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Microbial Analysis of Urine and Antibiotic Sensitivity Pattern of Isolated Infectious Agents from Patients Suffering in Urinary Tract Infection

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Abstract

This retrospective study was conducted at the National Institute of Neuroscience & Hospital, Agargaon, Dhaka from October, 2016 to March, 2017 with a view to finding out the incidence of UTI patients with aiming of testing & drug susceptibility. In order to conduct this study, consecutive urine samples of 200 of a total population of both sexes and various age groups were taken from both outpatient and inpatient departments. There were marked gender variations in all age groups, which comprised members between 2 and 80 years of age, and they were requested to provide urine samples for examination. Among them 22.50% of specimens yielded positive culture. UTI is more prevalent in females than in males. The most common isolates were *E. coli* (15%), *Enterococcus sspp.* (0.5%), *Staphylococcus* aureus (2.50%), *Staphylococcus* saprophyticus (3.50%) and *pseudomanas* (1.00%). Azithromycin and ceftriaxone showed the highest sensitivity against *E. coli* but Nitrofuranton, Gentamicin and Cefixime showed comparatively lower sensitivity against *E. coli*. Nitrofuranton, and Cefurixime showed the highest sensitivity against *S. saprophyticus* but Ceftazidime showed the lowest sensitivity against *S. saprophyticus*. Meropenem showed the highest sensitivity against *S. aureus* but Ceftriaxone, Ceftazidime, Cefurixime, Amoxyxillin, Gentamicin and Nitrofuranton showed comparatively lower sensitivity against *S. aureus*. Azithromycin, Ciprofloxacin, Imupenem, Doxycyclin, Co-trimoxazole and Meropenem showed the highest sensitivity against *Pseudomonas spp.* but Nitrofuranton, Gentamicin, Amoxycillin, Cefurixime, Ceftazidime and Ceftriaxone showed comparatively lower sensitivity against *Pseudomonas spp.* All the antibiotics used showed 100% sensitivity against *Enterococcus spp.*

Keywords

Specimen, Isolates, Antibiotic Sensitivity, Urinary Tract Infection

1. Introduction

A urinary tract infection (UTI) (also known as acute cystitis or bladder infection) is an infection that affects the urinary tract. When it affects the lower urinary tract, it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract, it is known as pyelonephritis (a kidney infection). Symptoms from a lower urinary tract include painful urination and either frequent urination or urge to urinate (or both), while those of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. In the elderly and the very young, symptoms may be vague or nonspecific. The main causal agent of both types is *Escherichia coli*, however other bacteria, viruses or fungi may rarely be the cause.

Bacterial infections of the urinary tract are the most common cause of both community acquired and nosocomial infections for patients admitted to hospitals in the United States. It is distressing and occasionally life threatening. However, the prognosis and management of urinary tract infections depend on the site of infection and any predisposing factors. It has been estimated that about six million patients visit outpatient departments and about 300,000 are treated in the wards every year for UTI worldwide [1]. With the constantly shifting trends in drug resistance, antibiotic options, and multiplying microorganisms, UTI implies both microbial colonization of the urine and invasion of the lower or upper urinary tract by microorganisms [2]. The traditional guideline that urine containing more than 100,000 bacteria/ ml is responsible for an incidence of UTI has been modified currently. Counting as low as 1000/ml of any single bacterial type or as few as 100/ml of coliforms such as *E. coli,* are now considered an indication of significant infection, especially if leukocytes appear in the urine [3] [4].

Uropathogens differ in terms of the virulence factors and pathogenic mechanisms that allow them to colonize and infect the urinary tract. Gram negative bacteria cause 80% - 85% whereas Gram positives cause 15% - 20% of the cases. Common Gram-negative species include *E. coli, Klebsiella, Proteus, Enterobacter, Pseudomonas,* and *Serratia spp*. and Gram-positive organisms, including group B *streptococci, Enterococcus spp*., and *Staphylococcus aureus* and *Staphylococcus saprophyticus*, have also been frequently isolated in both urban and rural areas [4] [5].

