



Incidence and antibiogram of UTIs at a tertiary teaching hospital of Chittagong city

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Abstract

Urinary Tract Infections (UTIs) are the common and topmost nosocomial infection, posing threat for both the patients and associated personnel in hospital. The study was conducted to determine the prevalence of bacteria in urine samples and the antibiotic sensitivity pattern of the clinical isolates of UTI causing uropathogens. A cross-sectional study was carried out on 186 patients admitted to Chattagram Maa-O-Shishu Hospital from November, 2017 to April, 2018. The mid-stream urine specimens were collected from the patients through aseptic way and then cultured on McConkey, Blood and HiChrome UTI media. The specimens that showed $\geq 10^5$ cfu/ml were regarded as positive for the bacterial infection and antimicrobial susceptibility tests (Kirby-Bauer disk diffusion method) were performed on those isolates. Among the 91 culture-confirmed positive UTIs (29.6 % male and 60.9% female), 21 and 70 UTIs were due to the gram positive and gram-negative bacteria, respectively. The main responsible pathogens were *Escherichia coli* (51.6%), *K. pneumoniae* (22%), *S. aureus* (13.2%), *P. mirabilis* (5.5%), *Enterococcus spp.* (4.4%) and *P. aeruginosa* (3.3%). It is suggested that four factors include awareness on UTI, recognition causative factors, prevention and treatment should be emphasized highly for ensuring safe public health.

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Introduction

Hospital infections have stood as serious global healthcare concerns for the growing antimicrobial resistance among the pathogens that cause hospital-acquired infections. This infection can develop symptoms during the hospital stay (Agbagwa and Ifeanacho-Enaka, 2015). Urinary Tract Infection (UTI) is one of such community-acquired and healthcare-associated infections due to the emergence of multi-drug resistant uropathogens (Alo *et al.*, 2015; Sanjee *et al.*, 2017). It is the most common bacterial infectious ailment and about 10% people may encounter it during their life time (Shanthi and Kayathri, 2012; Singh *et al.*, 2011). It is considered as one of the most important causes of morbidity among the mass people, leading to long term complications. Globally, it is the second most common cause of hospital visits (Kibret and Abera, 2014; Santosh and Siddiqui, 2017). UTI is defined as the invasion of pathogens to the urinary tract tissues outstretching from the renal cortex to the urethra. When a bacterium gets into the bladder and subsequently multiplies, then it may cause UTI (Onuoha and Fatokun, 2014; Sanjee *et al.*, 2017). All individuals may be susceptible to UTIs; however, the prevalence of infection differs with age, sex and certain predisposing factors (Noor *et al.*, 2013). The common mode of UTI is the ascending route, through which the organisms of the bowels flora contaminates the urethra, ascends to the bladder and migrates to the kidney (Amengialue *et al.*, 2013; Hines *et al.*, 2015).

The UTI occurrence depends on several factors those confirm the presence of bacteria ($\geq 10^5$ cfu/ml) in the urine. These bacteria cause UTI and if not treated, then the infection will spread and cause serious damage to the patient (Derese *et al.*, 2016; Mihankhah *et al.*, 2017). The boys are at greater risk but later the girls become sufferer of this infection. The pre-pubertal girls are more prone to UTI than the pre-pubertal boys (Onuoha and Fatokun, 2014). UTI is the most common infection in 20%-35% women, especially pregnant (Onuoha and Fatokun, 2014; Sanjee *et al.*, 2017). It occurs much more frequently in women than men with two exceptions, infants and the catheter related infections due to the proximity of

the urethra to the anus (Dielubanza *et al.*, 2011; Singh *et al.*, 2011). In young women, sexual activity is the cause of 75%-90% bladder infections, with the risk of infection related to the frequency of sex (Agbagwa and Ifeanacho-Enaka, 2015; Badri and Mohamed, 2017). The natural history of UTI in children has changed due to the introduction of antibiotics and the improvements in healthcare (Patel and Garala, 2014). The number of people diagnosed with UTI are approximately 150 million worldwide which costs healthcare facilities about USD6 billion for expense (Kashef *et al.*, 2010; Kibret and Abera, 2014). The empirical treatment of UTIs has become difficult and unpredictable due to the dearth of alternative effective antibiotics (Alo *et al.*, 2015; Badri and Mohamed, 2017). The delay in diagnosis and treatment of UTI can cause significant morbidity. Thereby, the physician's knowledge about the symptoms, UTI causing microbes and effective antibiotics is an urgent to combat against the multi-drug resistant bug (Patel and Garala, 2014; Pouladfar *et al.*, 2017).

