et al., 2013) which has become 100% resistance against *Escherichia coli* in 2021. Here it is obvious that the important organisms are becoming dramatically proof against maximum antibiotics in direction of time that is probably because of indiscriminate use of antibiotics by UTI sufferers and incomplete direction of antibiotic use by the sufferers displaying a remaining destiny of endangering the mankind in remedy perspective.

Antibiotic resistance has been called an emerging disease by The World Health Organization (WHO). Bacteria can be resistant inherently or may achieve it. Because of the rapid rise of antibacterial resistance, health workers are searching for new and more effective drugs. (Kalsoom BANO, 2012). For example, health workers are testing different types of new treatments to authorize non-carbapenem therapy of UTI which is caused by ESBL(Extended-spectrum beta-lactamases are a type of enzyme or chemical produced by some bacteria) producing organisms. Other reports showed that for non-bacteremic UTI carbapenems substitute like, piperacillin-tazobactam, gives good treatment result(Dhillon & Clark, 2012)The first crucial reason in raising microbial resistance is inaccurate use of antibiotics. The other reasons are faulty and illogical antibiotics instruction. The important conditions of balanced antibiotic prescription are considering time, the correct dose and presence of authority. Studies have reported that 30%–60% of the prescribing and use of antibiotics has been incorrect(Mihankhah et al., 2017; Prabakar & Rajasekaran, 2017). Antibiotic abuse because of smooth availability & working towards incomplete antibiotic routine because of poverty has significantly promoted the dissemination of multidrug-resistant bacteria. The Highest chances of resistance of E. coli inflicting UTI had been observed towards oral capsules like nalidixic acid 100%, amoxyclave100%, cotrimoxazole (94.81%), tetracycline (85.71%), and had less resistance towards much less normally used capsules like amikacin, gentamycin, imipenem, meropenem (100%), nitrofurantoin (92.20%), netilmicin (90.90%). The physicians need to have correct and recent information about the resistance percentage in that particular area where they are practicing for effective evaluation on of their patients of antimicrobial resistance.(Tarannum Haque et al., 2020). This is very important because the resistance of antibiotics can differ by institution, state and region, and also by other factors for example patient age, gender, outpatient or inpatient status, previous antimicrobial therapy,

catheterization, and/or recent hospitalization. But, this information is not available to the physicians leading to the wrong prescription of medicines(Karlowsky et al., 2001).

Untreated UTI can lead to complicated situations for patients. The main pathogenesis of UTI-causing bacteria is the complicated cooperation between an organism and, the environment and the host. The symptoms may vary with age and location. UTI can cause high blood pressure, kidney damage, and sometimes even death. UTI is categorized into two types: complicated and uncomplicated.(Arasa-Ouno et al., 2013). Complicated UTIs are pyelonephritis, cystitis, renal carbuncle, urethritis and prostatitis which may lead to hospitalization and intravenous antibiotic(Brown et al., 2005; Sammon et al., 2014). An uncomplicated UTI occurs with the person having normal urinary tracts.(Arasa-Ouno et al., 2013). If not treated the uncomplicated UTI can turn into complicated UTI. Uncomplicated cystitis, pyelonephritis causes high temperature, dysuria, persistent abdominal pain, nausea or vomiting and sometimes tachycardia, these are the indication that the patient need to be hospitalized(Foxman et al., 2003). More over in previous studies it is said that the ratio of pyelonephritis to cystitis was almost 1:28 in women age ranging from 15 to 35 years(Foxman, 2014; Tseng & Stoller, 2009). In case of male patients obstructive uropathy is more common(Tseng & Stoller, 2009), this combine with frequent and constant UTIs(Sammon et al., 2014; Tseng & Stoller, 2009).

In conclusion, we can say from this study the isolated bacteria in UTI have a higher antibiotic resistance ratio for antibiotics which are commonly used as oral drugs for UTI patients. This makes the treatment of UTI difficult, doctors are left with not so many options of drugs that can be used for the observational treatment of UTI. Accordingly, the doctors and other health care workers need to re-evaluate the empiric treatment of UTI. Urinary tract infections can be prevented by drinking plenty of water and maintaining hygiene in the first place.

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