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Original Research Article

Prevalence of Urinary Tract Infection among the Patients Admitted in the Brahmanbaria Medical College Hospital in Bangladesh

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Abstract

Urinary tract Infection has become on the top of the list of common infectious diseases worldwide especially in Bangladesh. This study attempted to emphasize the prevalence of UTI patients affected by uropathogenic strains admitted in Brahmanbaria Medical College, Bangladesh. The study was conducted among 150 patients of different ages and sex from November, 2019 to January, 2020 through conventional cultural, biochemical methods as well as disk diffusion methods (Kirby-Bauer methods) to determine the uropathogens and their antibiotic susceptibility pattern. Among 150 samples, 50 were male, 50 were female and the rest of the samples were children. About 33.33% cases were UTI positive among which 48% female patients were UTI positive. Most of the UTI were caused by *Escherichia coli* (48%). Moreover, *Enterobacter* spp, *Pseudomonas aeroginosa*, *Staphylococcus aures*, *Proteus vulguris*, *Citrobacter* spp, *Staphylococcus saprophyticus* were also estimated. The pathogens showed 100% resistance against Amoxicillin and the sensitivity (84%) was recorded against Meropename. Nonetheless, Nitrofurantion, Amikacin and Gentamicin were also effective against the isolates. The significant numbers of pathogens were found to be resistant against more than one antibiotics denoted as Multidrug Resistant (MDR) strain which might be hinder the proper treatment of the patients.

Keywords: UTI, Pathogens, Drug resistant, MDR

INTRODUCTION

Urinary Tract infection (UTI) is now very common disease throughout the world among the people of all ages and sex. However, UTI is the most familiar name of infection and the rate is so high among the women in all over the world. Many researchers reported that 60% women suffer from this infection at any phase of their lifetime and 20-30% people suffer from repeated infection (Rahman et al. 2004). About 25% of infections are UTI which is the major reason behind being the second most common reason of hospital visit and morbidity rate (Parveen and Rahim. 2017; Ronald AR, 1997). Though *Escherichia coli* is the most common causative agent of UTI (70%-79% of cases), some Gram negative *Enterobacteriaceae*, Gram positive *Enterococcus faecalis*, *Proteus spp*, *Klebsiella*,

