Uropathogenic Analysis and Commonly Used Drug Sensitivity Patterns of the Pathogens in Dhaka City, Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AS, KARS, AMJ and DS designed the study. Author TT managed the experimental process and analyses of the raw data. Author MA wrote the protocol and the first draft of the manuscript. Authors MA and IS managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Human urinary tract infections (UTI) are very common in Bangladesh. The objectives of the current study are to identify the uropathogenic agents infecting males and females of different age groups, and commonly used drugs sensitivity profiles.
Study Design: A total of 980 urine samples from both sex and different age groups, were collected for the study. Chemical analyses of the samples were done by Dipstick method. Cultural, microscopic and biochemical analyses were done to identify the isolates. Finally antibiotic sensitivity was tested against conventionally used antibiotics.
Place and Duration of Study: All the samples were collected from patients of both indoor patient department (IPD) and outdoor patient department (OPD) in the Laboratory Medicine Department,

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United Hospital, Dhaka, Bangladesh between January 2012 and May 2012 following published procedures.

Results: Only 29.6% of the urine samples yielded positive culture. UTI showed more prevalence among female than male. Female belonging to the age group of 41-50 years were detected as high risk groups for UTI. Most common Gram negative isolates were *Escherichia coli*, *Klebsiella* sp., *Candida* sp., *Pseudomonas* sp., *Proteus* sp., and *Acinetobacter baumannii*. Gram positive bacteria comprised *Enterococcus* sp. and non-hemolytic Streptococci. Like most of the previous reports, *E. coli* was predominant, which is also corroborated in this study. However, the sensitivity pattern of the organisms differed from the previous studies.

Conclusion: The isolates were found resistant to most common oral antibiotics used, such as cotrimoxazole, nitrofurantoin, and nalidixic acid. This finding, however, need further work to validate reliability.

1. INTRODUCTION

Urinary tract infections (UTI) are common and frequently encounter serious morbidity that badly affects its levy not only to all human population but also results in increasing antibiotic resistance due to persistence and unprofessional conduct of the ailment. Even today UTI is one of the most important causes of morbidity and mortality in the developing countries like Bangladesh and even in developed countries [1]. This may be attributed to lack of proper research, faulty diagnostic procedures, abuse of chemotherapeutic agents of the people and little or no preventive measures. The alarming phenomenon is that UTI does not restrict itself to the urinary tract only rather it can spread. UTI usually cause inflammation of the affected tissues of the urethra (urethritis) and urinary bladder. The most significant danger from lower urinary tract infections is that they can affect the kidney (causing pyelonephritis) and develop bladder infections subsequently [2]. Bacteria carried by blood stream can also infect the kidney and the infections can be very difficult to eradicate, are often chronic, and lead to marked damage of the kidney. Death promptly follows kidney failure unless the patient is lucky enough to be able to use artificial kidneys, or perhaps to receive a kidney transplant. UTI present as the clinical syndromes of acute, uncomplicated, urinary infection, including acute non obstructive pyelonephritis, complicated urinary tract infection, asymptomatic bacteriuria, and in men, bacterial prostatitis. Severe or life-threatening infection usually occurs with complicated urinary infection, which occurs in men and women with functional or structural abnormalities of the urinary tract. Obstruction or mucosal traumas are the most common precipitating events for urosepsis. Although 20% to 30% of women with acute non obstructive pyelonephritis or men with acute bacterial prostatitis have bacteremia, these syndromes seldom progress to severe sepsis or shock [3].

Any individual may be susceptible to UTI, however, the prevalence of infection differs with age, sex and certain predisposing factors such as diabetes, pregnancy, impaired voiding of the bladders etc [4]. The incidence of infection is greater in females than in males with two exceptions, infants and the catheter related infections [5,6]. Women are likely to get UTI frequently. The incidence of UTI is greater in women (20%) because of the anatomical predisposition or urothelial mucosa adherence to the mucopolysaccharide lining or other host factors. In children approximately 5% of girls and 1% of boys have a UTI by 11 years of age, in the neonates is 0.01-1% and can also be as high as 10% in low birth weight and preterm babies [7].

