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Address of Correspondence

Executive Editor, TMSS Medical College Journal
TMSS Medical College, Rangpur Road
Thengamara, Bogra, Bangladesh.
Phone: 051-61830, Fax : 051-61830
e-mail: editortmcjournal@gmail.com
Web: www.tmssmedicalcollege.com

Original Articles

Distribution and Antimicrobial Susceptibility Pattern of Commonest Bacteria Causing Urinary Tract Infection: A Study in A Tertiary Care Hospital of Bangladesh

Khan AH[1*], Yasmin T[2], Rahman A[3], Rahman A[4],Samad A[5], Akhter S[6].

1. Dr. Md. Abul Hossain Khan, Professor & Head, Department of Microbiology, TMSS Medical College, Bogra.
2. Dr. Taslima Yasmin, Assistant Professor, Department of Microbiology, North Bengal Medical College, Sirajgonj.
3. Dr. Md. Arifur Rahman, Assistant Professor, Department of Microbiology, TMSS Medical College, Bogra.
4. Dr. Md. Afzalur Rahman, Professor(cc) & Head, Department of Biochemistry, Prime Medical College, Rangpur.
5. Dr. Md. Abdus Samad, Associate Professor & Head, Department of Community Medicine, Prime Medical College, Rangpur.
6. Dr. Shamima Akhter, Assistant Professor, Department of Pathology, TMSS Medical College, Bogra.

* Corresponding Author

Abstract

Background: Urinary tract infection (UTI) is one of the common infections in the community and hospital. Bacterial distribution and susceptibility of UTI changes according to time and place. Objective: This study was conducted to determine the bacterial distribution and antimicrobial susceptibility of common uropathogen in a tertiary care hospital of Bangladesh. **Methodology:** Clean catch midstream urine samples were collected from 494 patients irrespective of the age and sex. Isolation of both gram positive and gram negative bacteria by standard culture technique and identification was done on the basis of their cultural and gram staining smear made from culture colonies and biochemical characteristics. Antimicrobial susceptibility was performed in common isolated bacteria by Kirby Bauer's disc diffusion method. **Results:** Among the 494 sample, 212 showed culture positive(42.9%) and Gram negative bacteria (94.3%) were found in high prevalence and Escherichia coli (58,96%) was the most prevalent gram negative isolates. Nalidixic acid(83.4%) and ciprofloxacin(88.4%) were found the most resistant and Imipenem and Nitrofurantoin were found the most susceptible drugs against isolated Escherichia coli which showed 94.1% and 87.5% susceptibility, respectively. **Conclusion:** Escherichia coli is the most common bacteria causing UTI in both sexes and irrespective of age. Imipenem, Nitrofurantoin and Amikacin are the most sensitive, Nalidixic acid, ciprofloxacin, cefuroxime and cephradine are the most resistant antibiotics.

Key words: UTI, Bacterial distribution, antibiotic susceptibility

Introduction

Urinary tract infection (UTI) is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that annually 150 million people were infected with UTI worldwide which costing global economy more than 6 billion US dollars[1]. UTI can affect lower and sometimes both lower and upper urinary tracts. The treatment of UTI can be classified into uncomplicated and complicated on the basis of their choice of treatment[2]. UTI is more common in females than in males as female urethra structurally found less effective for preventing the bacterial entry[3]. It may be due to the proximity of the genital tract and urethra[4] and adherence of urothelial mucosa to the mucopolysaccharide lining[5]. The other main factors which make females more prone to UTI are pregnancy and sexual activity[6]. In pregnancy, the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine[7].

The spectrum of bacteria causing complicated UTI is much broader than of those causing uncomplicated UTI. However, the most commonly encountered bacteria are gram negative of which Escherichia coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Pseudomonas aeruginosa, and Proteus spp., whereas gram

positive Staphylococcus aureus, and Candida spp. are found rarely[10].

Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem. Increasing drug resistance in UTI needs regular monitoring of the antibiotic susceptibility of uropathogens in a particular area. Various factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy of each UTI patient should also be considered to find out the correct global data on susceptibility[11]. The distribution of bacteria and their antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place[9]. The susceptibility data provided by regional microbiology laboratories helps to choose the empirical choice of antimicrobials to treat UTI; however, these conditions are limited to complicated UTI as the samples of uncomplicated UTI are rarely sent to laboratories[12,13]. Generally, the antimicrobial treatment is initiated before the laboratories results which may lead to the frequent misuse of antibiotics[14]. The resistance pattern of community acquired uropathogens has not been extensively increased in our country[15-17]. To the best of our knowledge, no data regarding the bacterial resistance in UTIs from Rangpur city has been documented. Since most UTIs are treated empirically. Therefore there is a need for periodic monitoring of etiologic agents of UTI and their resistance pattern in this community.

