Prevalence of Bacterial Urinary Tract Infections and Their Anti-microbial Susceptibility Test in a Tertiary Care Hospital in Bangladesh between April 2020 to April 2021

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Abstract

Urinary tract infection is the most common bacterial infection in Bangladesh and a common disease of women than men. An antimicrobial agent is used for the treatment of UTI. The increase in antibiotic resistance among uropathogens is a global problem. This study attempted to underline the prevalence of UTI patients affected by uropathogenic strains. The study was conducted among 3060 patients of different ages and sex from over a period of one year April 2020 to April 2021 through conventional cultural, biochemical methods as well as disk diffusion methods to determine the uropathogens and their antibiotic susceptibility pattern. Among 3060 samples, 1148 (37.5%) samples were found culture positive, 248 (22%) cases positive for male & 900 (78%) cases positive for females. Most of the UTI was caused by *Escherichia coli* 891 (60 %). Moreover Enterobacter spp (7.5%), *Pseudomonas aeruginosa* (11%), *Proteus vulgaris* (3.5%), *Proteus mirabilis* (2.5 %) *Citrobacter freundii* (1%), *Klebsiella pneumoniae* (3%), Staphylococcus spp (9.5%) *Enterococcus faecalis* (2%). 17 antibiotics were used against the isolated uropathogens. As it can be seen from (Table 2) *Escherichia coli* are sensitive to Meropenem 89%, Amikacin 80%, Gentamicin 74%, Nitrofuration 67%, Amoxiclave 71%, Ciprofloxacin 41%, Levofloxacin 37%, Doxycycline 40%. *Pseudomonas aeruginosa* sensitive to to Meropenem 72%, Amikacin 79%, Gentamicin 72%, Nitrofuration 54%, Amoxiclave 60%, Ciprofloxacine 43%, Levofloxacine 47%. *Klebsiella Pneumoniae* to Meropenem 82%, Amikacin 71%, Gentamicin 71%, Nitrofuration 50%, Amoxiclave 63%, Ciprofloxacin 32%, Levofloxacine 38%. Staphylococcus Spp Meropenem 83%, Amikacin 79%, Gentamicin 73%, Nitrofuration

60%, Amoxiclave 70%, Ciprofloxacine 46%, Levofloxacine 43%, Lenazolid 30%, Cotrimoxazole 43%. *Proteus vulgaris* Meropenem 84%, Amikacin 86%, Gentamicin 82%, Nitrofuration 56%, Amoxiclave 35%, Ciprofloxacine 28%, Levofloxacine 40%, Doxycycline 30%. *Proteus mirabilis* Meropenem 86%, Amikacin 84%, Gentamicin 84%, Nitrofuration 60%, Amoxiclave 63%, Ciprofloxacine 31%, Levofloxacine 43%, Doxycycline 30%. Enterobacter Spp Meropenem 78%, Amikacin 68%, Gentamicin 70%, Nitrofuration 47%, Amoxiclave 58%, Ciprofloxacin 38%, Levofloxacine 41%, Doxycycline 37%. *Enterococcus faecalis* Meropenem 80%, Amikacin 80%, Gentamicin 73%, Nitrofuration 59%, Amoxiclave 68%, Ciprofloxacine 30%, Levofloxacine 20%, Lenazolid 32%, Cotrimoxazole 43%. *Citrobacter freundii* Meropenem 88%, Amikacin 83%, Gentamicin 82%, Nitrofuration 60%, Amoxiclave 61%, Ciprofloxacin 33%, Levofloxacine 32%, Ceftriaxone 30%, Doxycycline 30%.

Keywords: Urinary Tract Infection (UTI), Uropathogens, Anti-microbial susceptibility test, Antibiotics, E.coli.

Introduction:

A urinary tract infection is an infection that affects any parts of the Urinary tract 29. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as a kidney infection 30. Symptoms from a lower urinary tract infection include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder 29. Symptoms of an upper urinary tract infection include fever and pain usually in addition to the symptoms of a lower UTI 30.