The prime etiological agent of UTIs is *Escherichia coli* followed by other Gram-negative bacteria such as *Klebsiella sp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter sp.*, and *Serratia sp.*; while Gram-positive bacteria like *Enterococcus sp.* and *Staphylococcus sp.* including fungus like *Candida sp.* (Bhatt *et al.*, 2017; Santosh and Siddiqui, 2017). *Enteric bacteria* are the most frequent cause of UTIs, although the distribution of the pathogens that cause UTIs is changing with time, however, about 50% of the infections are caused by *E. coli* (Shanthi and Kayathri, 2012; Swetha *et al.*, 2014). Antimicrobial resistance is an emerging global threat for public health due to the antibiotics abuse. It is considered as the curse of modern medicine (Bhatt *et al.*, 2017; Mihankhah *et al.*, 2017). Antibiotic resistance of UTI is aggravated by the increased use of muddled empirical treatments, mainly in the economically developing countries like Bangladesh (Gessese *et al.*, 2017; Okonko *et al.*, 2009). The selection of antibiotics should be based on the antibiotic susceptibility pattern and the periodic

evaluation of antimicrobial activity of different antibiotics is essential (Patel and Garala, 2014). Recently, UTI has become more complicated and difficult to treat because of the appearance of uropathogens resistant to the commonly used antimicrobial agents (Bhatt *et al.*, 2017; Gessese *et al.*, 2017). For the empirical treatment of UTIs, it is highly important to have knowledge on the antimicrobial resistance patterns of the common uropathogens and the subsequent treatment are thus required to minimize the urinary diseases (Noor *et al.*, 2013; Sanjee *et al.*, 2017). Therefore, the study was designed to determine the prevalence of bacteria in urine samples of the patients and the antibiotic resistance and sensitivity pattern of UTI causing microbes in Chattagram Maa-O-Shishu Hospital (CMOSH), Chittagong.

Materials and methods

Study design and sampling

A well-designed experiment was performed on collected urine specimens at tertiary care and teaching hospital “Chattagram Maa-O-Shishu Hospital (CMOSH)” of Chittagong city between November, 2017 and April, 2018. A total of 186 admitted patients (male 71 and female 115) with and without symptoms detected were picked from different age and sex groups from different wards of CMOSH. The sex and age of the patients, the organism isolated and the antimicrobial susceptibility profiles were collected from the culture plate records following the standards of the Clinical and Laboratory Standards Institute (CLSI). The universal sterile holders were handed over to the patients in which the clean catch midstream urine was collected and labelled precisely. Then the collected urine specimens were cultured and examined.

Ethical considerations

The ethical clearance was approved by the National Research Ethics Committee (NREC) of Bangladesh Medical Research Council (BMRC). Further, the ethical clearance was also approved by the ethic committee of the CMOSH and the Department of Microbiology, University of Chittagong. Then the

permission was also taken from the administrators of the hospital before the implementation of the study. Else, the entire antenatal patients selected for the investigation agreed to a written consent after the clear explanation of the rationale for the investigation.

Urine culture and bacterial analysis

The collected urine samples from different wards were directly inoculated on McConkey agar, Blood agar and Hi Chrome UTI agar (Fig.1). For differentiating the samples to find out the infected one and to reject the contaminated one – bacteriuria sample which had $\geq 10^5$ cfu/ml was considered and put through tests. Therefore, the cultures were incubated at 37 °C for 24–48 h.

The cultures having positive traits were identified depending on their media, following their biological profile patterns. Furthermore, the reference strains were used to confirm the isolates.

