Isolation, Identification and Antibiotic Susceptibility Analysis of Bacterial Pathogens from Suspected Urinary Tract Infected Patients of Tertiary Medical Centre

Md. Robeul Islam[1], Avijit Banik[1], Md. Abu Zihad[1], Kumkum Rahman Mouree[1*] and Suvamoy Datta[1]

Abstract

An infection of the urinary tract (kidneys, ureters, bladder, and urethra) is known as a urinary tract infection (UTI). The lower urinary tract, the bladder, and the urethra are the most often infected areas. Women are more likely than males to have a bacterial infection. The aim of the research was to determine the causative agent of UTI in patients and see how they responded to standard treatments. A total of 435 urine samples were examined using the culture technique. The samples were streaked evenly on blood agar, MacConkey, and then incubated for 24 hours at 37°C. The morphological characteristics of the colony on culture media were used to identify the presumptive bacterium. Gram staining and routine biochemical assays were also used to confirm the findings. On Muller-Hinton agar, the disk diffusion technique was employed to assess susceptibility to 12 different antibiotics. The most prevalent uropathogen was E. coli (44%) samples. The second isolate was Staphylococcus aureus (21%), Klebsiella species (13%) and Proteus species (12%), Enterobacter species (10%). In overall, the uropathogens were highest susceptible to Meropenem (82.2%), Amikacin (63.6%) and Cefixime (59.8%) highest resistance to Azithromycin (84.2%), Gentamycin (75.6%) and Nalidixic acid (64.4%), (84.2%), Gentamycin (75.6%) and Nalidixic acid (64.4%) was the least effective. The present Study can be helpful for the clinicians in finding proper drugs in the developing countries In both genders and age groups, E. coli was the most common uropathogen (44%). The most successful medications for the treatment of UTI Meropenem (82.2%), Amikacin (63.6%), and Azithromycin (93.15 percent), whereas Azithromycin like Bangladesh where multi-drug resistance problem has just complicated the treatment of UTIs.

Keywords: UTI, Uropathogen, Antibiotic Susceptibility, Tertiary Medical Centre.

Significance | Urinary tract infections (UTIs) are common, especially in women. Identifying bacteria causing UTIs helps choose effective treatments, crucial for managing infections.

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Introduction

The most frequent life-threatening and community-acquired bacterial illness, urinary tract infection (UTI), has a high rate of morbidity and financial cost (Akram, Shahid, & Khan, 2007; Akter et al., 2016a, 2016b). UTI is a bacterial infection that affects one or more sections of the urinary system and occurs when bacteria overcome the natural host defense mechanism (Akter et al., 2016b). A bladder infection or cystitis occurs when infections occur in the lower urinary system, and kidney infection or pyelonephritis occurs when infections occur in the upper urinary tract (Al-Dujiaily, 2000). Urinary urgency, dysuria, pyuria, inflammation of the