Urinary Tract Infection is the most common bacterial infection occurring in women than men through males over the 60 years with prostatic hypertrophy are the exceptions 1. Many researchers reported that 60% of women suffer from this infection at any phase of their lifetime and 20-30 % of people suffer from repeated infection 2. UTI are infections caused by the presence and growth of microorganisms anywhere in the urinary tract and is perhaps the single commonest bacterial infection of human 3. The urinary tract consists of the kidney, ureter, bladder, and urethra and any part of these structures can become infected and UTI is the infection of any part of this tract. Where bacteria can enter into the sterile urinary system through the urethra or more rarely through the bloodstream 4. Women are more susceptible to UTI than men because the urethra is much shorter and closer to the anus in females 5. Major risk factors behind this common UTI are an unhygienic lifestyle, using contaminated water to clean after urination, sexual activation like sex with multiple partners without condoms 5, 6

Escherichia coli is the most common Uropathogen of all forms of UTI and is responsible for 80% of Cases 7,8. Though *Escherichia coli* is the most common causative agent of UTI but, Pseudomonas spp, Enterobacter spp, Gram-positive *Enterococcus faecalis*, Proteus spp, Klebsiella, and *Staphylococcus saprophyticus* are also responsible for UTI 2,9.

UTI was verified by burning or pain, dark urine, strong smell, and urination urgency 10. UTIs can occur as asymptomatic bacteriuria, acute cystitis, and acute pyelonephritis 11. Fever and

chills are common along with other systemic symptoms and board spectrum therapy is required 12.

The aim of a microbiology laboratory is to diagnose UTI accurately and timely. Aim also includes appropriate antimicrobial sensitivity testing 13. The development of resistant strains is a common problem in antimicrobial chemotherapy. The frequency of resistance to antibiotics and is directly linked to the consumption of antibiotics 14. Due to improper use of antibiotics the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide 15, 16.

Consequently, the aim of this research was to identify the uropathogenes isolated from the local patient of district of Brahmanbaria in Bangladesh as well as evaluate the antimicrobial susceptibility patterns of the causative UTI agent in recent time.

Methodology

This study was carried in the Department of Microbiology of Brahmanbaria Medical College over a period of one year from April 2020 to April 2021. This is a very well recognized and governmentally approved 400 beds hospital in the District of Brahmanbaria under the city of Chittagong in Bangladesh where all the patients were registered by their personal information according to the rules of the organization. Midstream urine samples (MSU) were collected in sterile wide-mouthed screw-capped containers from the 3060 patients including males & females. Culture the urine sample was inoculated on 5% sheep blood agar and MacConkey's agar media using calibrated loop following standard bacteriological technique and incubated at 37°c for 24 hours. After the incubation period, the plates were examined for the bacterial pathogen. Pure bacterial colony counting $\geq 10^5$ bacteria/ml were considered as significant and was subjected to identification based on colony characters and biochemical test, Gram staining 17,18. Isolated colonies from MacConkey's agar media & blood agar media were processed for Gram staining by following the steps (primary stain-crystal violet, mordanting – grams iodine, decolorizer- acetone, counterstain- safranin. After the staining colonies of different isolated microorganisms were observed under the microscope and identified 19. Finally, the standard biochemical test which includes catalase, coagulase, indole, urease, oxidase, citrate, and test were performed aimed to identify the bacterial isolate (Table-2).

Antimicrobial susceptibility testing:-

All the isolates were tested for antibiotic susceptibility against 17 antimicrobial drugs (including first, second & third generation drugs) by disc diffusion assay on Mueller- Hinton agar with antibiotic discs according to the modified Kirby-Bauer method. A single colony of each isolate was inoculated into 2 ml Mueller–Hinton broth and incubated at 37°c for 24 hours. The culture turbidity was adjusted to 0.5McFarland standard. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire surface of Muller-Hinton agar. Antibiotic discs of appropriate concentration were placed aseptically over the surface at an appropriate spatial distance of 5mm. Plates were then inverted & 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 20,21.