Antimicrobial susceptibility test

The antimicrobial susceptibility tests were conducted on Mueller-Hinton agar by using Kirby-Bauer disk diffusion method. The used antibiotics were ampicillin (10µg), sulphamethoxazole (25µg), amoxicillin (30µg), augmentin (Amoxicillin/clavulanic acid) (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), chloramphenicol (30 µg), cloxacillin (1µg), tetracycline (30µg), gentamicin (10µg), and norfloxacin (10µg), penicillin and others. The identified morphologically confirmed bacterial colonies were kept in 5ml nutrient broth and incubated further for few hours and these turbid growth suspensions were matched to 0.5 McFarland standards. The antimicrobial discs were put on Mueller Hinton agar using a sterile needle 5 min later, after being inoculated on that agar by cotton swab using that prepared suspension. Following the incubation period, the diameter of zone of the inhibition was observed and measured, and further interpreted as sensitive, MS or resistant according to CLSI standards. Besides, the reference strains were used as controls.

Identification of isolates

The organisms isolated from the tested urine specimen were detected by using the standard techniques. A couple of biochemical tests, for instance – Gram staining, catalase test, coagulase test, urease test, citrate, indole test, sugar fermentation and oxidase test were used to identify the isolates which are shown in Table 1.

Data analysis

All the experimental data were compiled and arranged in the MS excel file. Then the Microsoft Office Excel (version: 2010) was used to analyse the collected and to create the required graphs.

Results

Prevalence of UTI based on sex and gender

CMOSH is a Child Care Hospital, so the diagnosis of UTI is prominent in female patient in comparison

with male patient admitted. As far as the age groups are concerned, female are at higher threat than male.

Out of 186 urine specimens from UTI affected children, young and adults; 62% are female and 38% are male. Women in the age group 20–24 and 30–34 years are found most likely to suffer from UTIs (Fig. 2). Among the elderly, after 45 years males become more prone to UTI than female. The number of male patients increases significantly in the age group 30–34 years and above 45 years.

The highest rate of infection is recorded in the patients between the age group 30–34 and the least infection recorded in the patients above 45 years. However, the prostatic gland enlargement and the decrease of bacteriostatic prostatic secretions might account for such infections.

Table 1. Biochemical test to differentiate isolates present in urine specimen (Agbagwa and Ifeanacho-Enaka, 2015).

Colony Morphology	Pink and mucoid colonies	Bluish to greenish Round colonies	Colonies raised and cream	Yellow and slightly Raised colonies	White small colonies	Colourless small colonies
Gram Rxn	-	-	+	-	-	+
Catalase	+	+	+	+	+	+
Coagulase	-	-	-	NA	-	-
Oxidase	-	-	-	+	-	-
Citrate	+	-	+	+	-	+
Indole	-	+	-	-	+	-
Urease	-	+	-	-	+	-
Methyl-Red	-	+	-	-	+	-
V. proskauer	+	-	+	+	-	+
H ₂ S	-	+	-	-	-	-
Gas	+	+	+	+	+	+
Slope	Y	R	R	Y	Y	R
Butt	Y	Y	R	Y	Y	R
Lactose	+	-	+	+	+	+
Glucose	+	+	+	+	+	+
Motility	-	+	-	+	+	NA
Tentative Microorganism	<i>Klebsiella spp</i>	<i>Proteus spp</i>	<i>S. aureus</i>	<i>Pseudomonas spp</i>	<i>E. coli</i>	<i>Streptococcus spp</i>

Note: R = Acid, Y = Alkaline, and NA = Not available.

UTI incidence by individual pathogen

In the study, the occurrence of UTI and isolated pathogens irrespective to the age groups and sex in the test subjected to show that *E. coli* is the highest occurring UTI pathogen both in male and female patients (Fig. 3). Then *Klebsiella sp.* and *S. aureus* are

the second highest occurring pathogens in both sexes. Hospital-acquired UTI show a variety of organisms, such as *Proteus sp.*, *Enterococcus sp.*, and *Pseudomonas sp.*, but the infection by them are significantly lower compared to other studies pathogens.