UTI is considered significant and requires treatment when more than 10^5 microorganisms per ml of urine are present in a properly collected sample [8]. Uropathogenic *E. coli* causes 90% of the UTIs in anatomically-normal, unobstructed urinary tracts [9]. After *E. coli*, the most common UTIs pathogens include *Staphylococcus saprophyticus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Candida* spp., *Klebsiella pneumonia*, *Proteus* spp. and *Enterobacter* spp. Group B streptococci are rare pathogens in UTIs in young healthy women [10]. Interestingly, the pathogens traditionally associated with UTI are known to change many of their features, particularly due to their antimicrobial resistance patterns [11]. Though antibiotics are the mainstay treatment for all UTIs, the increasing trend of resistance in bacterial pathogens is of worldwide concern that

can vary according to geographical and regional locations [12].

Since the initiation of antimicrobial therapy in UTI is empirical, a huge need demand for antimicrobial resistance exists at local, national and international levels [13]. Knowledge on the antimicrobial resistance patterns of common uropathogens and the subsequent treatment are thus required to minimize urinary diseases [14]. The current study aimed to identify the uropathogenic agents of UTI in males and females of different age groups, their sensitivity and resistance patterns against locally available antibiotics frequently prescribed by the physicians in order to find suitable antimicrobial agents to treat UTI.

2. MATERIALS AND METHODS

2.1 Sample Collection

Urine samples were collected aseptically from out-patient department (OPD) and in-patient department (IPD) patients of all age groups including age 0 to 90 from United Hospitals Ltd, Gulshan, Dhaka, Bangladesh, having clinical symptoms of microbial infection. The study was conducted over a period of five months from January 1, 2012 to May 31, 2012 at the hospital setting. A total of 980 clinical isolates were tested from OPD and IPD patients.

2.2 Chemical Analysis

Chemical analysis of the urine specimens were done using COMBINA 11S dipstick. The analysis was done immediately within one hour of specimen collection. The specimens were collected from the midstream flow of the first morning urine. Specimens were thoroughly mixed by inversion several times and no centrifugation was done before analysis.

2.3 Microscopic Analysis

12 mL of each specimen was centrifuged at 1500 rpm for 5 minutes. After discarding the supernatant leaving only around 1 mL of concentrated specimen pellet. The pellet was mixed well and 20 µL of the suspension was placed over glass slide with cover slip on top for microscopic observation. Average number of casts were scanned and counted at low power field (LPF). RBCs, WBCs, crystals, yeast, bacteria, epithelial cells, mucus, and other formed elements were also scanned and counted at high power field (HPF).

2.4 Microbiological Analysis

10 µL of the specimens were directly streaked on blood agar and macConkey agar plates and incubated for 24-48 hours at 37ºC. After incubation the colony characteristics were observed and recorded. Gram staining was also done for all the colonies obtained on blood agar and macConkey agar plates [15].

2.5 Biochemical Analysis

Microgen GnA+B-ID system containing two separate micro well test strips GnA and GnB, was used for biochemical analysis of the isolates following their instruction manual. Each Micro well test strip contains 12 standardized biochemical substrates which have been selected on the basis of extensive computer analysis. The dehydrated substrates in each well are reconstituted with a saline suspension of the organism to be identified. If the individual substrates are metabolized by the organisms, a color change occurs during incubation or after addition of the specific reagents. The permutation of metabolized substrates was interpreted using the Microgen Identification System Software (MID60) to identify the test organism.