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urinary tract, uncomfortable pressure, bloody urine with a strong odor, and weariness are all symptoms of cystitis (Andreu et al., 2005). Fever, flank discomfort, and cystitis symptoms are all signs of a kidney infection (Al-Dujiaily, 2000). UTI can be caused by a variety of microorganisms, although Gram-negative bacteria cause the majority of UTIs. In both inpatients and outpatients, E. coli, a common member of the Enterobacteriaceae family, is responsible for 75.0-90.0 percent of all UTIs (Barnes, Roddy, Daifuku, & Stamm, 1986). Pseudomonas species, Proteus species, Klebsiella species, and Citrobacter species are among the Enterobacteriaceae bacteria that cause UTI. Group B Streptococci, Staphylococcus aureus, and Staphylococcus saprophyticus are the most common Gram-positive bacteria that are frequently detected (Beyene & Tsegaye, 2011). Nonresident infectious organisms invade structurally and functionally normal urinary tracts in uncomplicated urinary tract infections, whereas infections in severe urinary tract infections occur in patients with physically and functionally aberrant urinary tracts or both (Bauer, Kirby, Sherris, & Turck, 1966). Potential urinary pathogens from the intestine, or in rare circumstances from the vagina of women as a result of sexual activity, are examples of nonresident infectious organisms (Beyene & Tsegaye, 2011). Organisms colonize the periurethral mucosa and then climb to the bladder via the urethra and, in rare circumstances, the kidney via the ureter (Bauer et al., 1966). Bacterial adhesion to the bladder mucosal membrane is inhibited by antimicrobial secretions, polymorphonuclear cells, and the Tamm- Horsfall glycoprotein of the host (Bauer et al., 1966). Uropathogens, on the other hand, colonize and infect the urinary system through various virulence factors and processes. Uropathogenic E. coli is the most prevalent pathogen in simple urinary tract infection, and it has improved virulence factors such adhesins and fimbriae (pili), which allow it to adhere to particular uroepithelial receptors (Beyene & Tsegaye, 2011). Because of anatomical predisposition, urothelial mucosa adhesion to the mucopolysaccharide lining, or other host characteristics, uncomplicated UTI is more prevalent in women (Clinical and Laboratory Standards Institute, 2013). Recent research suggests that simple UTI can arise in males as a result of insertive anal intercourse, absence of circumcision, having a sexual partner with vaginal colonization by uropathogenic bacteria, or a lack of immunity (Cunha et al., 2016; Dromigny et al., 2005; Farajnia et al., 2009). The vast majority of UTIs do not pose a life-threatening or irreversible hazard. With the predominance of bacteremia, however, there is a danger of significant tissue damage when the kidneys are implicated (Ferry, Burman, & Holm, 1988). The clinical indications, symptoms, and urinalysis findings are all significant in determining whether or not you have a UTI. Urine culture results are critical in the diagnosis of UTI because they reveal the identity of the invading bacteria and their antibiotic susceptibility (Hvidberg et al., 2000). Only a few investigations on the susceptibility pattern of community-acquired UTI pathogens in various countries have been described (Lane & Takhar, 2011; Sobel, 1997; Spach, Stapleton, & Stamm, 1992).

Materials and methods

Study Area and Study Population

The study was carried out in a different tertiary care hospital in Dhaka city, Bangladesh. This study includes 435 patients with suspected urinary tract infections during the period from January (2021) to October (2021). 435 clinical samples were collected from different age and sex groups, among which, 128 samples were from males and 307 samples from female patients.

Preparation of Culture Media

A batch of general culture media was prepared per the company's instructions and sterilized by autoclaving at 121°C for 15 minutes. Such as CLED Agar, MacConkey Agar, Nutrient agar, Blood Agar. Muller-Hinton agar, Simmons' citrate Agar, MR –VP Medium and Peptone water medium.

Isolation and identification

Bacterial identification was accomplished by phenotypic assessment of culture and biochemical properties on an uropathogen-specific selective medium. 100µl of urine were inoculated onto sterilized and solidified Blood agar and MacConkey agar medium, then incubated aerobically for 24 hours at 37°C. The colony number was counted after incubation to confirm the diagnosis of UTI. The appearance, size, color, and morphology of the colonies were recorded macroscopically.

Standard culture and biochemical profiles of the isolates were used to isolate and identify bacteria. Standard biochemical assays (Spach et al, 1992) were used to identify Gram-negative bacteria. Gram-positive pathogens, on the other hand, were identified using laboratory tests such as catalase, and the mannitol test for Staphylococcus aureus (Stamm et al, 1993). It was deemed positive culture and recorded as "significant growth" if the number of colonies was higher than >105 CFU/mL, but it was termed "non-significant growth" if the number of colonies was less than 105 CFU/mL (Sobel et al. 1997).

Determination of Antibiotic Resistance Using Kirby Bauer Method

The Kirby Bauer disk diffusion technique has been used to determine antibacterial susceptibility to various antibiotics following Clinical Laboratory and Standards Institute (CLSI) standards (Stratchounski & Rafalski, 2006). Ciprofloxacin, Cefuroxime, Cefixime, Azithromycin, Sulphamethaxazol, Meropenem, Amoxicillin, Ceftriaxone, Nalidixic Acid, Amikacin, Gentamycin, and Nitrofurantoin were administered as antibiotics in this study. The inoculum adjusted to 0.5 McFarland standards, a sterile cotton swab was dipped in the solution and smeared across the Muller Hinton agar plate's surface. The plate was then allowed