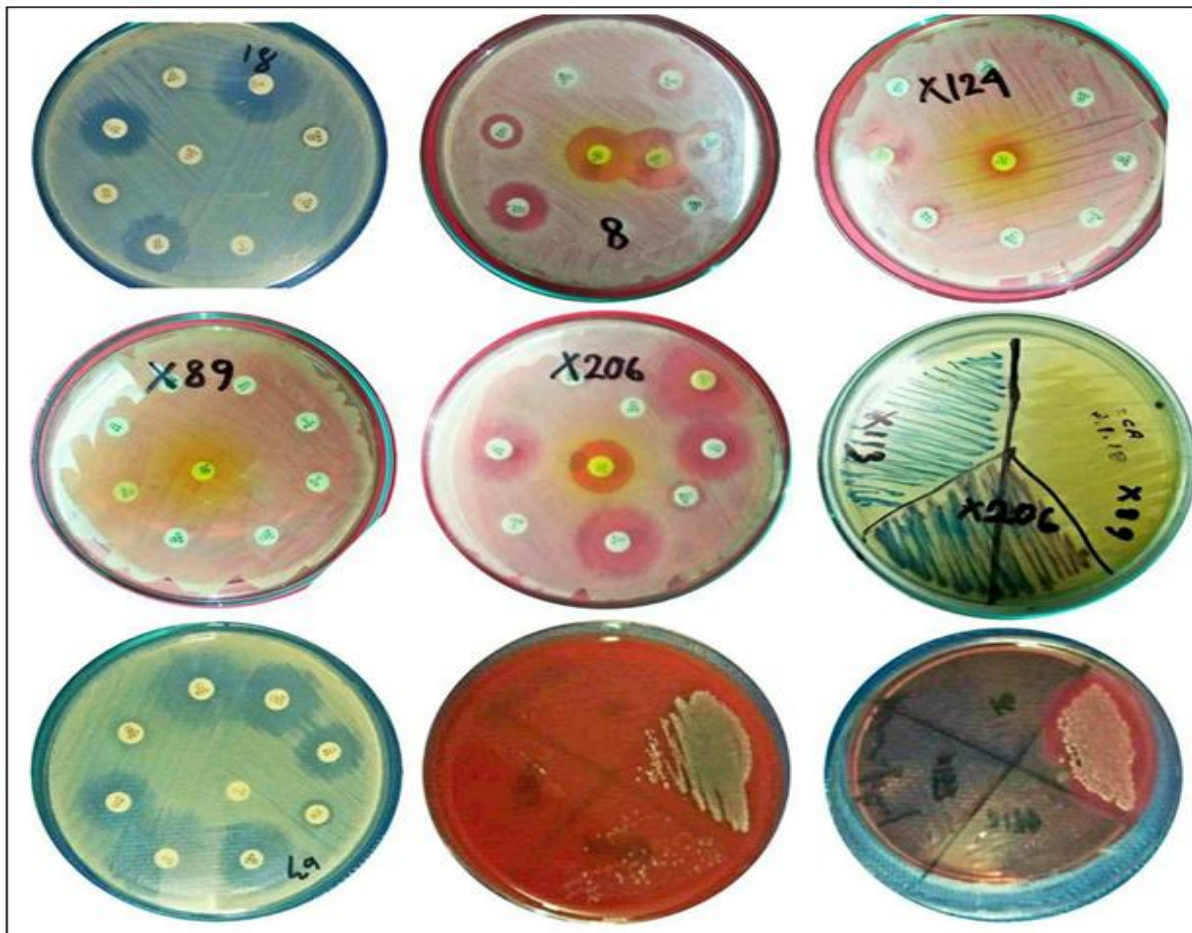
Table 2. Antibiotic susceptibility and resistance pattern of UTI isolates.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	67(73.6%)	4(4.4%)	20(22%)
Cefixime	39(42.9%)	4(4.4%)	48(52.7%)
Amoxicillin	63(69.2%)	8(8.8%)	20(22%)
Azithromycin	65(71.4%)	7(7.7%)	19(20.9%)
Ciprofloxacin	34(37.4%)	5(5.5%)	52(57.1%)
Sulphamethoxazole	64(70.3%)	5(5.5%)	22(24.2%)
Chloramphenicol	30(33%)	8(8.8%)	53(58.2%)
Ceftriaxone	60(65.9%)	6(6.6%)	25(27.5%)
Meropenem	40(44%)	5(5.5%)	46(50.5%)
Cinoxacin	55(60.4%)	4(4.4%)	32(35.2%)
Fluconazole	55(60.4%)	6(6.6%)	30(33%)