2.6 Antibiotic Susceptibility

Antibiotic susceptibility test for the isolates were done by disc diffusion method on muller-hinton agar plates [16]. Antibiotic discs of amoxyclave (AMC), amikacin (AK), ceftezidime (CAZ), ceftriaxone (CRO), ciprofloxacin (CIP), meropenem (MEM), imipenem (IPM), gentamicin (GN), cefixime (CFM), cefepime (FEP), netilmycin (NET), cotrimoxazole (CXT), nitrofurantion (F), nalidixic Acid (NA), polymyxin B (PB), tazobactum (TZP) were used for the susceptibility test. After placing the discs, the plates were inverted and incubated overnight at 35ºC.

3. RESULTS AND DISCUSSION

Identification of the etiological agents and their susceptibility to antimicrobial agents is very important for choosing proper drug to treat the patient in their early stage of UTI. It is therefore, recommended that routine uropathological

analysis and antibiotic sensitivity test of mid stream urine samples of the patients be carried out before the treatment of UTI [17].

A total number of 980 specimens were collected and analyzed for uropathogenic analysis. 288 specimens (29.4%) were positive for growth of urinary pathogen, 60 specimens (6.1%) showed growth of non pathogenic organisms and 632 specimens (64.5%) showed no apparent growth.

A total of 288 patients of UTI of either sex with the respective ratio of 27:73 (male: female) between age group of 1-90 years was studied (Fig. 1). Schaeffer and Dielubanzan [18] and Ahmed et al. [19] also found similar results in their study. Among the 288 UTI patients, 185 patients were from In Patient Department (IPD) and 103 patients were from Out Patient Department (OPD). Fig. 1 showed the number of UTI suspected cases according to different age group where higher UTI suspected age groups were from 41-50 for female and 0-10 and 11-20 age groups for male. However, maximum UTI patients were from age group of 30-35 years according to Ahmed et al. [19]. For male and female UTI patients, age group of 11-20 and 41-50 were found to be high risk group, respectively (Fig. 1). For females, after the age of 40, they became prone to UTI.
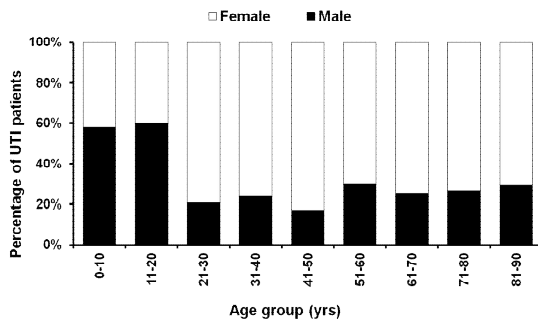

Fig. 1. Gender wise UTI patients (%) by age group

The current study indicated that *E. coli* is the primary etiological agent of UTI (Fig. 2). Besides, *Klebsiella* spp. and *Candida* spp. were also predominant among the UTI patients. *Acinetobacter baumanni* was detected from male patients mostly (80%), whereas *E. coli* and *Morganella morgannii* were detected mostly from female (80%) patients (Fig. 3). Other than *A. baumanni*; *Enterococcus* sp., *Candida* spp. and *Klebsiella* sp. were predominant causal

agent of UTI among male patients. *Klebsiella* sp., *Pseudomonas, Proteus* and others were predominant for female UTI (Fig. 3), which is similar to previous findings [18].
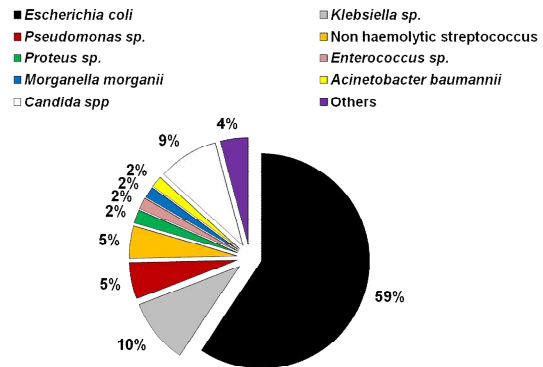