Result:

According to this research Table -1, 1148 (37.5%) positive cases of UTI were found from a total of 3060 urine samples where the Male participant was 972 (32%) & the Female participant was 2088 (68%). Out of 1148 UTI-positive patients, 248 cases were positive for males which is 22 % & 900 positive cases were positive for females which 78 % (Figure -1).

Table 1 Sex distribution among susceptible population (n-3060).

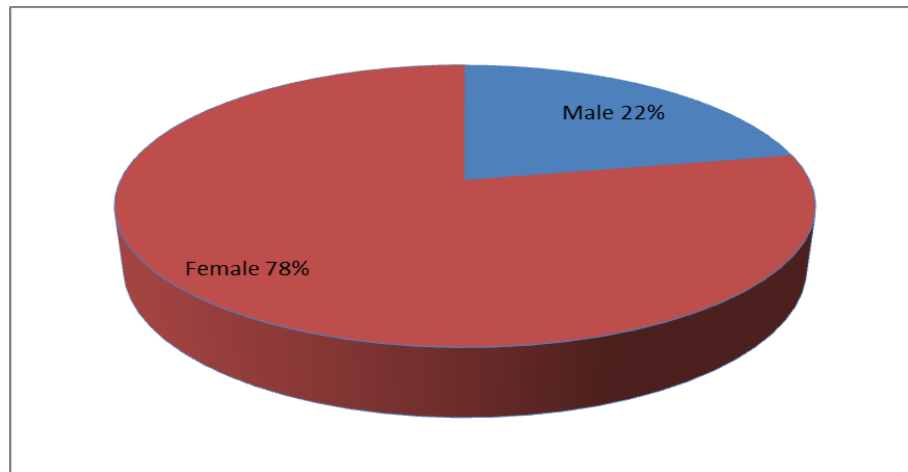| Gender	Total	Growth	
		Positive	Negative
Male	972 (32 %)	248 (22%)	724 (38)
Female	2088 (68 %)	900 (78%)	1188 (62)
Total	3060 (100%)	1148 (37.5)	1912 (62.5)

Figure 1: Pie chart showing sex distribution of isolated UTI patients. Out of 1148 positive females were 900 (78%) and males were 248 (22%).
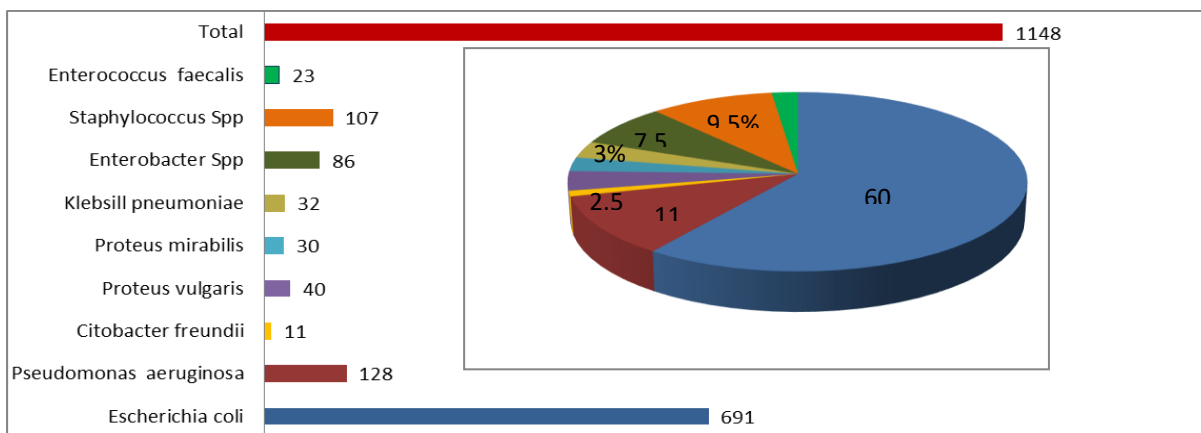
Figure 2: The bar diagram showing the frequency distribution of different organisms among the positive cases & pie chart showing the percentage of total isolation. Our finding pathogens are *Escherichia coli*, Enterobacter spp, *Pseudomonas aeruginosa*, *Proteus Vulgaris*, *Proteus mirabilis, Citrobacter freundii, Klebsiella pneumoniae,* Staphylococcus spp, *and Enterococcus faecalis.*