Antibiogram profile of the isolates

In this study, the antibiotic resistance and susceptibility testing of UTI isolates showed that the most active antimicrobial is Amikacin (67%) followed by Azithromycin (65%) and Sulphamethoxazole (64%) which shown in Table 2. The resistance rates to Chloramphenicol (53%), Ciprofloxacin (52%) and Cefixime (48%) are, respectively well-supported by

the study. Increasing resistance rate to all the antibiotics used in the study may be explained as uncontrolled consumption of these antibiotics during the past decade in the studied area. Sometimes discontinuation of the treatment due to negligence of the patient or unavailability of the drugs develops antibiotic resistance.

**Fig. 1.** Isolated uropathogens that obtained from the urine culture plates.

Discussion

Influence of sex and gender on UTI

Less than most of the people suffer from UTIs during their life span in the world, especially in the developing countries. Therefore, Asian developing countries are more prone to UTIs due to the unhygienic health condition and poor medical facilities. UTI is increasingly rising in Bangladesh due

to the development of resistance of causative pathogens against commonly prescribed antibiotics. It is the most common nonsurgical nosocomial infection in the postoperative patients in Bangladesh. 52.69% urine samples were identified as UTIs affected where women (mainly 20-24 and 30-34 years age groups) were higher sufferers in this study which is agreed with (Sanjee *et al.*, 2017).

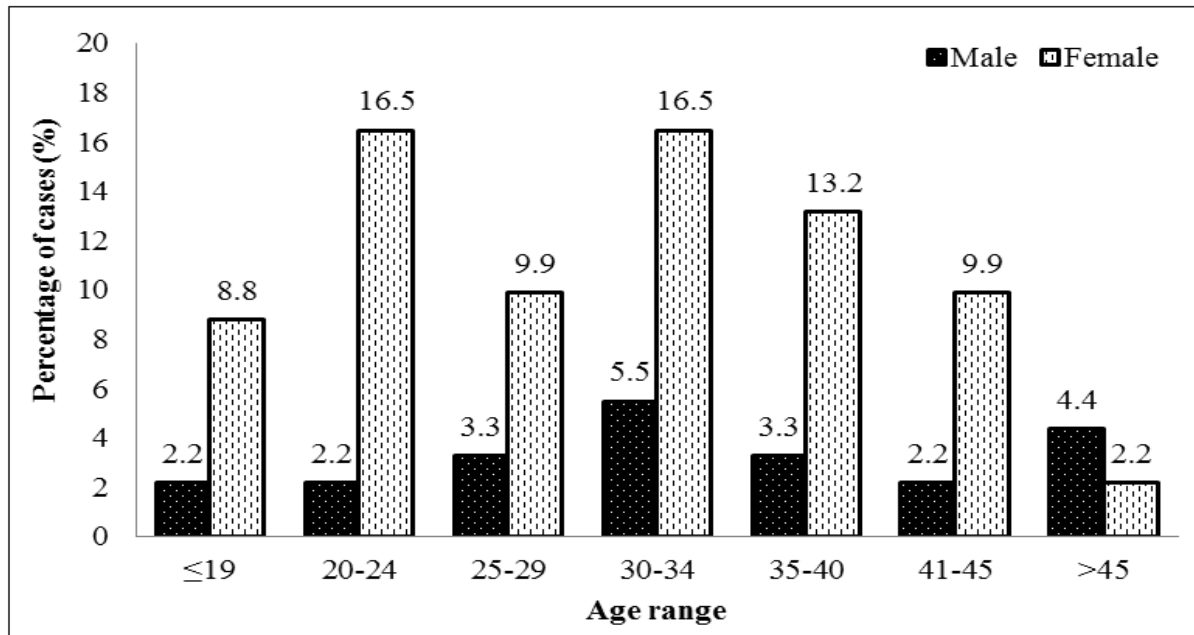


Fig. 2. Sex and age specific prevalence of UTI in tested urine specimens.