and *Staphylococcus saprophyticus* are also responsible for UTI (Rahman et al. 2004; Lee and Neild, 2007; Haque et al. 1976; Nahar et al. 1989) . Moreover, some pathogenic bacteria like *Pseudomonas*, *Staphylococcus aureus*, Group B *Streptococcus* have virulence properties such as capsule production, lipopolysaccharide, siderophore activity, alginate (algD), pilus, lipoteichoic acid that causes UTI or Recurrent Urinary Tract Infection (RUTI) (Rahman et al. 2004; Abdul, 2009). Although women are considered as the common main target as host for UTI but men over the age of 60 years with prostatic hypertrophy may also face this problem (Calvin, 1994). Nowadays, people of all ages from neonate to adult male or female are prone to UTI (Yasmeen et al. 2015). Major risk factors behind this common UTI are unhygienic lifestyle, using contaminated water to clean after urination, sexual activities like sex with multiple partners without condoms. (Jawetz and Melninick, 1995, Bardhan et al. 2015). Females are more susceptible to UTI than male because of the difference in their genetical organ (Jawetz and Melninick, 1995). Urinary Tract Infection could be life-threatening to children as it can cause sepsis and damage the normal function of kidney. Meanwhile, UTI causes a severe urge to urinate and mostly a burning sensation during urination (Abdul, 2009). Though, different antibiotics have been already manufactured depending on several factors such as of metabolic activities of gut microbes, types of urinary tract infection caused by different microbes and the physical appearance of urine (alkaline pH of urine) to resist bacterial growth. To treat UTI patients in U.K. calcium supplements and alkalizing agents are already used as inducer to raise the urine pH so that the alkaline pH can reduce the growth of pathogenic microflora (Nahar et al. 1989; Bardhan et al. 2015). Another cellular protein - siderocalin, activates at the first stage of infection and blocks microbial (such as *E.coli*) growth. Sometimes it takes two to three weeks to recover but in some complicated UTI cases, antibiotic therapy might have to be taken more than 6 months (Abdul, 2009). Reportedly, different serotypes of *E.coli* were found to be associated with UTI and some of those strains were Extended-Spectrum-Beta-Lactamases (ESBL) producing pathogens (Rahman et al. 2004; Graham and Galloway, 2001; Wilson and Gaido, 2004). Due to the improper therapeutic treatments and incomplete medication, overuse of antibiotics - some uropathogens become resistant against the previously used antibiotics over time (Bonadio et al. 2001; Grude et Al. 2001). Regular and exact diagnosis is needed to minimize the risk of increasing drug resistance which would help the patients physically and financially (Bacheller and Bernstein, 1997). Therefore, antibiotics selection should be based on the susceptibility patterns of the isolated pathogens. Different diagnosis laboratory should provide the doctors the periodic evolution of the resistance patterns of the isolated pathogens from the local patients. For example, doctors used to prescribe cotrimoxazole once against which urinary pathogens; especially *E. coli* has become resistant globally. After realizing this fact, other drugs like Nitrofurantion, Amikacin and Gentamicin are now being prescribed (Begum et al. 2017). Physicians need to know local patterns of microbial susceptibility and cost of those drugs to prescribe proper antibiotic treatment (17). Consequently, the aim of this research was to identify the prevalence of UTI in Brahmanbaria, common uropathogens isolated from the local patients of Brahmanbaria as well as to evaluate the antimicrobial susceptibility patterns of the causative UTI agents in recent time.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology Culture Lab of Brahmanbaria Medical College, Bangladesh over a period of November 2019 to January 2020. This is very well recognized and governmentally approved hospital in Bramhmanbaria district where all the patients were registered by their personal information according to the rules of the hospital. The sample collection, data processing and laboratory experiment of this hospital maintained all the ethical protocol. During this period the urine samples of children, teenage, adult male and female patients were collected aseptically to examine the condition. The patients who were facing different symptoms like fever, painful micturition, increased frequency of urine discharge, burning sensation those were suspected as a UTI patient therefore their urine samples were collected for the microbiological analysis. (Rahman et al., 2004).

Study population

A total 150 urine samples were collected from the suspected cases of UTI patients and transported aseptically to the experimental laboratory by following thermostable condition for analyzing the microbiological status.

Collection of urine samples

The mid-stream Urine samples were collected in sterile wide mouthed screw capped containers from the individual patients. The collected samples were labeled

including proper information of the patients such as name, age, sex, previous history of infection and treatment. The microbial analysis of the samples was done according to the standard protocol within 2 hours of collection (Begum et al., 2017; Sultana et al., 2019).

Sample Processing

Cultural identification

A sterile micropipette was used to transfer 0.1 ml of urine sample on different selective media such as MacConkey agar plates and CLED (cysteine-, lactose- and electrolyte-deficient), eosin methylene Blue (EMB) agar plates to detect mostly coliform group such as *E. coli*, *Enterobacter* spp. *Klebsiella* spp. and *Citrobacter* spp. As lactose non fermented bacteria, the growth of Proteus vulguris was also found to appear on MacConkey agar. Subsequently, Blood agar and Cetrimide agar were used for the detection of *Staphylococcus* spp. and *Pseudomonas* spp. respectively. The raw samples were spread onto Nutrient Agar (NA) for the detection of total bacterial count. All the culture media were incubated at 37°C for 18 hours or 24 hours. After the incubation period, the plates were examined and colonies were counted by using semi-quantitative method and colony forming unit (cfu) by following the standard formula: Number of colony multiplied by dilution factor divided by amount of samples (Rahman et al., 2004, Majumder et al., 2019).

Identification of pathogenic microbes:

For the identification of specific microbes several identification procedures were applied such as gram staining, morphological identification and biochemical tests (Rahman et al. 2004; Parveen and Rahim. 2017; Sultana et al. 2019).