Table 1. Presumptive Organism

Features	*E. coli*	*Klebsiella* spp.	*S. aureus*	*Proteus* spp.	*Enterobacter* spp.
Colony on Blood agar	Non-hemolytic, large, gray colonies	Non-hemolytic, Mucoid colonies	β-hemolysis colonies	Non-hemolytic, swarming colonies	Non-hemolytic, Rough, white colonies
Colony on MacConkey agar	Smooth, Lactose fermenter colonies	Mucoid, Lactose fermenter colonies	No growth	Smooth, non-Lactose fermenter colonies	Small, Lactose fermenter colonies
Gram staining	Pink color, rod-shaped,	Pink color, rod-shaped,	Violet color, round shape	Pink color, rod-shaped,	Pink color, rod-shaped,
Indole	+	-	-	+	-
Methyl red	+	+	+	+	-
Voges-Proskauer	-	+	+	-	+
Citrate utilization	-	+	-	+	+
Motility	+	-	-	+	+
H_2S production	-	-	-	+	-
Gas production	+	+	-	+	+
Oxidase	-	-	-	--	-
Catalase	+	+	+	+	+
Urease	-	+	-	+	-

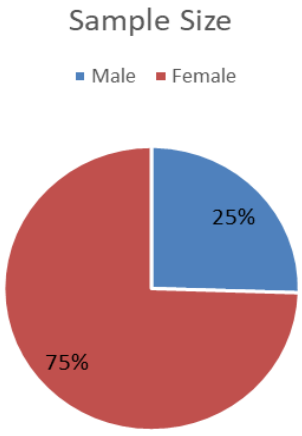

Figure 1. Total Sample size (Male and Female)

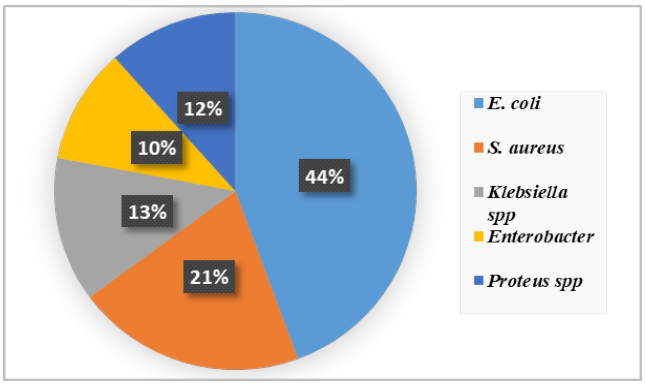

Figure 2. Distribution of isolated uropathogens.