Almost similar results were found in other Asian countries, e.g. – Pandey and Timalisina (2016) found the UTIs in female close to this study in Nepal. Swetha *et al.* (2014) found about 1.5 times higher UTIs in female in India and 29.8% UTI prevalence among paediatric patients by (Patel and Garala, 2014). Shanthi and Kayathri (2012) and Banerjee (2009) also found close results of UTIs in India but little higher in female patients. Most of the African countries are either developing or least developing countries, therefore the scenarios of UTIs is similar to Asian countries. Alo *et al.* (2015) and Amengialue *et al.* (2013) found the similar results of this study in Nigeria but Agbagwa and Ifeancha-Enaka (2015) found about 1.5 times higher UTIs in female patients. Gessese *et al.* (2017) found 18.7% UTIs in pregnant in Ethiopia which is lower than (Patel and Garala, 2014). Kibret and Abera (2014) found little lower UTIs in female than this study. The prevalence of UTIs was

4.78 times higher in pregnant whose monthly income ≤USD21.18 than those monthly income >USD84.79 (Gessese *et al.*, 2017). Battikhi and Battikhi (2015) showed 66.7% UTIs in the age groups 38-42 years in Jordan while only 15% patients showed positive UTIs in Iran (Mihankhah *et al.*, 2017) and significantly higher in girls by (Pouladfar *et al.*, 2017). Hidalgo-Tenorio *et al.* (2004) found 36% UTIs in lupus patients in Spain whereas risk factors were age, previous cases of UTI and antinuclear antibodies. Shapiro and Donald (2002) found that 12 microbiology employees were exposed to *Francisella tularensis* where the identification was delayed due to the dearth of alerting in Massachusetts, United States.

The main responsible pathogens of UTI

UTI is an infection caused by the presence and growth of microorganism anywhere in the urinary tract and perhaps the single commonest bacterial infection of

mankind. It is a native gut flora in human intestine and has some traits like virulence factors to overcome the new environment. Both Gram-negative and Gram-positive bacteria along with fungus are the responsible microbes for UTIs occurrence. In the study, the most common uropathogens for UTIs is *E. coli* both in male and female patients, followed by

Klebsiella sp. and *S. aureus*. Noor *et al.* (2013) showed significantly higher UTIs by *E. coli* (70%), and *Klebsiella sp.* (11%) and *Enterococcus sp.* (6%) in Bangladesh that was close to this study. Sanjee *et al.* (2017) also reported *E. coli* as the leading etiological agent; however, the succeeding *Enterococcus sp.* (36.1%) was about 9 times higher than this study.