This study was undertaken in view of paucity of reports of UTIs in patients of Rangpur city. The aim of the study is to determine the UTI-causing microorganisms distribution and antimicrobial susceptibility will also be determined against most common bacteria.

Materials and Methods

This cross sectional study was carried out in the microbiology laboratory of the Department of Microbiology Prime Medical College Hospital, Rangpur from January 2013 to December 2013. A total of 494 urine samples from OPD and IPD irrespective of age and sex at Prime Medical College hospital were examined by standard laboratory methods. Relevant history, laboratory record and findings was recorded in a predesigned data sheet and subsequently analyzed manually.

Sample Collection

Clean catch midstream urine samples of 494 patients were collected from each patient into a 20 mL calibrated sterile screw-capped universal container which was distributed to the patients, comprised of 192 males and 302 females, who attended the outpatient departments (OPDs) and IPD of this hospital with clinical evidence of urinary tract infection, determined by treating physicians. The specimens were labeled, transported to the laboratory, and analyzed within 6 hours. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Verbal informed consent was obtained from all patients prior to specimen collection. The study was conducted after due ethical approval which was subjected to the hospital administrations.

Sample Processing

A calibrated loop method was used for the isolation of bacterial pathogens from urinary samples. A sterile 4.0 mm platinum wired calibrated loop was used which delivered 0.001 mL of urine. A loopful urine sample was plated on Hi-chrome, Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar, and blood agar medium (Hi Media Laboratories, Mumbai, India). The inoculated plates were incubated at 37°C for 24 h and for 48 h in negative cases. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. A specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$ cfu/mL or when an organism was cultured

at a concentration of 10^4 cfu/mL and >5 pus cells per high-power field were observed on microscopic examination of the urine[18].

Identification Bacterial Isolates

Identification of both gram positive and gram negative bacterial isolates was done on the basis of their cultural and biochemical characteristics and gram staining smear made from culture colonies. Gram negative bacteria were identified by the standard biochemical tests[10,19] and Gram positive microorganisms were identified with the corresponding laboratory tests: catalase, coagulase, and subculture on mannitol salt agar for Staphylococcus aureus[20]. Identified and pure isolates were maintained in nutrient agar slants and incubated at 37°C for 24 hrs. The isolates were subcultured periodically.

Antibiotic Susceptibility Testing

Isolates were tested for antimicrobial susceptibility testing by the standard Kirby Bauer's disc diffusion method[21]. Standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and was allowed to soak for 2 to 5 minutes. After that antibiotic disks were placed on the surface of media and pressed gently. Mueller Hinton agar plates were then incubated at 37°C for 24 h. After 24 h the zone of growth inhibition were measured and interpreted by the recommendations of clinical and laboratory standards[22]. The following standard antibiotic discs were used for the isolates, amoxicillin, cephradine, co-trimoxazole, ciprofloxacin, nitrofurantoin, nalidixic acid, ceftriaxone, amoxicillin-clavulonic , ceftazidime, cefuroxime, amikacin, azithromycin, imipenem and vancomycin. Standard strains of Esch. coli (ATCC 25922) was used routinely in this study as control.

Results

In total 494 urine samples, 212 samples showed the significant bacterial growth (table-1) which were comprised of 42.9% samples.

Table 1: Result of culture in suspected urine samples from UTI patients.

Number of samples	Culture positive	Culture negative
494	212 (42.9%)	282 (57.1%)

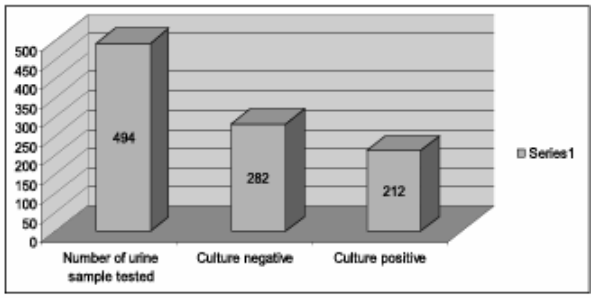

Figure-I: Bacterial isolation rate in the study sample

Among the total of 212 bacterial uropathogens comprised of 200 (94.3%) Gram negative and 12 (5.7%) Gram positive (table-2) were isolated from positive urine samples.

Table 2: Frequency of gram reaction among isolates.

Number of positive samples	Gram negative organisms	Gram positive organisms
212	200 (94.3%)	12 (5.7%)

Escherichia coli (58.96%) was found the dominant bacteria among all isolated uropathogens. The second most prevalent isolate was Enterobacter spp. (10.85%), followed by Klebsiella spp.(10.38%), Enterococcal spp. (9.43%), Pseudomonas spp.(4.71%), Staphylococcus aureus (3.30%), and Candida spp.(2.36%) (table-3).

Table 3: Bacterial distribution in positive culture in all age groups and genders.