Table 2. Morphologically and Biochemical test results of the isolated pathogens

Isolated pathogens	Microscopic observation				Biochemical test											
	Gram-reaction	Cell color	Cell shape	Cell arrangement	Lactose Fermentation	TSI				MIU						
						Slant	Butt	H2S	Gas	Motility	Indole	Urease	Oxidase	Citrate	Catalase	Coagulase
Escherichia coli	Gram negative	pink	Rod	Single	+	Y	Y	-	+/-	+	+	-	-	-	+	-
Enterobacter spp	Gram negative	Pink	Rod	Single	+	Y	Y	-	+	+	-	-	-	+	+	-
Proteus vulgaris	Gram - negative	Pink	Rod	Swarm	-	Y	R	+	-	+	+	+	-	-	+	-
Proteus mirabilis	Gram - negative	Pink	Rod	Swarm	-	Y	R	+	-	+	-	+	-	-	+	-
Citrobacter freundii	Gram - negative	pink	Rod	Single	+	Y	Y	-	+/-	+	+	-	-	-	+	-
Pseudomonas aeruginosa	Gram - negative	Pink	Rod	Single	-	R	R	-	-	+	-	Slow	+	+	+	-
Klebsiella pneumoniae	Gram-negative	Pink	Rod	Single	+	R	Y	-	+	-	-	Slow	-	+	+	-
Staphylococcus spp	Gram-positive	Purple	Cocci	Cluster	+	Y	Y	-	-	-	-	+	-	+	+	Not done

Table 3. Antibiotic sensitivity pattern on isolated uropathogenes

Antibiotic	*Escherichia coli* (691)	*Pseudomonas aeruginosa* (128)	*Klebsiella pneumoniae* (32)	Staphylococcus Spp (107)	*Proteus vulgaris* (40)	*Proteus mirabilis* (30)	*Enterobacter spp* (86)	*Enterococcs faecalis* (23)	*Citrobacter freundii* (11)
Amoxicillin (10 µg)	02 %	07 %	02 %	20 %	03 %	05 %	05 %	25 %	00%
Amoxiclav (30 µg)	71 %	60 %	63 %	70 %	35 %	63 %	38 %	68 %	61%
Azithromycin (15 µg)	06 %	02 %	00 %	07 %	15 %	16 %	17 %	10 %	00%
Cefixime (05µg)	15 %	12 %	14 %	05 %	05 %	07 %	05 %	02 %	03%
Ceftazidime (30 µg)	18 %	14 %	20 %	08 %	08 %	05 %	11 %	07 %	06%
Cefuroxime (30 µg)	14 %	09 %	13 %	07 %	03 %	04 %	08 %	05 %	02%
Ciprofloxacin (05µg)	41 %	43 %	32 %	46 %	28 %	31 %	38 %	30 %	33%
Cotrimoxazole (25µg)	15 %	21 %	08 %	43 %	25 %	28 %	07 %	02 %	02%
Ceftriaxone (30 µg)	33 %	36 %	28 %	30 %	15 %	18 %	25 %	15 %	30%
Nitrofurantoin (30 µg)	67 %	31 %	50 %	60 %	56 %	60 %	47 %	59 %	60%
Amikacin (30µg)	80 %	79 %	71 %	79 %	86 %	84 %	68 %	80 %	83%
Gentamicin (10 µg)	74 %	72 %	71 %	73 %	82 %	84 %	70 %	73 %	82%
Meropenem (10 µg)	89 %	72 %	82 %	83 %	84 %	84 %	78 %	80 %	88%
Doxycycline (30 µg)	40 %	38 %	40 %	53 %	30 %	36 %	37 %	20 %	30%
Levofloxacin (05µg)	37 %	47 %	38 %	43 %	40 %	43 %	41 %	20 %	32%
Linezolid (30µg)	10 %	03 %	02 %	30 %	00 %	00 %	09 %	45 %	00%
Nalidixic Acid (30 µg)	07 %	15 %	02 %	10 %	15 %	18 %	15 %	00 %	00%