Microscopic examination

Isolated colonies from the selective media - Mac Conkey agar and Blood agar were processed for Gram Staining by following the standard steps (primary stain: crystal violet, grams iodine, decolorizing agent: ethyl alcohol, counterstain: safranin). After the staining, colonies of different isolated micro-organisms were observed under the microscope and identified (Zinnat et al. 2011)

Biochemical tests

Finally, the standard biochemical tests were performed for the identification of the pathogens found in the urine samples. For this specific purpose, Kliglar Iron Agar (KIA) was prepared to determine the capability of bacteria for the utilization of different carbohydrate. Methyl Red (MR) test was performed to ensure the ability of organisms to produce stable acids as end product. Voges Proskauer (VP) test was executed to detect the ability of organisms to produce acetylmethyl carbinol and the Simmons' Citrate agar media was used to perceive the capability of bacteria to utilize citrate as a sole source of carbon. The Oxidase test was also performed to evaluate the ability of bacteria to produce cytochrome c oxidase and the coagulase test was also performed to evaluate either *Staphylococcus spp*. can produce coagulase enzyme or not. Finally, Motility Indole Urease test was executed to determine the bacterial motility (Sultana et al. 2019, Yasmeen et al. 2015; Zinnat et al. 2011).

Antimicrobial susceptibility testing

All the isolates were tested for antibiotic susceptibility against 19 antibacterial drugs (including first, second and third generation drugs) by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) according to the modified Kirby-Bauer method. A single colony of each isolate was inoculated into 2 ml of Mueller-Hinton broth, and incubated at 37 °C for 4 hours. The culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire surface of Muller-Hinton agar. Antibiotic discs of appropriate concentrations (Amoxicillin 10µg, Cefuroxime 10µg, Azithromycin 15µg, Cefepime 10µg , Ceftazidime 10µg, Nalidixic acid 30µg, Cifixime 5µg, Ceftriaxone 30µg, Cotrimoxaxole 800mg, Linezolid 30µg, Ciprofloxacin 5µg, Doxycycline 30µg, Levofloxacin 5µg, Tetracycline 30µg, Aztreonam 30µg, Gentamycin 30µg, Amikacin 30µg, Nitrofurantion 300µg, Meropename 10µg) were placed aseptically over the surface at appropriate spatial distance of 5 mm. Plates were then inverted and incubated at 37 °C. After 24 hours, plates were examined and the diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant (Rahman et al. 2004; Yasmeen et al. 2015).

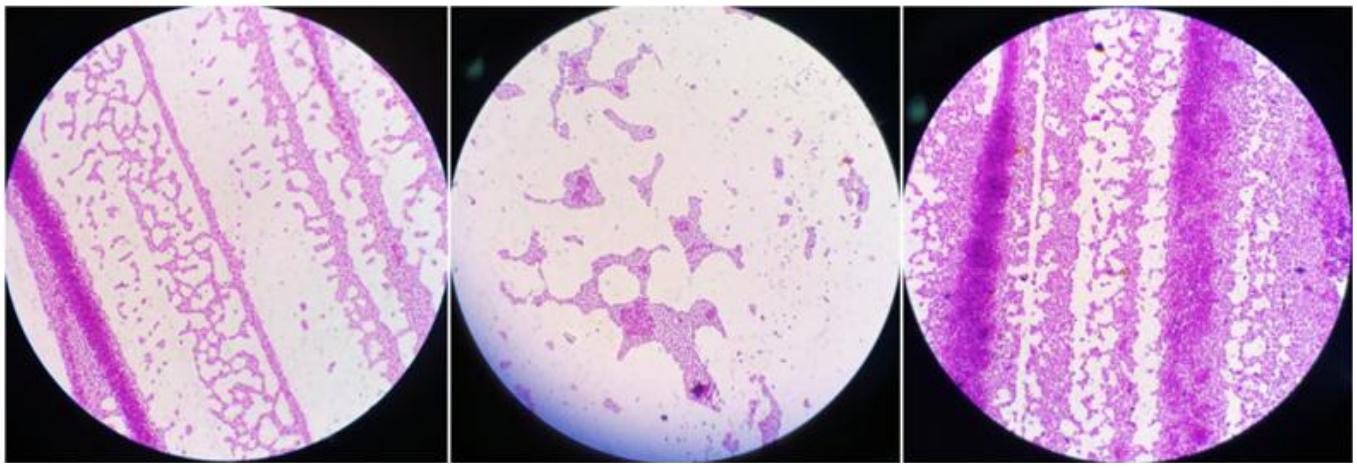

Figure 1. Microscopically observed the pathogens after gram staining based on their color, size, shape and arrangement.