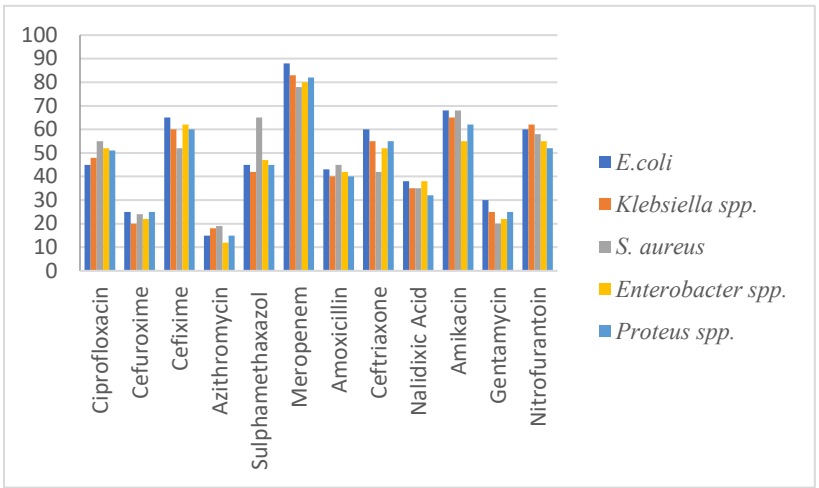

Figure 3. Sensitive pattern of Antibiotics against isolated Microorganisms

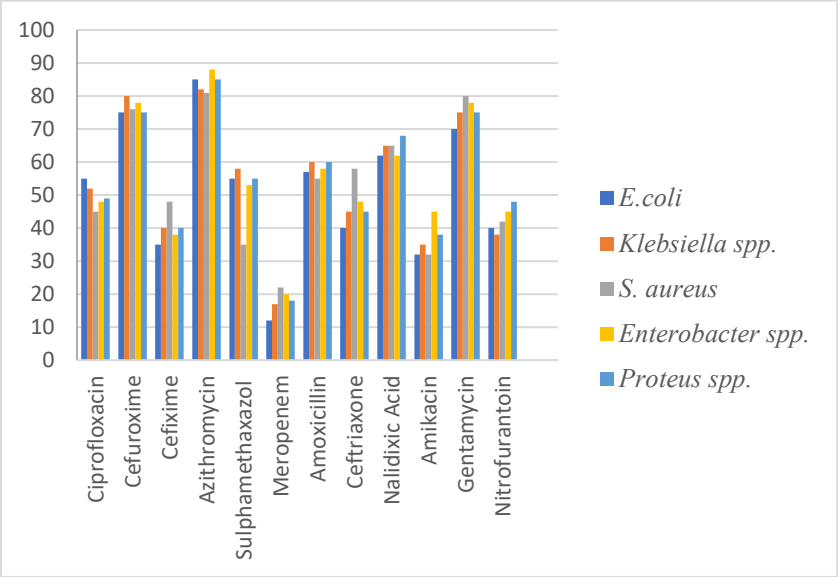

Figure 4. Resistance pattern of Antibiotics against isolated Microorganisms

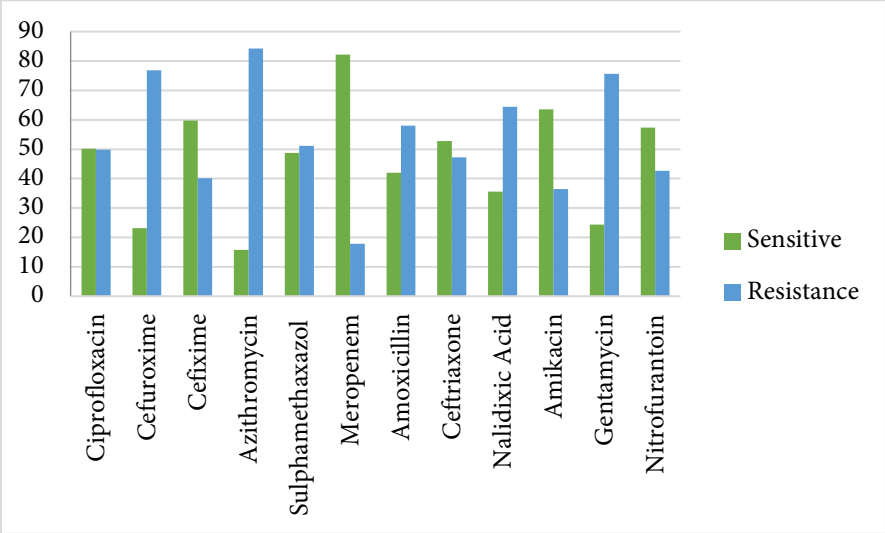

Figure 5. Total percentage of Antibiotics.

to dry for a few minutes at room temperature. Antibiotic discs were aseptically inserted on the surface of agar with sterile forceps, and plates were subsequently incubated for 24 hours at 37°C. Millimeter calipers were used to measure the widths of the zones of complete inhibition after incubation.