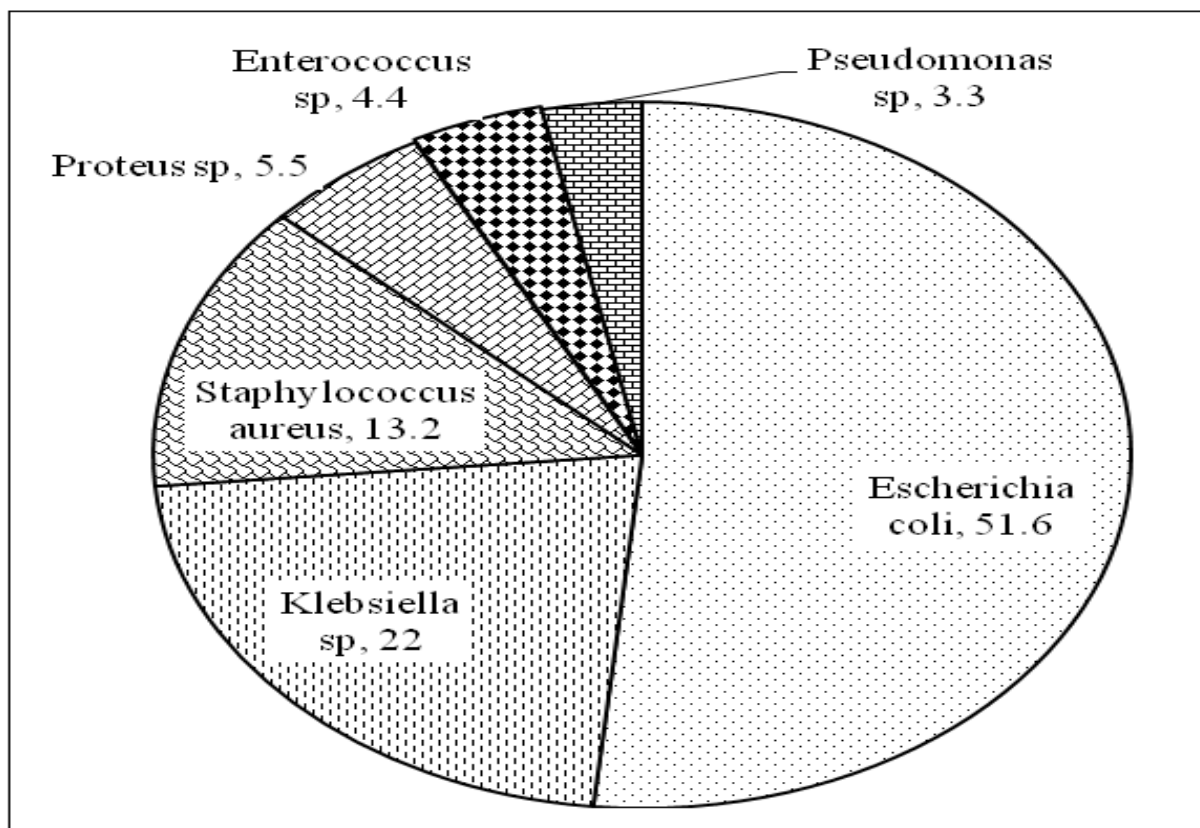


Fig. 3. The frequency (%) of microorganisms prevalent in the urine specimen of the patients.

Pandey and Timalisina (2016) found almost similar results of this study in Nepal. Patel and Garala (2014) and Swetha *et al.* (2014) found little different results from this study in India. Chatterjee *et al.* (2016) found catheterized UTIs were occurred by *E. coli* (64%) which is little higher than this study and other microbes also higher. Bhatt *et al.* (2017) also found close values of this study in India. Alo *et al.* (2015) found lower UTIs by *E. coli* (35.8%) including more bacteria species responsible for UTIs in Nigeria; however, it is opposite based on sex of this study. Amengialue *et al.* (2013) found *S. aureus* (28%) as the main responsible microbes for UTIs in Nigeria which is disagreed with this study; however, their findings were further little different by (Onuoha and

Fatokun, 2014) but close to (Nasir, 2017). In contrast, Agbagwa and Ifeancha-Enaka (2015) found *Klebsiella sp.* as the highest UTIs occurring pathogen in Nigeria, followed by *E. coli* which is disagreed with this study. Derese *et al.* (2016) found UTIs by *E. coli* (34.6%) in Ethiopia that is lower as-well-as other microbes UTIs occurrence sequence is also higher than this study. However, Kibret and Abera (2014) found higher UTIs by *E. coli* (63.6%) but lower *E. coli* (46.4%) by (Gessese *et al.*, 2017) in Ethiopia than this study. Badri and Mohamed (2017) found almost close results of this study in Sudan but differed by (Battikhi and Battikhi, 2015) in Jordan. Mihankhah *et al.* (2017) and Pouladfar *et al.* (2017) found similar results of this study in Iran. Hidalgo-Tenorio *et al.*

(2004) and Kocak *et al.* (2016) found UTIs by *E. coli* (60%-80.3%) in Turkey and Spain which is higher than this study but significantly lower *E. coli* (20%) in Maryland, United States (Hines *et al.*, 2015).