Table 1. Morphologically and Biochemical test results of the isolated pathogens

Isolated pathogens	Microscopic Observation					Biochemical test										
						TSI								Motility		
	Gram-staining result	Cell color	Cell shape	Cell arrangement	Lactose fermentation	Slant	Butt	H2S	Gas	Oxidase	Citrate	Catalase	Coagulase	Motility	Indole	Urease
Escherichia coli	Gram negative	Pink	Rod shaped	Single	+	Y	Y	-	+	-	-	+	-	+	+	-
Enterobacter spp	Gram negative	Pink	Rod shaped	Single	+	Y	Y	-	+	-	+	+	-	+	+	-
Citrobacter freundii	Gram-negative	Pink	Rod shaped	Single	+	Y	R		-	-	-	+	-	+	+	+
Proteus vulgaris	Gram-negative	Pink	Rod shaped	Swarm	-	Y	R	+	+	-	-	+	-	+	-	+
Pseudomonas aeruginosa	Gram-negative	Pink	Rod shaped	Single	-	R	R	-	-	+	+	+	-	+	-	Slow
Staphylococcus aureus	Gram-positive	Purple	Cocci shaped	Cluster	+	Y	Y	-	+	-	+	+	+	-	-	+
Staphylococcus saprophyticus	Gram-positive	Purple	Cocci shaped	Cluster	+	Y	Y	-	+	-	+	+	-	-	-	+

RESULTS

Identification and confirmative results of isolated pathogens from urine samples

After performing the cultural and microscopic observation the existence of pathogens in urine samples were determined such as *Escherichia coli, Enterobacter* spp*, Pseudomonas aeroginosa, Staphylococcus aures, Proteus vulguris, Citrobacter* spp and *Staphylococcus saprophyticus* (Figure 1). Further confirmation of the isolates were recognized according to the results of different biochemical tests (Table 1).

