International Journal of Chemical and Biomedical Science
2018; 4(1): 7-10
http://www.aascit.org/journal/ijcbs
ISSN: 2472-9574 (Print); ISSN: 2472-9590 (Online)

AASCIT American Association for Science and Technology

Keywords
Urinary Tract Infection,
Antimicrobial Drug Susceptibility,
Uropathogens

Received: August 14, 2016
Accepted: February 3, 2017
Published: January 18, 2018

Study of Common Bacterial Pathogen Causing Urinary Tract Infection in Assam Medical College and Hospital, India

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Citation
Trishna Nath, Sharanya K., Vanlaldina K, Vimala Gandhi. Study of Common Bacterial Pathogen Causing Urinary Tract Infection in Assam Medical College and Hospital, India. *International Journal of Chemical and Biomedical Science.* Vol. 4, No. 1, 2018, pp. 7-10.

Abstract
Urinary tract infection (UTI) is one of the most common infections encountered by clinicians and approximately 1 in 3 women will be subjected to antimicrobial treatment for urinary tract infection before age 25. UTI is most predominant in female than in male. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for urinary tract infections and their resistance patterns may help the clinician to choose the correct empirical treatment. Therefore, the aim of this study is to determine the type and antibiotic resistance pattern of the urinary pathogens isolated from patients attending Assam Medical College and Hospital in Dibrugarh, Assam, India, from November 2013 to January 2014. In the present study, samples were collected from 40 patients showing various clinical symptoms were suspected to UTI. Clean-Catch midstream urine of the patients was collected. Urine samples were cultured for isolation of the microbial agents of UTI. The isolated bacteria were identified using biochemical tests. Disk diffusion susceptibility test was used to determine susceptibility of bacterial agents to antibiotics. Retrospective analysis was carried out for 40 urine samples, 55% UTI was found in case of female and 45% in case of male and also 35% UTI was found in age group 1-15. *Escherichia coli* were the most common cause of UTI. Isolated and diagnosed bacteria were *Escherichia coli (45%), Pseudomonas (25%), Staphylococcus (15%), and Klebsiella (15%).* These uropathogens showed different pattern of antimicrobial drug susceptibility against Amikacin, Cefotaxime, Ciprofloxacin, Kanamycin, Tetracycline and Ampicillin. Almost all organisms are sensitive to Amikacin than other antibiotics.

1. Introduction

Urinary tract infection is considered to be one of the most common bacterial infections [17]. Over 150 million people are infected with urinary tract infection each year [7]. Diagnosis depends upon the symptoms and urine culture. Reports show that the infection occurs more frequently in women than men. Half of all women would have a urinary tract infection during their life time [18]. The anatomy of organs including the urethra, bladder, ureters, prostrates and kidneys greatly influenced the pathogenesis and course of the urinary tract infection where the renal parenchyma (pyelonephritis) or the urethras

(urethritis) involve the upper urinary tract infection. Lower urinary tract infection involve the bladder (cystitis), the urethra (urethritis) and in males the prostrate (prostatitis) [13].

Bacteria gain access to the urinary tract by three routes involving the ascending route, the hematogenous route and the lymphatic pathways [22]. The most common bacterial pathogens that cause urinary tract infections are *Escherichia coli*, *Pseudomonas, Proteus and Klebsiella* [18]. In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas* spp. are comparatively more common. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization.

Neonates and children younger than 2 years of age with urinary tract infections usually have nonspecific symptoms, including failure to thrive, vomiting, and fever. Children older than 2 years of age are more likely to display localized symptoms such as dysuria, frequency and abdominal or flank pain [3]. Adults with uncomplicated urinary tract infection limited to the urethra or bladder present primarily with dysuria often in combination with frequency, regency suprapubic pain and hematuria [22].

Patients with upper urinary tract infection such as pyelonephritis present with flank pain, nausea, vomiting, fever, chills, night sweats and cost vertebral angle tenderness [14]. There symptoms may occur in the absence of symptoms of cystitis. At times, the lower urinary tract symptoms precede the appearance of fever and upper urinary tract symptoms by 1 or 2 days. Bacteremia, when present, may help confirm diagnosis of pyelonephritis [12 and 19].