Results and Discussion

A total of 435 urine samples were collected and analyzed. Among the cultures screened, Out of 435 urine samples, 259 (59%) samples were found positive for bacterial infection. Of these 259 positive cases, 66 (25%) isolates were male, and the rest 193 (75%) isolates were female (Figure 1). The positive growth of E. coli, S. aureus, Enterobacter spp. Klebsiella spp. and Proteus spp were confirmed by cultural, microscopic, and various biochemical tests which results are presented in Table 1 . This result indicated that the female patients had a higher prevalence of UTI than males. This result is consistent with other studies performed in Dhaka city (Tammana et al., 2016). The most prevalent uropathogen was E. coli 115(44%) samples (Figure 2). The second isolate was Staphylococcus aureus 53(21%), followed by Klebsiella species 34(13%) and Proteus species 30(12%), Enterobacter species 27(10%) (Tammana et al., 2016). The results showed that the organisms isolated from urine samples were E. coli, Enterobacter species, Klebsiella species, Proteus species, and Staphylococcus aureus. Also, the most frequent causative agent of UTI was found to be E. coli (44%) in both sex groups. This result is consistent with another study conducted in Dhaka, the capital of Bangladesh (Tammana et al., 2016). In Bangladesh, where E. coli (59%) was found as the primary etiological agent of UTI. Our report was higher than reports conducted in India and Southwest Ethiopia, where E. coli was 31.5% and 33.3%, respectively. However, our report is lower than the report carried out in Russia where E. coli (85.9%) was also found as the predominant isolate (Stratchounski & Rafalski, 2006; Tammana et al., 2016; Weber et al., 1995; Wing et al., 2000; Wong & Stamm, 1983; Wullt et al., 2000).

The antimicrobial susceptibility test showed that, E. coli was susceptible to Meropenem 88%, Amikacin 68%, Cefixime 65%, Ceftriaxone 60%, Nitrofurantoin 60% and moderate level of susceptible to Amoxicillin 43%, Nalidixic acid 38%, Sulphamethaxazol 45%, Ciprofloxacin 45% and least susceptible to Azithromycin 15 %, Cefuroxime 25% and Gentamycin 30%. The second uropathogen S. aureus was susceptible to Meropenem 78%, Amikacin 68%, Sulphamethaxazol 65%, Nitrofurantoin 58%, Ciprofloxacin 55%, Cefixime52%, and moderate level of susceptible to Ceftriaxone 42%, Amoxicillin 45%, Nalidixic acid 35% and least susceptible to Azithromycin 19 %, Cefuroxime 24% and Gentamycin 20%. Klebsiella species was 83% susceptible to Meropenem, Amikacin 65%, Nitrofurantoin 62%, Cefixime 60%, Amoxicillin 40%, Ciprofloxacin 48%, Sulphamethaxazol 42%, Nalidixic acid 35% and 55% susceptible to Ceftriaxone, followed by 18% susceptible to Azithromycin and 22% Cefuroxime, Gentamycin 25%. Proteus species was susceptible to Meropenem 82%, Amikacin 62%, Cefixime 60%, Ceftriaxone 55%, Nitrofurantoin 52%, Ciprofloxacin 51% and moderate level of susceptible to Sulphamethaxazol 45%, Amoxicillin 40%, and least susceptible to Azithromycin 15 %, Nalidixic acid 32% Cefuroxime and Gentamycin both are 25%. Enterobacter species was 80% susceptible to Meropenem, 55% to Amikacin and Nitrofurantoin 55%, Cefixime 62%, Amoxicillin 42%, Ciprofloxacin 52%, Sulphamethaxazol 47%, Nalidixic acid 38% and 52% susceptible to Ceftriaxone, followed by 12% susceptible to Azithromycin and 22% to Cefuroxime and Gentamycin. (Figure 3, 4) In overall, the uropathogens were highest susceptible to Meropenem (82.2%), Amikacin (63.6%) and Cefixime (59.8%) and lowest susceptible to Azithromycin (15.8%) and Cefuroxime (23.2%) In overall, the uropathogens were highest resistance to Azithromycin (84.2%), Gentamycin (75.6%) and Nalidixic acid (64.4%) and lowest resistance to Meropenem (17.8%) and Amikacin (36.4%) (Figure: 5)

Conclusion

Current study confirmed that E.coil, S. aureus, Klebsiella species, Enterobacter species and Proteus species are common pathogenic organisms that are usually associated with UTI. The result of total samples volume showed that females are more frequently .infected with UTI infections than males. The bacterial sensitivity profile reveals that only fourth-generation drug Meropenem was shown sensitive against more than all isolates otherwise most of the third and second-generation drugs were highly resistant against the isolated UTI organisms. It is recommended that for appropriate treatment and prevention of bacterial resistance, the Physicians should prescribe antibiotics after having the culture sensitivity test.

Author contribution

K.R.M., conceptualized and developed the methodology, M.R.I. and A.B., prepared the original draft and collected data, M.A.Z., reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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