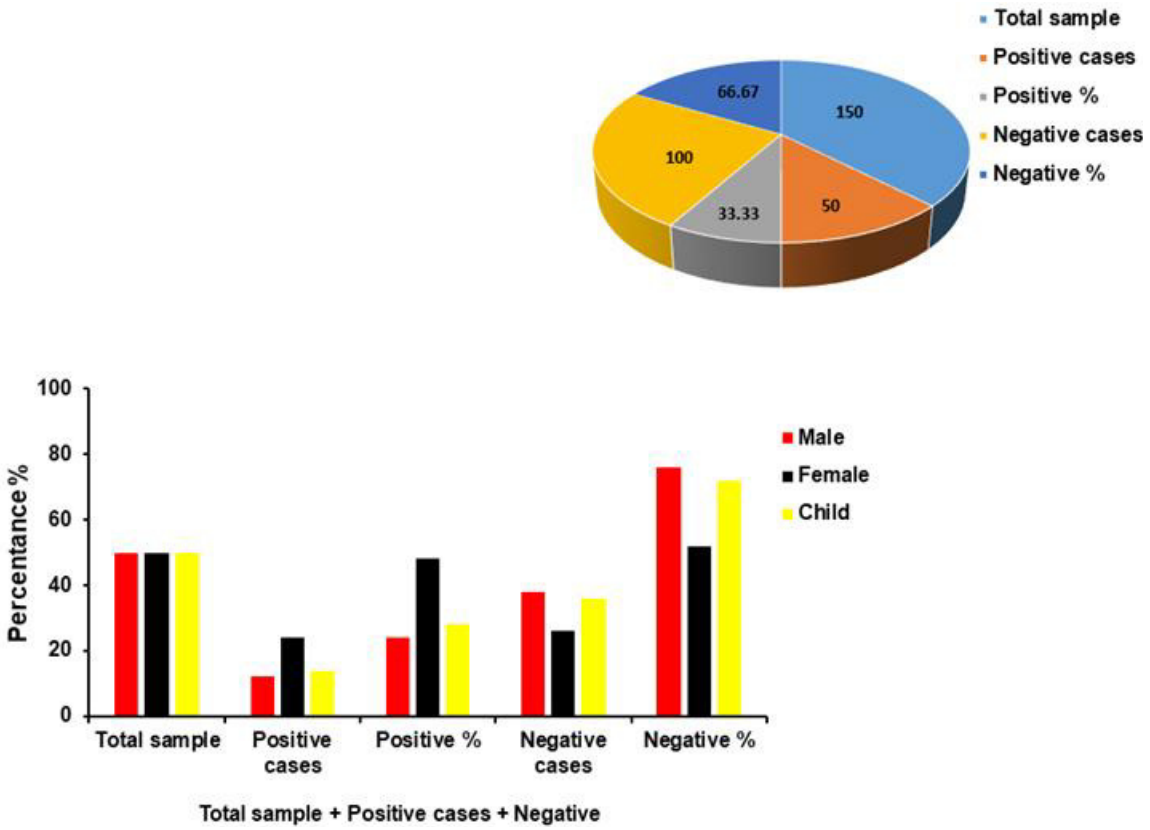

Figure 2. Positive cases in total number of suspected sample and sex distribution of the suspected patients: The pie chart in the figure explains the 150 (blue) total samples, 50 (orange) samples were UTI positive and 100 (yellow) samples were negative. It means about 33.33% (Ash) people had UTI. The bar graph demonstrates percentage of positive and negative cases among male, female and children. The red bar, black bar and yellow bar indicate the number of male, female and children respectively. The percentage of positive cases in female was higher than children and male.

Positive cases in total number of suspected sample and sex distribution of the suspected patients

According to this research (Table 1), 33.33% positive cases of UTI were found which means 50 UTI positive samples among the 150 total urine samples. Out of these 50 UTI positive patients, 12 cases were positive for male which is 24% of total and 14 cases were positive for children which is 28% of total. The highest number of Urinary Tract infected patients was women which almost about half of the total positive cases (24 out of 50) of UTI were found in women**.** (Figure 2).

Isolated Common causative agents of UTI in patients of different ages and sex

In our study most of the patients of different ages and sex were found to be infected with common uropathogens such as *Escherichia coli, Pseudomonads aeroginosa, Citobacter freundii, Proteus vulgaris, Enterobacter* spp*, Staphylococcus* spp. Almost half (48%) of the total UTI cases were found to be caused by the most common uropathogen *E.coli*. In terms of different sex, women were more prone to this infection than male and children. *Enterobacter* spp. (18%) was found to be estimated as the second highest causative agent of total UTI cases while, in case of child almost 8% was UTI positive. On the other hand, *Staphylococcus saprophyticus* was absent in male patients and only 2% female patients were found to be infected. However, UTI caused by *Staphylococcus aureus* was found to be estimated as 4% for female and 6% for child respectively. All of the pathogens were found in female urine samples as causative agent of UTI. Most importantly, *E.coli* and *Enterobacter* spp. were found in all types of patients (Table 2).

Table 2. Percentage of causative uropathogen isolated from UTI patients including sex distribution

Identified Causative pathogens of UTI	Number of cases caused by this uropathogen	Male patients		Female patients		Child and neonates	
Escherichia coli	24 (48%)	06	12%	11	22%	07	14%
Pseudomonas aeruginosa	05 (10%)	02	4%	03	6%	00	0%
Citobacter freundii	03 (6%)	01	2%	02	4%	00	0%
Proteus vulgaris	03 (6%)	01	2%	02	4%	00	0%
Enterobacter Spp	09 (18%)	02	4%	03	6%	04	8%
Staphylococcus saprophyticus	01 (2%)	00	0%	01	2%	00	0%
Staphylococcus aureus	05 (10%)	00	0%	02	4%	03	6%
Total	**50 (100%)**	**12 (24%)**		**24 (48%)**		**14 (28%)**	

Table 3. Antibiotic susceptibility Tests of the isolated uropathogens

Antibiotic	Total number of antibiotic use	Total number of antibiotic sensitive cases	Percentage of totally antibiotic sensitive cases(%)	Number of resistant cases	Percentage of totally resistant cases against this antibiotic (%)
Amoxicillin	50	00	00 %	50	100%
Cefuroxime	50	03	6 %	47	94%
Azithromycin	50	05	10 %	45	90%
Cefepime	50	05	10%	45	90%
Ceftazidime	50	05	10%	45	90%
Nalidixicacid	50	05	10 %	45	90%
Cifixime	50	06	12 %	42	88%
Cetriaxone	50	09	18 %	41	82%
Cotrimoxaxole	50	15	30 %	35	70%
Linezolid	50	15	30 %	35	70%
Ciprofloxacin	50	22	44 %	28	66%
Doxycycline	50	22	44 %	28	66%
Levofloxacin	50	18	36%	32	64%
Tetracycline	50	20	40 %	30	60%
Aztreonam	50	25	50%	25	50%
Gentamicin	50	36	72 %	14	28%
Amikacin	50	38	76 %	12	24%
Nitrofurantion	50	39	78 %	11	22%
Meropename	50	42	84 %	08	16%