Fig. 2. Etiological agents of UTI

The distribution of abnormal findings such as presence of pus cell, RBC, albumin, glucose, nitrite and ketone bodies were analyzed and the findings are presented in Fig. 4. Nitrite, pus cells, RBC, ketone bodies and albumin were found predominantly among female patients. Glucose was reported equally for patients of either sex (Fig. 4). Pus cells were found in urines of all UTI patients no matter what pathogen was involved (Fig. 5). Patients infected with *Enterococcus* sp. showed highest pus cells as well as albumin in urine (Fig. 5). As *Enterococcus* spp. are frequently encountered uropathogens in complicated UTIs [20], pyuria and albuminuria might have a relationship with that. However, patients infected with other uropathogens also have higher pus and albumin in urine [20], which indicated that these two abnormalities are very common in UTI.

A total of 20 antibiotics were tested on the isolates for their sensitivity pattern. Amikacin showed maximum 62% sensitivity against the isolates whereas, Nalidixic acid showed minimum 22% sensitivity, which is similar to the findings of Amdekar et al. [21] and Nicolle [22]. Gram positive isolates showed highest (72%) sensitivity against Vancomicin and Linuzolid (Table 1) whereas; lowest (13%) sensitivity was obtained against Ciprofloxacin. Gram negative isolates showed 79% sensitivity against Amikacin [23] and around 27% against Ciprofloxacin and Cotrimoxazole presented in Table 1.
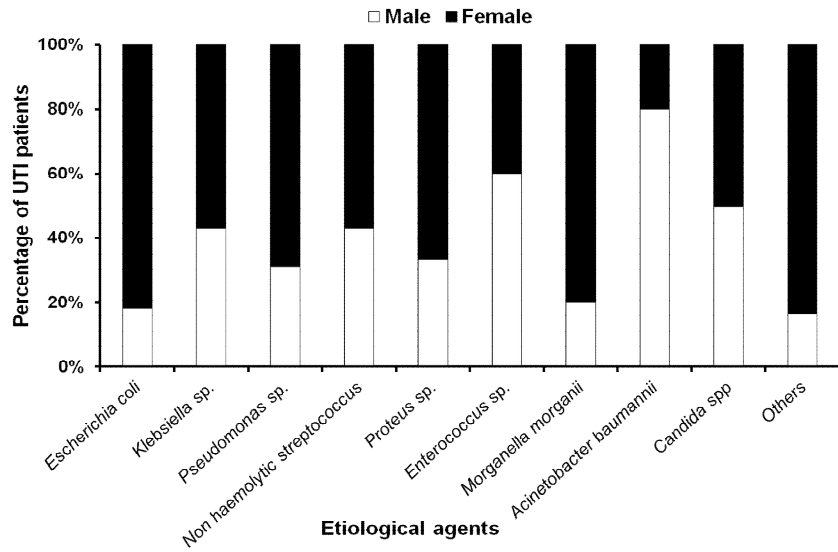
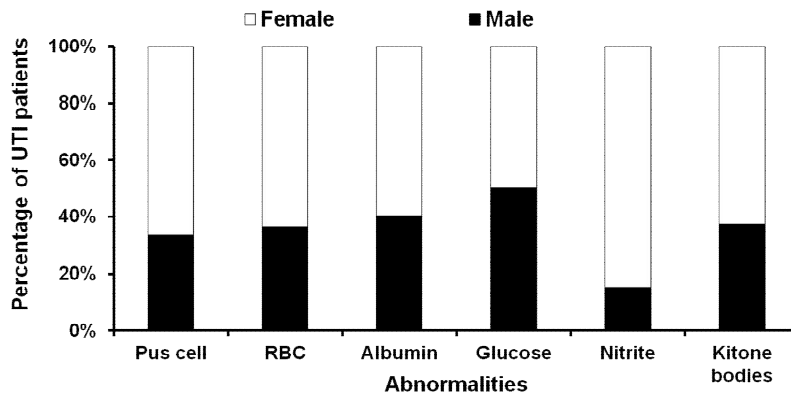**Fig. 3. Etiological agents for UTI in male and female**