Name of Isolates	Number	Percentages
Escherichia coli	125	58.96
Enterobacter spp.	23	10.85
Klebsiella spp.	22	10.38
Enterococcal spp.	20	9.43
Pseudomonas aeruginosa,	10	4.71
Staphylococcus aureus	07	3.30
Candida spp.	05	2.36

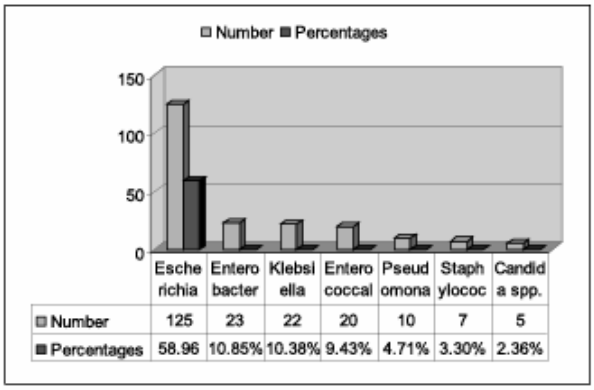

Figure 2: Bacterial distribution in positive culture in all age groups and genders.

Antibiotic susceptibility results (table-4) showed the resistant and susceptible antibiotics for the tested uropathogen. Overall Ciprofloxacin was found the most resistant drug as 94 (88.4%) against Escherichia coli. The second most resistant drug was Nalidixic acid 100 (83.4%) followed by Cefuroxime 94 (78.4%) and Ceftriaxone 93(77.5%); however, the most sensitive drug against Escherichia coli was Imipenem(IMP) 113(94.1%) followed by Nitrofurantoin 105(87.5%) and Amikacin 95 (79.0%) (Figure 3).

Table 4: Resistant and susceptibility rates (%) for isolated Escherichia coli.

Antibiotic	Sensitive	Resistant
Amoxicillin	32(26.6%)	88(26.6%)
Cephradine	27(22.8%)	91(77.2%)
Co-trimoxazole	45(37.5%)	75(62.5%)
Ciprofloxacin	26(21.6%)	94(88.4%)
Nitrofurantoin	105(87.5%)	15(12.5%)
Nalidixic acid	20(16.6%)	100(83.4%)
Ceftriaxone	27(22.5%)	93(77.5%)
Amoxicillin-clavulonic acid	77(64.1%)	43(35.9%)
Ceftazidime	38(31.6%)	82(68.4%)
Cefuroxime	26(21.6%)	94(78.4%)
Amikacin	95(79.0%)	25(21.0%)
Azithromycin	57(47.5%)	63(52.5%)
Imipenem	113(94.1%)	07(5.9%)
Vancomycin	36(30.0%)	84(70.0%)

Discussion

This study provides valuable data to compare and monitor the status of antimicrobial resistance against most common uropathogens to improve efficient empirical treatment. Increasing antimicrobial resistance has been documented globally[23-26]. The prevalence of UTI was found 42.9% in this study and this rate of prevalence is higher than in savar, Dhaka and the other studies in which accounts for 25.6%28, 22%29, 38.6%30, 35.5%41, 4.2%31, 17.19%16, 10.86%17, 34.5%32, and 36.68%33, however, the prevalence rate of UTI in our study correlates with other studies done in South Trinidad[34], and in the Mexican population[35] which showed such more highly significant uropathogens 49% and 97.3%, respectively.

In this study, the Gram negative bacilli constituted 94.3% of the total bacterial isolates while Gram positive organisms constituted 5.7%. Escherichia coli (42.58%) was found the most prevalent gram negative bacteria in the positive urine samples of UTI. This result is consistent with reports from other studies[32,36,37] but differs from the reports in which P. aeruginosa[39] and Klebsiella spp.[40] were recorded as the predominant bacteria in UTI. Other isolated bacteria from UTI cases in this study were Enterobacter spp. (7.10%), Klebsiella spp. (18.71%), P. aeruginosa (12.90%), Staphylococcus aureus (9.68%), and Candida spp.. These findings were not correlate with other reports in which P. aeruginosa was reported as the second most common bacterial isolate in UTI studies in India[14] and Lafia, Nigeria[8] ; however, these results correlates with others in which Klebsiella spp. was reported as the second most frequently isolated organism in UTI[27,38]. The tudies on UTI in other places of the world also showed that E. coli and Klebsiella spp. are the commonest uropathogens in UTI[16,17]. Higher incidence of gram negative bacteria, related to Enterobacteriaceae, in causing UTI.

Conclusion

Against the background of paucity of reports of UTI in PMC Rangpur, this is the first study conducted to determine the prevalence of UTI, the effect of gender and age on its prevalence, and their susceptibility profile in the community of Rangpur city. This study provides valuable laboratory data to monitor the status of antimicrobial resistance among uropathogens and to improve treatment recommendations in this geographical area.

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