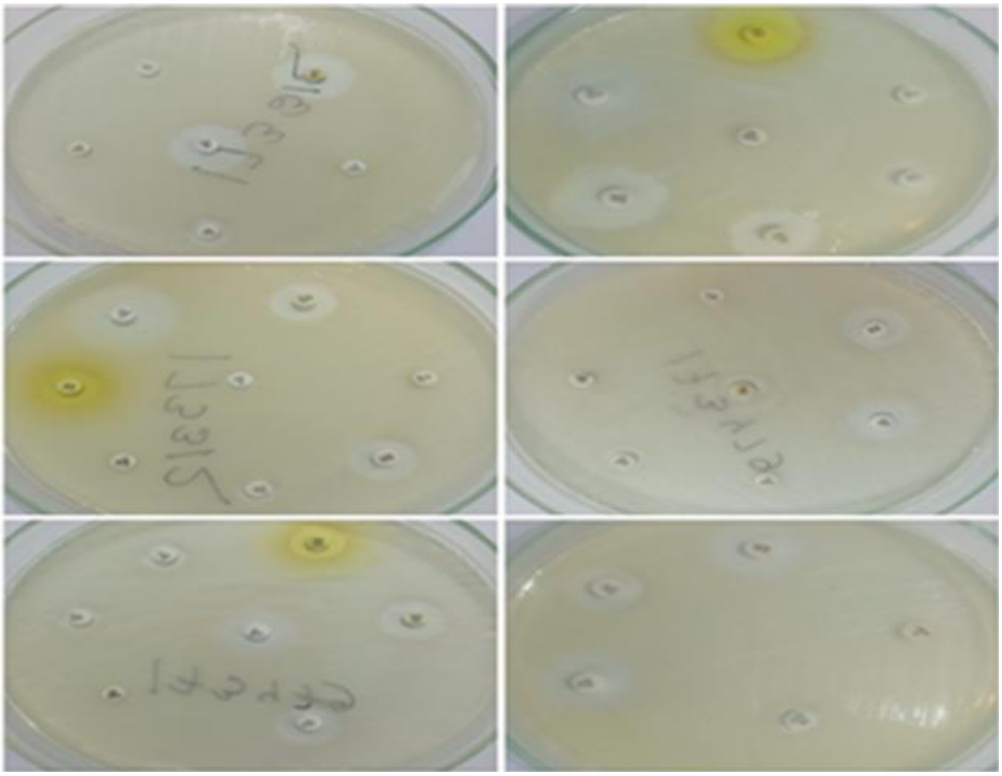

Figure 3. Anitibiogram test results of the isolated pathogens against different antibiotics: The figure shows the antibiotics sensitivity patterns of different isolated uropathogens. The zone of inhibition in some pictures indicate the pathogenic bacteria was sensitive against those drugs.

Antibiotic sensitivity Tests of the isolated uropathogens

Total 19 antibiotics were used against the isolated uropathogens. The Table 3 indicates maximum numbers of pathogens were found to be resistant against the drugs while some of the antibiotics were effective against the isolated pathogens. According to the result, the isolated pathogens were found to be resistant against the most commonly used drug - Amoxicillin. Cefuroxime, Azithromycin, Cefepime, Ceftazidime and Nalidixicacid. About 82% - 88% UTI cases, Cifixime and Cetriaxone were incapable to kill the isolated pathogens. Likewise Linezolid and Cotrimoxaxole were ineffective against the pathogens. Ciprofloxacin (66% cases), Doxycycline (66% cases), Levofloxacin (66%) and Tetracycline (60%) were also found ineffective against the pathogens. Aztreonam were effective against the isolated pathogens in 50% cases and ineffective against 50% cases. Meanwhile, Nitrofurantion (78%), Amikacin (76%) and Gentamicin (72%) could be considered as the secondly effective drugs against all pathogens. The most useful drug was Meropename which was able to kill all the pathogens in 84% cases (Table 3) (Figure 3).