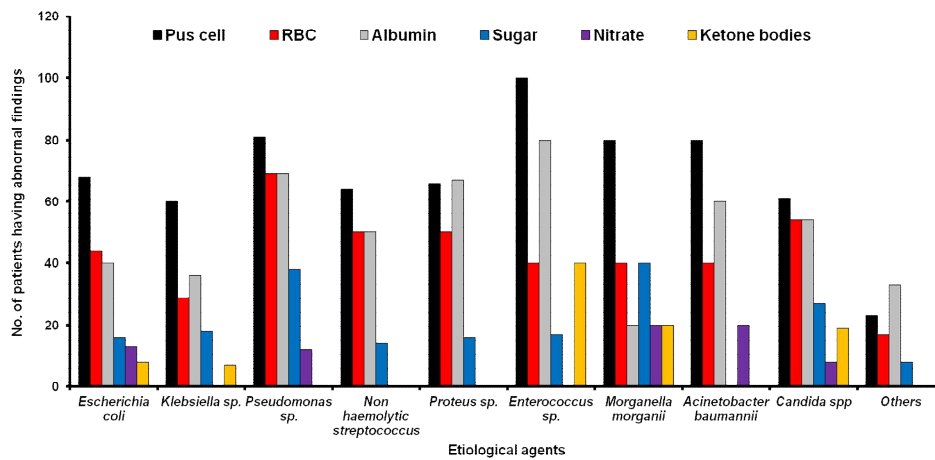**Fig. 4. Presence of other abnormalities in UTI patients**

Fig. 5. Correlation between abnormal findings and etiological agents

Table 1. Degree (%) of antibiotic sensitivity of the pathogen isolates

Antobiotics	*E. coli* (n=171)	*K. pneumoniae* (n=28)	*Pseudomonas* sp. (n=16)	*Proteus* sp. (n=6)	*A. baumanni* (n=5)	*M. morganni* (n=5)	NH *Stretococcus* (n=14)	*Enterococcus* sp. (n=5)
Amoxyclave	49	36	25	50	20	0	21	20
Amikacin	88	54	50	83	40	60	-	-
Ceftazidime	51	36	38	83	-	60	-	-
Ciprofloxacin	35	32	31	-	20	20	7	20
Cotrimoxazole	36	32	13	-	20	40	14	20
Ceftriaxone	41	36	-	67	-	60	-	-
Cefixime	45	39	38	50	20	60	21	20
Gentamicin	68	36	-	67	-	20	-	-
Imipenem	81	39	56	-	-	-	-	-
Meropenem	71	46	56	67	20	60	-	-
Nalidixic acid	15	36	19	-	20	20	-	-
Nitrofurantoin	69	18	13	-	20	40	21	80
Netilmicin	72	36	44	83	-	60	-	-
Tazobactum	51	25	44	33	20	20	-	-
Colistin	80	68	69	33	40	-	-	-
Cefixime	42	39	19	83	20	60	43	20
Vancomycin	-	-	-	-	-	-	64	80
Linezolid	-	-	-	-	-	-	50	80
Penicillin G	-	-	-	-	-	-	21	0
Doxycycline	-	-	-	-	-	-	43	80