Treatment of UTIs cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections. As a result, the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide [8]. Resistance rates to the most common prescribed drugs used in the treatment of UTIs vary considerably in different areas world-wide. The estimation of local etiology and susceptibility profile could support the most effective empirical treatment.

Therefore, investigating epidemiology of UTIs (prevalence, risk factors, bacterial isolates and antibiotic sensitivity) is fundamental for care givers and health planners to guide the expected interventions. Thus, the aim of this study is to determine the common bacterial pathogen causing urinary tract infection and to find out the pattern of antimicrobial drug susceptibility to commonly used antimicrobial drugs.

2. Materials and Methods

2.1. Sample Collection

Urine samples were collected from 40 patients identified from various symptoms and different age groups in Assam Medical College and Hospital in Dibrugarh, Assam, India. The study was carried out between November 2013 and January 2014. There were 22 (55%) females and 18 (45%) males, with an age range of 1-65 years. Clean-Catch midstream urine of the patients was collected in a sterile tube (4-5ml) and immediately transported to the laboratory. Guidelines for proper specimen collection were given to all patients on a printed card [5]. For the isolation of UTI causing microorganisms; loop full of urine sample was streaked on nutrient agar plate and incubated on 37°C for 24 hours.

2.2. Staining of Isolated Culture

To check morphological characteristics, isolated cultures are subjected to Gram staining and motility test. Reagents required were methylene blue, Gram's iodine, alcohol/acetone, safranin and distilled water.

2.3. Culture Method

Urine sample was inoculated by calibrated urine loop method. With the calibrated loop, transfer 0.01 ml of the urine specimen to the center of a nutrient agar plate and streak. The loop was touched to the center of the nutrient agar plate, from which the inoculum was spread in a line across the diameter of the plate. Without flaming or reentering urine, loop was drawn across the entire plate crossing the first inoculum streak numerous times to produce isolated colonies. Plates were incubated aerobically at 37°C for 24 hours, and those cultures which become negative at the end of 24 hours incubations were further incubated for 48 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/ml [5].

2.4. Cultivation of Bacteria

The isolated bacteria were cultivated in Mac Conkey agar and Blood agar. MacConkey agar and blood agar are differential media. In Mac Conkey agar, colonies observed were spherical and pink color. Whereas, in blood agar the colonies observed were spherical. [5]

2.5. Biochemical Analysis

The morphology and biochemical tests were conducted according to the methods described by Bergey's manual of determinative bacteriology. Biochemical characterization of isolates were done by Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Catalase test, Amylase test and Protease test [4 and 15].

2.6. Antimicrobial Susceptibility Testing

In the present study antimicrobial susceptibility testing was done on Mueller-Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique. This method was

done according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility of UTI agents [6]. The antibiotic discs and concentration were ampicillin (30µg), kanamycin (30µg), ciprofloxacin (30µg), tetracycline (30µg), amikacin (30µg), and cefotaxime (30µg) [9].

3. Results and Discussion

The symptomatic cases are shown in table 1, were out of 40 patients 20% had fever, 30% had loin pain, 15% had retention of urine, 35%had frequency of micturition.

Table 1. *Symptomatic cases.*

Main symptoms	Total NO. of cases	Percentage
Fever	8	20%
Loin pain	12	30%
Retention of urine	6	15%
Frequency of micturition	14	35%

A urine culture with $\geq 10^5$ colony-forming units/mL (CFU/mL) was considered to be positive, a culture with $\geq 10^4$ but $\leq 10^5$ CFU/mL was considered to be border line, and a culture with $\leq 10^4$CFU/mL was considered to be negative. 40 culture positive urine samples were isolated as positive by calibrated loop culture method. The isolated bacteria were cultivated in Mac Conkey agar and Blood agar. MacConkey agar and blood agar are differential media. In Mac Conkey agar, colonies observed were spherical and pink color. Whereas, in blood agar the colonies observed were spherical. All the microorganisms grown in the culture were identified by biochemical tests.

Escherichia coli were the predominant pathogen in all categories with the total percentage being 45% as shown in table 2. The next three pathogens of importance were *Pseudomonas* (25%), *Staphylococcus* (15%) and *Klebsiella* (15%). Farrell *et al.,* 2003 [7] reported that *Escherichia coli* were found to be observed predominantly in all the categories, with the total percentage in each category varying between 56.3-77.3%. Following *Escherichia coli*, other pathogens of importance were *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Proteus mirabilis* varying slightly from category to category.