The incidence of UTI is greater in women (20%) as compared to men, which may be either due to anatomical predisposition or urothelial mucosa adherence to the mucopolysaccharide lining or other host factors and vice versa [5] [6]. Most UTIs are preceded by an association of sexual activity with colonization and vaginal acquisition, pregnancy and obstruction which are responsible for the increasing

frequency of UTI in females. Though UTI susceptibility may occur to anybody, the prevalence of infection normally differs with age, sex and certain predisposing factors such as diabetes, pregnancy, and impaired voiding of the bladders [7].

For this reason, the present study aimed to evaluate the prevalence of uropathogens and observe the patterns of antibiotics against uropathogens in different groups ages of both genders.

2. Materials and Methods

2.1. Ethical Concern

The protocol was approved by the Ethical Review Committee of the National Institute of Neurosciences & Hospital, Dhaka, Bangladesh.

2.2. Study Area and Population

The study was carried out at the National Institute of Neurosciences & Hospital, Dhaka, Bangladesh from October, 2016 to March, 2017. This retrospective analysis included 200 consecutively collected midstream and/or catheter-catch urine samples from clinically suspected patients of UTI of different ages and sexes who were admitted to the hospital.

2.3. Sample Collection

The clean catch midstream urine samples were collected and transported from clinically suspected patients following CLSI guidelines 10. The samples were taken in clean, sterile 50 mL screw-capped amber colored universal containers with an opening of at least 4 cm. The consent was obtained from all patients by verbal information, and they were advised to follow the proper aseptic collection procedure for urine prior to collection. All the samples were collected before the start of antibiotic therapy.

2.4. Chemicals and Media

Several cultural media were used in this study. These are MacConkey's Agar, Muellar Hinton and Blood Agar.

2.5. Isolation and Identification of Uropathogens

Bacterial identification was done by phenotypic examination of the organisms on culture media followed by biochemical tests specific for uropathogens. One loop full of urine samples was inoculated onto sterilized and solidified Blood agar and MacConkey agar media followed by incubation at 37˚C for 24 h aerobically. After incubation, the numbers of bacterial colonies were counted.

2.6. Antibiogram

Mueller–Hinton agar was used for antimicrobial susceptibility testing (AST) following Kirby-Bauer disc diffusion method, against a panel of 13 antibiotics (Biomaxima, Poland) including Azythromycin, Ciprofloxacin, Ceftriaxone, Ceftazidime,

Cefixim, Imipenem, Cefuroxime, Amoxycillin, Gentamicin, Doxycyclin, Co-trimoxazole, Nitrofurantion, Meropenem as per the Clinical Laboratory Standard Institute (CLSI) guidelines 13, susceptibility was noted as sensitive, resistant and intermediate based on the diameter of zone of inhibition.

2.7. Data Analysis

Data obtained were analyzed by SPSS version 20 and Excel 2016. The percentage of frequencies was generated for categorical variables such as rate of isolation, type of bacteria, rate of antibiotic sensitivity, resistance, and intermediate of the organisms.

3. Result & Discussion

A total of 200 patients of UTI of either sex (male-60 and female-140) with the respective male ratio of 30% and female 70% provided urine specimens (see Tables 1-9).

Table 1. Total number of specimens 200 (N).

Criterion of specimen	Number
Specimens showing positive case	45
Percentage of positive culture for urinary pathogen	22.50%
Specimens showing negative case	155
Percentage of negative culture for urinary pathogen	77.5%

We found 45 cases positive among a total of 200 cases in this study.

Table 2. Sample size and distribution: The total number of respondents sample was 200.

Sample study	Number
Patient attending	**200**
Total Sample	**200**
Sample collect from adult male	**45**
Sample collect from adult female	**100**
Sample collect from male child	**15**
Sample collect from female child	**40**
Negative case in adult male	**38**
Positive case in adult male	**07**
Negative case in adult female	**70**
Positive case in adult female	**30**
Negative case in male child	**13**
Positive case in male child	**02**
Negative case in female child	**34**
Positive case in female child	**06**
Total negative case	**155**
Total positive case	**45**

The following type of distribution was done according to age and sex in this study.

Table 3. Age and sex distribution of UTI patient.