After performing the cultural & biochemical test, Gram staining, microscopic observation the existence of total pathogens 1148 (100%) in urine sample were determined as *Escherichia coli* 691 (60%), *Pseudomonas aeruginosa* 128 (11%), *Citobacter freundii* 11 (01%), *Proteus vulgaris* 40 (3.5%) , *Proteus mirabilis* 30 (2.5%), *Klebsilla pneumoniae* 32 (3%), Enterobacter Spp 86 (7.5%), Staphylococcus Spp 107 (9.5%), *Enterococcus faecalis* 23 (2 %) Figure 2. 17 antibiotics were used against the isolated uropathogens. As it can be seen from (Table 3) *Escherichia coli* are sensitive to Meropenem 89%, Amikacin 80%, Gentamicin 74%, Nitrofuration 67%, Amoxiclave 71%, Ciprofloxacine 41%, Levofloxacin 37%, Doxycycline 40%. *Pseudomonas aeruginosa* sensitive to to Meropenem 72%, Amikacin 79%, Gentamicin 72%, Nitrofuration 54%, Amoxiclave 60%, Ciprofloxacine 43%, Levofloxacine 47%. *Klebsilla Pneumoniae* to Meropenem 82%, Amikacin 71%, Gentamicin 71%, Nitrofuration 50%, Amoxiclave 63%, Ciprofloxacine 32%, Levofloxacine 38%. Staphylococcus Spp Meropenem 83%, Amikacin 79%, Gentamicin 73%, Nitrofuration 60%, Amoxiclave 70%, Ciprofloxacine 46%, Levofloxacine 43%, Lenazolid 30%,Cotrimoxazole 43%. *Proteus vulgaris* Meropenem 84%, Amikacin 86%, Gentamicin 82%, Nitrofuration 56%, Amoxiclave 35%, Ciprofloxacine 28%, Levofloxacine 40%, Doxycycline 30%. *Proteus mirabilis* Meropenem 86%, Amikacin 84%, Gentamicin 84%, Nitrofuration 60%, Amoxiclave 63%, Ciprofloxacine 31%, Levofloxacine 43%, Doxycycline 30%. Enterobacter Spp Meropenem 78%, Amikacin 68%,Gentamicin 70%, Nitrofuration 47%, Amoxiclave 58%, Ciprofloxacine 38%, Levofloxacine 41%, Doxycycline 37%. Enterococcus faecalis Meropenem 80%, Amikacin 80%, Gentamicin 73%, Nitrofuration 59%, Amoxiclave 68%, Ciprofloxacine 30%, Levofloxacine 20%, Lenazolid 32%, Cotrimoxazole 43%. Citobacter freundii Meropenem 88%, Amikacin 83%, Gentamicin 82%, Nitrofuration 60%, Amoxiclave 61%, Ciprofloxacine 33%, Levofloxacine 32%, Ceftriaxone 30%, Doxycycline 30%.

Discussion:

Urinary tract infection is an emerging issue as a common clinical problem in both the community and healthcare-associated settings. Epidemiological UTI accounts for seven million outdoor visits, one million emergency visits, resulting in 100000 hospitalization visits yearly 31. Moreover, antimicrobial resistance to uropathogenic creates major health problems worldwide. The study describes the relation between sex, isolated bacterial pathogens, and antibiotic susceptibility patterns. Among the total number of patients 3060, 1148 (37.5%) were culture positive include males & females. Among 1148 positive samples of UTI of those 248 (22%) male and 900 (78%) female. In our study, 37.5 % positive that is similar with 290 (36.1%) positive out of 804 susceptible participants 22 and 49 (31.4%) positive case out of 156 is not similar with our study 25. Our finding *Escherichia coli* 691 (60%), *Pseudomonas aeruginosa* 128 (11%), *Citrobacter freundii* 11 (01%),
Proteus vulgaris 40 (3.5%) , *Proteus mirabilis* 30 (2.5%), *Klebsiella pneumoniae* 32 (3%),
Enterobacter spp 86 (7.5%), Staphylococcus spp 107 (9.5%), *Enterococcus faecalis* 23 (2%).Two hundred and sixty-seven (267) morning clean catch midstream urine samples were collected from patients attending three hospitals in Bushenyi district. Significant bacteriuria was observed in 86/267 (32.2%). *Escherichia coli* was the most prevalent bacterial uropathogen with 36/86 (41.9%) followed by *Staphylococcus aureus* 27/86 (31.4%), *Klebsiella pneumoniae* 10/86