4. CONCLUSION

This study intended to ascertain the existing situation of UTIs and drug resistance among different age groups of patients in Dhaka City. The study concluded that the incidence of UTI is higher in females and infection is higher among the patients of 41-50 age group of females and 11-20 age group of males as compared to other groups. However, UTI can affect anyone at any age. UTI is mainly caused by Gram negative organisms whereas Gram positives can also cause the infection. The study also revealed that patients of UTI cases were associated with abnormal count of pus cells, RBC, albumin, glucose, nitrite and ketone bodies. Most of the isolates were found resistant against commonly used antibiotics such as Cotrimoxazole, Nitrofurantion, Nalidixic acid. *E. coli* were found sensitive to Amikacin, Colistin and Imipenem ranging from 80- 90%, followed by Netilmycin and Meropenem from 70-80%. Further research is needed to better understand the real situation of UTI and treatment efficacy in Dhaka City.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rahman F, Chowdhury S, Rahman MM, Ahmed D and Hossain A. Antimicrobial resistance pattern of gram-negative bacteria causing urinary tract infection. Stamford J Pharm Sci. 2009;2(1):44-50.
2. Nahar SJ, Khanum H, Shimusaki K. Occurrence of *Escherichia coli* among the women of Dhaka city. ARPN J Agri and Bio Sci. 2010;5(6):68-73.
3. Nicolle LE. Urinary tract infections in long-term–care facilities. Infec Cont. 2001; 22(03):167-175.
4. Griebling TL. Urinary tract infection in women. In: urologic diseases in America. (Ed. Litwin MS, Saigal CS.). US Government Printing Office, Washington, DC; 2001.
5. Kunin CM. Detection, Prevention and Management of Urinary Tract Infections. 4th ed. Lea & Febiger, Philadelphia; 1987.
6. Pezzlo M. Detection of urinary tract infections by rapid methods. Clin Microbiol. Rev. 1988;1(2):268-80.
7. Foxman B. Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. Am J Med. 2002;113(1): 5-13.
8. Travis LB, Bruhard BH. Infections of the urinary tract. In: Rudolph's Pediatrics (Ed. Rudolph, A. M.). 20th ed. Appleton & Lange, Stanford, Connecticut; 1996.
9. Todar K. Pathogenic *Escherichia coli*. In: Todar's Online Text Book of Bacteriology. University of Wisconsin-Madison; 2008.
10. Singleton P, Sainsbury D. Dictionary of microbiology and molecular biology. 3rd ed. John Wiley & Sons, New York; 2001.
11. Ronald A. The etiology of urinary tract infection: Traditional and emerging pathogens. Dis Mon. 2003;49(2):71-82.
12. Mathai D, Jones RN, Pfaller MA. Epidemiology and frequency of resistance among pathogens causing urinary tract infection in 1,510 hospitalized patients: A report from the SENTRY antimicrobial surveillance program (North America). Diagn Microbiol Infect Dis. 2001;40: 129-136.
13. Bassetti D, Bassetyti M, Mantero M. Strategies for antibiotic selection in empirical therapy. Clin Microbiol Infect. 2000;6:98-100.
14. Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. Arch Dis Child. 2003;88(3):215-18.
15. Hucker GJ, Harold JC. Methods of Gram Staining; 1923.
16. Bauer AW, Kirby WMM, Sherris JCT, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493.
17. Popescu OE, Landus SK, Haas GP. The spectrum of eosinophilic cystitis in males: Case series and literature review. Arch and Pathol and Lab Med. 2009;133(2): 289-94.
18. Schaeffer AJ, Dielubanza EJ. Urinary tract infection in women. The Med Clin of North Am. 2011;29(3):539-52.

19. Ahmed B, Akhter M, Hasan M, Alam MK. Sensitivity pattern of urinary tract pathogens to anti-microbial drugs at a tertiary level hospital in Bangladesh. J Dhaka National Medical College & Hospital. 2012;17(1):18-21.
20. Orenstein R, Wong ES. Urinary tract infections in adults. Am Fam Physician. 1999;59(5):1225-34.
21. Amdekar S, Singh V, Singh DD. Probiotic therapy: Immunomodulating approach toward urinary tract infection. Curr microbial. 2011;63(5):484-90.
22. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am. 2008; 35(1):1-12.
23. Eves FJ, Rivera N. Prevention of urinary tract infections in persons with spinal cord injury in home health care. Home Healthcare Nurse. 2010;28(4):230-241.