Sl. No	Age	Total patient	Male	Percentage	Female	Percentage
1	0 - 10	28	07	25%	21	75%
2	11 - 20	18	06	33.33%	12	66.66%
3	21 - 30	25	09	36%	16	64%
4	31 - 40	36	10	27.77%	26	72.235
5	41 - 50	32	11	34.37%	21	65.63%
6	51 - 60	15	06	40%	09	60%
7	61 - 70	35	09	25.72%	26	74.28%
9	71 to above	11	03	27.28%	08	72.72%

We found 41 - 50 and 31 - 40 aged people among male are more infected compared to other raged people and 31 - 40, 0 - 10, 41 - 50 aged people among female are more infected compared to other people in this study. But in some other studies, among positive cases, 5.77% cases were in people aged 0 - 12 years old, 36.54% cases were in between 13 - 24 years, 32.7% were in between 25 - 36 years, 13.46% cases were 37 - 48 years, 7.70% cases were 49 - 60 years, 3.83% cases were 61 - 72 years of age [8].

Table 4. Infectious agent and their number with percentage (N = 200).

Finding infectious agent of the study	Number of isolation	Percentage
E. Coli	30	15%
Staphylococcus saprophyticus	7	3.5%
Staphylococcus aureus	5	2.5%
Pseudomonas spp	2	1.0%
Enterococcus spp.	1	0.5%

We found *E. coli* was most prominent in this study but *Staphylococcus saprophyticus, Staphylococcus aureus, Pseudomonas spp* and *Enterococcus spp* was also found in the observed specimens. But in some other studies, out of 100 urine samples 60 were positive for pathogenic organisms. *Escherichia coli* was isolated in 68.3% of the positive samples, followed by *Klebsiella sp* 21.6%, *Pseudomonas sp* 5% *Proteus* sp 3.3% *Staph. aureus* 1.66% [7].

Table 5. Antibiotic sensitivity pattern of *E. Coli*.

Name of antibiotic	Sensitivity	Percentage	Resistance	Percentage
Azithromycin	24	80%	6	20%
Ciprofloxacin	20	66.6%	10	33.3%
Ceftriaxone	24	80%	6	20%

Continued

Ceftazidime	21	70%	9	30%
Cefixim	19	63.3%	11	36.6%
Imipenem	18	60%	12	40%
Cefuroxime	22	73.3%	8	26.6%
Amoxycillin	19	63.3%	11	36.6%
Gentamicin	21	70%	9	30%
Doxycyclin	22	73.3%	8	26.6%
Co-trimoxazole	20	66.6%	10	33.3%
Nitrofurantion	17	56.6%	13	43.3%
Meropenem	23	76.6%	7	23.3%

Azithromycin, Ceftriaxone showed highest sensitivity against *E. coli* but Nitrofuranton, Gentamicin and Cefixime showed comparatively lower sensitivity against *E. coli*.

Table 6. Antibiotic sensitivity pattern of *Staphylococcus saprophyticus*.

Name of antibiotic	Sensitivity	Percentage	Resistance	Percentage
Azithromycin	4	57.1	3	42.8%
Ciprofloxacin	3	42.8%	4	57.1
Ceftriaxone	5	71.4%	2	28.5%
Ceftazidime	2	28.5%	5	71.4%
Cefixim	3	42.8%	4	57.1
Imipenem	6	85.7%	1	14.2%
Cefuroxime	7	100%	0	-
Amoxycillin	3	42.8%	4	57.1
Gentamicin	4	57.1	3	42.8%
Doxycyclin	5	71.4%	2	28.5%
Co-trimoxazole	3	42.8%	4	57.1
Nitrofurantion	7	100%	0	-
Meropenem	5	71.4%	2	28.5%

Nitrofuranton, and Cefurixime showed the highest sensitivity against *S. saprophyticus* but Ceftazidime showed lowest sensitivity against *S. saprophyticus.*

Table 7. Antibiotic sensitivity pattern of *Staphylococcus aureus.*

Name of antibiotic	Sensitivity	Percentage	Resistance	Percentage
Azithromycin	4	80%	1	20%
Ciprofloxacin	4	80%	1	20%
Ceftriaxone	3	60%	2	40%
Ceftazidime	3	60%	2	40%

Continued

Imipenem	4	80%	1	20%
Cefuroxime	3	60%	2	40%
Amoxycillin	3	60%	2	40%
Gentamicin	3	60%	2	40%
Doxycyclin	4	80%	1	20%
Co-trimoxazole	4	80%	1	20%
Nitrofurantion	3	60%	2	40%
Meropenem	5	100%	0	-

Meropenem showed highest sensitivity against *S. aureus* but Ceftriaxone, Ceftazidime, Cefurixime, Amoxyxillin, Gentamicin and Nitrofuranton showed comparatively lower sensitivity against *S. aureus*.