(11.6%), *Klebsiella oxytoca* (16.3%), Proteus spp (12%), *Enterococcus faecalis* (4.1%) 23. that is not similar with our study *Escherichia coli* (55.1%), *Pseudomonas aeruginosa* (4.1%), *Citobacter freundii* (02%), *Proteus vulgaris*

(3.5%), *Proteus mirabilis* (2.5%), *Klebsiella pneumoniae* (3%), Enterobacter Spp (7.5%) , Staphylococcus Spp (9.5%) , *Enterococcus faecalis* (2 %)25 that few match with our study. *Escherichia coli* (52.9%)24 was found to be the predominant pathogen that is similar with our study And Klebsiella spp (22.9%), Proteus spp. (4.7%), Pseudomonas spp. (4.4%), Enterobacter spp (2.4%), Citrobacter spp (3%)24 are not similar with our study E. coli is the most prominent bacterial isolate that similar with 22,23,24,25. Among the 1148 positive sample of UTI 248 (22%) was male and 900 (78%) female that are not similar with male (49.3%) & female (66.67%) that is few match with our study. All gram-negative uropathogens are most effective to Meropenem, Gentamicin. Amikacin, Nitrofuraton, Ciprofloxacin, Levofloxacin, Doxycycline and Gram-positive uropathogens are effective to Meropenom, Gentamicin, amikacin, doxacyclin, linezolid that is similar with22. Our finding Gam-negative uropathogens antibiotic sensibility pattern similar with 27. In our study, Amoxiclave are effective on all pathogens that we can compare with 28. Increasing drug resistance is a great concern to common bacterial infections including UTI. Still, antimicrobial agents like Amoxicillin, Azithromycin, Nalidixic Acid, Cefixime, Cotrimoxazole are in place to treat many Gram-positive & Gram-negative bacterial infection including UTIs in many developed and developing country including south Asian countries like Bangladesh, India. These findings are alarming to the choice of antimicrobial agent. In November 2019 one of our studies showed 33% positive and E.coli 48% and Amoxicillin was showed 100% resistant and Meropenem was 84% sensitive

32, our recent study so far change from that. Antibiotic resistance among uropathogens has become a public health concern in Bangladesh. The pattern of antibiotic resistance of the microorganism causing infection including UIT varies in their antibiotic susceptibility from place to place & time to time. Antibiotic abuse due to easy availability & practicing an incomplete course of antibiotics during infection has promoted antibiotic resistance. The highest number of resistance found in commonly used oral drugs and lower resistance developed in less common use drugs.

Conclusion:

UTI is a serious health problem all over the world including Bangladesh, also affecting millions people every year. The prevalence of antimicrobial resistance become increasing worldwide and is a major factor in the selection of antibiotics for treatment and misuse of antibiotics. To avoid this danger we have must take an antibiotic as prescribed by a doctor and complete the dose according to the correct rules. And there is a need to create awareness among the people about antibiotic resistance. Regular monitoring is required to establish reliable information about the susceptibility pattern of uropathogenic for optimization of urinary tract infection. The emergence and spread of resistance can be reduce through appropriate use of antimicrobial agents. If it is possible to increase the research and reach the result to physician that helps to select the right antibiotic and reduce the misuse of many antibiotic.

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