Antimicrobial resistance of microbes

The consumption of antibiotics and antimicrobial resistance is simultaneously increasing due to the modern medical science. Antimicrobial resistance is emerging as an important public health problem in both the hospital and the community. The emergence of antibiotic resistant *E. coli* presents a challenge for the urinary tract health management. Untreatable infections are being recognized more frequently and the bacterial pathogens become increasingly resistant. UTI bacteria demonstrated the most active antimicrobial to amikacin (67%), following azithromycin (65%), sulphamethoxazole (64%), chloramphenicol (53%), ciprofloxacin (52%) and cefixime (48%) in this study. Noor *et al.* (2013) found about 75% uropathogens were sensitive against amikacin and the resistance rate of *E. coli* and *Klebsiella sp.* against ampicillin was 98.5% and 100%, respectively. Sanjee *et al.* (2017) showed remarkable amount of sensitivity of uropathogens against gentamicin and ciprofloxacin.

Swetha *et al.* (2014) found that *E. coli* was highly sensitive to amikacin (71%) while *Klebsiella sp.* to ampicillin (13%) in India. Singh *et al.* (2011) found gentamycin, chloramphenicol and ceftriaxone sensitive while penicillin resistant to Salmonella. Shanthi and Kayathri (2012) found the lowest percentage of susceptibility of *E. coli* against ampicillin. Bhatt *et al.* (2017) found most of the Gram-positive bacteria resistant to penicillin but Banerjee (2009) found various antimicrobials maximum sensitivity against amikacin. Alo *et al.* (2015) found more than 60% of the bacteria susceptible to ceftriaxone, while more than 40% to gentamycin and ciprofloxacin. *S. saprophyticus* exhibited the highest multi-drug resistance in Nigeria while most of the bacteria exhibited the prevalence of multi-drug resistance (47.8%) by (Nasir, 2017). Derese *et al.* (2016) found that most of the bacterial

isolates were sensitive to ciprofloxacin, ceftriaxone and gentamicin; however, resistant against ampicillin, amoxicillin, tetracycline, sulfamethoxazole and chloramphenicol, and 100% multidrug resistance in Ethiopia. Kibret and Abera (2014) found the overall resistance to amoxicillin (88.9%) and tetracycline (76.7%) along with 47.9% multidrug resistance in Ethiopia. Gessese *et al.* (2017) found most of the Gram-negative bacteria resistant to ampicillin (70%), ceftriaxone (66%) and gentamicin (68%) while 75%-100% of the Gram-positive bacteria resistant to ampicillin. Badri and Mohamed (2017) found that *E. coli* and *K. pneumonia* exhibited the highest resistance to ampicillin, amikacin and ciprofloxacin, succeeding gentamycin in Sudan. Mihankhah *et al.* (2017) found 87.5% isolated bacteria resistant to at least one antibiotic in Iran and the highest resistance to ampicillin (89.3%) and sensitivity to amikacin (91.6%). Compared to Emergency Department antibiogram, notably lower resistance to ciprofloxacin was observed in Maryland, United States (Hines *et al.*, 2015). Noor *et al.* (2013) showed 29.6% of *E. coli* isolates were resistant to trimethoprim-sulfamethoxazole. Song (2009) found that prior exposure to ciprofloxacin raised the risk of resistance in Taiwan. *Proteus spp.* was identified as resistant against most of the antibiotics.

Conclusion

The advancement of medical science has made the antimicrobial drugs pretty much convenient for the patients worldwide. UTIs are still predominant and the most common diagnosed phenomenon around the world. As for this particular healthcare facility the vicious factor that has chained the proper management of UTI is none other than the rise of antimicrobial resistance. *E. coli* were the most predominant organisms followed by *Klebsiella sp.* and *S. aureus*. Most of the uropathogens were sensitive to amikacin, azithromycin and sulphamethoxazole. A good number of the isolates were resistant to chloramphenicol, ciprofloxacin and cefixime. The multi-drug resistant pathogens were also found. It recommended that the routine microbiological analysis and antibiotic sensitivity test

of mid-stream urine of pregnant women and other patients should be carried out before the administration of the drugs for the treatment of UTIs. Regular and continuous follow-up are mandatory to curb the effects of asymptomatic bacteria and multi-drug resistance bacteria in pregnancy and also for health of unborn and new-born child that receive treatment from this healthcare facility.

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