Table 2. *Organisms found in the urinary tract infection.*

Organisms	Total NO. of cases	Percentage
Escherichia coli	18	45%
Pseudomonas	10	25%
Staphylococcus	6	15%
Klebsiella	6	15%

Approximately 35% UTI out of 40 samples occurred in the age between 1 to 15, 22.5% UTI occurred in the age between 15 to 25, 15% UTI occurred between 25 to 35, 7.5% UTI occurred in the age between 35 to 45, 7.5% UTI occurred in the age between 45 to 55 and 12.5% UTI occurred in the age between 55 to 65 (Table 3). Priyadharsini *et al*., 2014 [21] reported that age group of 21 -30 were showed maximum infection. Female patients were more when compared with male patients.

Table 4 discusses about the UTI in sex wise which reports UTI mostly occurred in female than in male. Out of 40 samples 55% UTI was found in case of female and 45% in case of male. Foxman, 2003 [10] reported that approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will have a UTI during their lifetime. The sex distribution of patients in our study is consistent with those of other reported studies, showing a statistically predominance of females with UTI (68% of the positive cultures). This result is similar to those reported from many other centers (Abu Shaqra, 2000) [1].

Table 3. *UTI in age wise.*

Age group	Number	Percentage
1-15	14	35%
15-25	9	22.5%
25-35	6	15%
35-45	3	7.5%
45-55	3	7.5%
55-65	5	12.5%

Table 4. *UTI in sex wise.*

Sex	No. of cases	Percentage
MALE	18	45%
FEMALE	22	55%

The most effective antimicrobial agents in our study (table 5) were *E. coli* sensitive to Amikacin (90.5%), Cefotaxime (89.6%), Ciprofloxacin (85.3), and Kanamycin (76.1%). Amikacin was more effective against Pseudomonas (77.5%). *Klebsiella* was noted to be more sensitive to Amikacin (90.6%), Ciprofloxacin (85.9%) and Cefotaxime (75%). The high prevalence of resistance to the commonly used antibiotics such as ampicillin, cefotaxime and tetracycline has caused considerable alarm. The most effective antimicrobial agents in our study were amikacin and ciprofloxacin for Gram negative Bacilli (81%-100%) which was in accordance with the research carried out by Orrett and Davis, 2003 [20]. However, isolated Gram-positive cocci were fully sensitive to kanamicin and tobramicin. This study was comparable with the results reported by Astal *et al., 2002* [2] and McIsaac *et al., 2005* [16]. Based on the results of this study, it was revealed that the susceptibility of bacteria to ciprofloxacin and other antibiotics was similar to many studies by Orrett and Davis, 2003 [20]; Mclsaac *et al*., 2004 [16] and Gupta et al., 2001 [11]. According to our results, the efficacy of amikacin was comparable to other reports by Kothari and Sagar, 2008 [13]

Table 5. *Antimicrobial sensitivity pattern of bacterial agents isolated from urine specimens.*

Sl. No	Isolated organisms	Ampicilin	Kanamicin	Cefotaxime	Ciprofloxacin	Tetracycline	Amikacin
1	*E. coli*	4.90	76.10	89.6	85.3	25.5	90.5
2	*Pseudomonas*	10.0	22.50	27.5	60.0	10.0	77.5
3	*Klebsiella*	18.8	62.50	75.0	85.9	32.5	90.6
4	*Staphylococcus*	0.00	100.0	25.0	75.0	50.0	0.00

4. Conclusion

The present study concluded that the organism majorly responsible for urinary tract infection is gram negative Bacilli, with most of the strains being multi-drug resistant. E. coli is observed to be the most common isolated bacteria, with the most effective antimicrobial agents being Amikacin (90.5%), Cefotaxime (89.6%), Ciprofloxacin (85.3%) and Kanamycin (76.1%) against Gram-negative Bacilli. In conclusion, the isolation of bacterial uropathogens with a higher resistance rates for commonly used antimicrobials leaves the clinicians with very few options to choose drug used for empirical treatment of UTIs. Therefore, it is important to urge physician and other health worker in the field on the need of re-evaluation of empirical treatment of UTI. It is also noted in our study that almost all organisms are sensitive to Amikacin, so we can suggest Amikacin to be prescribed as the empirical treatment for UTI.

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