DISCUSSION

Due to high rate of population in Bangladesh, people are leading a poor, unhygienic lifestyle with poor basic facilities in every sectors. From slum dwellers to rich people, female in every sectors have faced UTI infection at least one time in any stage of their lives (Yasmeen et al., 2015). Nowadays, the number of children and male patients infected with UTI are gradually increasing. Most importantly, people who are sexually active with different partners without using condoms, wearing tight undergarments could be the major victims of UTI (Rahman et al. 2004; Abdul, 2009). Additionally, the causative agents of UTI are becoming resistant against different antibiotics day by day which are also a major cause of higher frequency of morbidity and mortality (Blanco et al. 1997; Rahman et al. 2004; Yasmeen et al., 2015). Inadequate antibiotic dose consumption or overdose consumption could be a reasonable issue behind the growing number of antibiotics resistant cases which might be the reason of increasing the multidrug resistance by sharing their resistant genes or plasmids (Wang et al. 2004, Woodford et al., 2007). Antibiotics resistant Uropathogens poses different invasive virulence

factors and spread community acquired UTI or nosocomially acquired UTI (Lin et al., 2010). Though females are more prone to UTI than man, children are also becoming Infected with UTI during renal diseases and congenital abnormalities as regular (Najib et al., 2009). The high morbidity rates due to UTI are found in children (Begum et al. 2017). Our current investigation revealed that *E. coli, Enterobacter, Staphylococcus saprophyticus, Staphylococcus aureus, Pseudomonas* spp. were the common uropathogens and those were found to be highly sensitive to Meropename. Though doctors in Bangladesh use to prescribe the drugs like Amoxicillin, Cefuroxime, Azithromycin, Cefepime, Ceftazidime, Nalidixic acid, Ciprofloxacin, Tetracycline which showed highly ineffective (60% - 100%) against different pathogens isolated from these UTI infected patients (Yasmeen et al., 2015). It can easily be said that the patients of this area in Bangladesh, harbors a wide range of multidrug resistant uropathogens. The doctors need to be aware of the current drug resistant stage of the uropathogens or local susceptibility patterns, after that treatment should be based on known susceptibility and resistance as reported in the literature (Hassali et al. 2018). To be updated with the current stage of susceptibility patterns, regular research on the isolated pathogens from all types of UTI patients in different hospitals in our country - is urgently needed. The collected data might be small in size but gives a proper example of increasing rate of antibiotic resistant uropathogens in different aged patients. These findings will help to create awareness among the physicians about the effective and ineffective drugs for the local patients in this area. Further experiments should be done to understand the resistant genes responsible for the resistance ability of the pathogens, additionally to enhance awareness among all. The doctors in different hospitals should arrange community awareness program on the proper use of antibiotics to minimize the resistant cases among the patients including others. This would be great if proper regular medical education will be given and shared with the physicians as a best experimental antimicrobial research and practices.

CONCLUSION

Being an ordinary infection, the simple treatment procedure for UTI is becoming more complicated due to the involvement of multi drug resistant pathogenic bacteria. The sensitivity of pathogenic bacteria towards the antibiotics are decreasing remarkably. To become completely resistant toward these antibiotics is a matter of time only. Our study explains that *Escherichia coli* was the most common causative uropathogen among UTI patients. However, some other pathogens like *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus vulgaris*, *Citrobacter* spp., and *Enterobacter* spp. were also found among male, female and children patients. All of these pathogens were found to be resistant against Amoxicillin. Cefuroxime, Azithromycin, Cefepime, Ceftazidime, Nalidixic acid that may create serious health threat towards general people. However, some of the antibiotics such as Meropename, Nitrofurantion, Amikacin and Gentamicin were found to be effective against the isolated pathogens. Development of alternative drugs is necessary for such conditions and patients should be advised properly to take the antibiotic medication to control the condition of rapidly occurring resistance traits. Overall, educational programs regarding this topic should be arranged regularly to create awareness in not only among the physicians but also the general people of country.

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