Table 8. Antibiotic sensitivity pattern of *Pseudomonas spp.*

Name of antibiotic	Sensitivity	Percentage	Resistance	Percentage
Azithromycin	2	100%	-	-
Ciprofloxacin	2	100%	-	-
Ceftriaxone	1	50%	1	50%
Ceftazidime	1	50%	1	50%
Imipenem	2	100%	-	-
Cefuroxime	1	50%	1	50%
Amoxycillin	1	50%	1	50%
Gentamicin	1	50%	1	50%
Doxycyclin	2	100%	-	-
Co-trimoxazole	2	100%	-	-
Nitrofurantion	1	50%	1	50%
Meropenem	2	100%	-	-

Azithromycin, Ciprofloxacin, Imupenem, Doxycyclin, Co-trimoxazole and Meropenem showed highest sensitivity against *Pseudomonas spp*. but Nitrofuranton, Gentamicin, Amoxycillin, Cefurixime, Ceftazidime and Ceftriaxone showed comparatively lower sensitivity against *Pseudomonas spp*.

Table 9. Antibiotic sensitivity pattern of *Enterococcus Spp.*

Name of antibiotic	Sensitivity	Percentage	Resistance	Percentage
Azithromycin	_	_	1	100%
Ciprofloxacin	1	100%	_	_
Ceftazidime	1	100%	_	_
Cefixime	_	_	1	100%
Imipenem	1	100%	_	_

Continued

Amoxycillin	_	_	1	100%
Gentamicin	1	100%	_	_
Doxycyclin	1	100%	_	_
Co-trimoxazole	_	_	1	100%
Nitrofurantion	1	100%	_	_
Meropenem	1	100%	_	_

All the antibiotics used showed 100% sensitivity against *Enterococcus spp*.

4. Conclusions

The present study concluded that the incidence of UTI is higher in females than males. Of course, this is also a foregone conclusion. The study found that Gram negative bacteria such as *E. coli*, *Pseudomonas* and Gram positive bacteria such as *staphylococcus saprophyticus* and *staphylococcus aureus* are the more common etiological agents for UTI in female subjects and in case of male patients. *E. coli* is found to be a principal etiological agent of UTI in this study.

Azithromycin and ceftriaxone showed the highest sensitivity against *E. coli* but Nitrofuranton, Gentamicin and Cefixime showed comparatively lower sensitivity against *E. coli*. Nitrofuranton and Cefurixime showed the highest sensitivity against *S. saprophyticus* but Ceftazidime showed the lowest sensitivity against *S. saprophyticus.* Meropenem showed the highest sensitivity against *S. aureus* but Ceftriaxone, Ceftazidime, Cefurixime, Amoxyxillin, Gentamicin and Nitrofuranton showed comparatively lower sensitivity against *S. aureus*. Azithromycin, Ciprofloxacin, Imupenem, Doxycyclin, Co-trimoxazole and Meropenem showed the highest sensitivity against *Pseudomonas spp*. but Nitrofuranton, Gentamicin, Amoxycillin, Cefurixime, Ceftazidime and Ceftriaxone showed comparatively lower sensitivity against *Pseudomonas spp*. All the antibiotics used showed 100% sensitivity against *Enterococcus spp.*

This phenomenon has arisen due to the indiscriminate use of antibiotics. This is due to the fact that even when no antibiotics are needed, they are given. Here, people can buy antibiotics without any prescription, take them for a few days, and then stop. No proper dose or duration of treatment is followed. All these have produced resistance to the antibiotics by the causative agents of UTI. The etiological agents of UTI and their sensitivity patterns vary between health institutions and different seasons, even within the same area. Therefore, we suggest that each health institution should determine the causative agents of UTI and their susceptibility to guide the management of UTI.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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