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Antibiotic Susceptibility Patterns of Bacteria among Urinary Tract Infection Patients in Chittagong, Bangladesh

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Abstract

Analyzing antibiotic susceptibility pattern of uropathogens help to overcome the therapeutic difficulties created by the rising antimicrobial resistant bacteria and guides in choosing appropriate antibiotics. The aim of the study was to estimate the prevalence of various bacterial isolates and to understand the susceptibility patterns of the uropathogens. Midstream urine samples were collected, cultured and subjected to microscopical and appropriate biochemical tests for proper identification. Antimicrobial susceptibility tests were carried out by disc diffusion technique using Muller Hinton Agar as culture media. The prevalence of urinary tract infection was 25.91%. It was more prevalent in woman than men (68% vs. 32%). The most common isolated uropathogens were *Escherichia coli* (82.6%) and *Klebsiella pneumoniae* (14.6%). Other bacterial species, named *Morganella morganii*,

Enterobacter spp., *Citrobacter freundii*, *Pseudomonas spp*., *Yersinia enterocolitica*, were also found in patients with urinary tract infection, although they were least frequent. High level of sensitivity was found to imipenem, nitrofurantoin, meropenem, ceftazidime, netilmicin, gentamicin, chloramphenicol and amikacin in most of the isolates. Most of the bacterial isolates showed a higher percentage of resistance against amoxicillin, cefradine, ciprofloxacin, levofloxacin, ceftriaxone, co-trimoxazole and nalidixic acid. Multiple antibiotic resistance indexes suggested that almost all the test organisms exhibited multiple antibiotic resistances. The high multiple antibiotics resistance identified makes it necessary for antibiotic susceptibility testing to be conducted prior to antibiotic(s) prescription.

Key words: urinary tract infection; uropathogens; antibiotic; susceptibility; resistance.

Introduction

Urinary tract infection (UTI) is the infection of any part of body's excretory system which includes two kidneys, two ureters, a bladder, and a urethra. It is the second most common type of infection in the body, accounting for about 8.1 million visits to health care providers each year [1]. Because of its favorable chemical composition human urine can support growth of several different strains of bacteria [2, 3]. *E. coli* is the cause of 80–85% of urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–10% [4]. Other bacterial species that causes the UTI include *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*. UTI may also be due to fungal or viral infections [5], although these are uncommon and typically related to abnormalities of the urinary system or urinary catheterization [6]. Urinary tract infections due to *Staphylococcus aureus* typically occurs secondary to blood borne infections [7]. When bacteria affect 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). Bacterial infections of urinary tracts are generally classified as uncomplicated or complicated [8]. Uncomplicated UTIs occur in sexually active healthy female patients with structurally and functionally normal urinary tracts. UTIs in male patients are considered complicated. Complicated UTIs are those that are associated with co-morbid conditions that either prolong the necessity of treatment or increase the chances for therapeutic failure. These conditions include abnormalities of the urinary tract that slow down urine flow, the existence of a foreign body (e.g., indwelling catheter, stone), or infection with multidrug resistant pathogens [9, 10]. Bacterial infections of the urinary tract are the commonest cause of both community acquired infections and hospital-acquired infections in patients admitted to American hospitals [11]. UTI accounts for about

6% of new consultations in general practice in Europe and Scandinavia [12]. In general, UTI patients are treated with different antibiotics. Doctor may ask patient to take the antibiotics for a week or two to make sure the infection has been cured. If infection has spread to kidneys, patient may need several weeks of antibiotic treatment [13]. Local antimicrobial susceptibility patterns of *E. coli* in particular should be considered in sensible antimicrobial selection for uncomplicated UTIs. Since the resistance patterns of *E. coli* strains causing uncomplicated UTI varies considerably between regions and countries, a specific treatment recommendation may not be universally suitable for all regions or countries. Four large studies reporting *in vitro* susceptibility of *E. coli* causing uncomplicated UTI in North America and Europe were conducted [14-16]. All of these demonstrate considerable geographic variability in susceptibility toward the antibiotics. For example, resistance rates for all antimicrobials were higher in US medical centers than in Canadian medical centers and were usually higher in Portugal and Spain than other European countries. In general, 20% of resistance is reported in all regions for ampicillin. The same percentage of resistance is also found in many regions for trimethoprim with or without sulfamethoxazole. Resistance rates were still 10% for fluoroquinolone in most parts of North America and Europe, but there is a clear trend for increasing resistance compared with previous years. Moreover, data for nalidixic acid in these studies suggest that 10% (in some countries 20%) of the *E. coli* strains have acquired resistance genes for quinolones [15, 16]. First and second generation oral cephalosporin and amoxicillin-clavulanic acid also show regional variability, but the resistance rates are generally 10%. Some bacterial strains are sensitive to a specific antibiotic whether they may show resistance to a different one. Thus it is utmost need to culture the strain and observe their sensitivity pattern. Physicians need to be conscious of the strains that are prevalent locally and also about their susceptibility pattern against antibiotics for proper medication of the patients. Current study was carried out to investigate the prevalence and antibiotics susceptibility patterns of bacteria isolated from urine samples of some residents in Chittagong, Bangladesh.

Methodology

Study subjects:

In total, 1957 patients with the clinical symptoms of UTI were investigated that were referred to Popular diagnostic centre limited, one specialized clinical and diagnostic centre of Chittagong, during the period of June 2011- June 2012.

Specimen Collection:

Suspected UTI patients were instructed to collect their clean-catch midstream urine in a sterile tube (20 ml) and to fill half of the container. A label was attached to the outside of the container with patients' name and identification number prior collecting the sample. The date and time of collection of each specimen was also recorded. Every sample was transported to the laboratory for further proceedings immediately after collection.

Media:

Blood agar, MacConkey agar, Mueller-Hinton agar, Nutrient agar were used for bacterial culture and colony counts (Oxoid Ltd., Basingstoke, Hampshire, England).

Method of bacterial culture:

Urine specimens were cultured for isolation of the microbial agents of UTI on blood and MacConky agar media. Petri dishes containing approximately 15 ml of blood agar and MacConkey agar media were prepared in a total sterile condition prior to the culture of the sample and were preserved at 0-8°C. But before inoculating the sample these petri dishes were made to be in room temperature. The samples were plated out on MacConkey and blood agar media and incubated overnight at 37ºc [17-19]. Samples were then plated out on nutrient agar and Mueller-Hinton agar media for colony count. Samples that showed pure growth of isolate in a count of $\geq 10^5$ colony forming units (CFU) per milliliter of urine after overnight incubation were considered to indicate significant bacteriuria [20]. The characteristic bacteria on the culture media were aseptically isolated and subjected to microscopical and appropriate biochemical tests for proper identification [17].

Antibiotic susceptibility:

In the present study, antimicrobial susceptibility testing was done on Mueller-Hinton agar (Oxoid Ltd. Basingstoke, Hampshire, England) using disk diffusion (Kirby Bauer's) technique. This method was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility of UTIs agents [21]. The antibiotic disks (Oxoid Ltd. Basingstoke, Hampshire, England) comprised amoxycillin (10µg), cephradine (30µg), ceftriaxone (30µg), ceftazidime (30µg), imipenem (10µg), meropenem (10µg), sulphamethoxazole/trimethoprim (co-trimoxazole) (25µg), gentamicin

(10µg), netilmycin (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), levofloxacin (5µg), nitrofurantoin (300µg), amikacin (30µg), and chloramphenicol (30µg) [22]. The diameter of the zone of inhibition produced by each antibiotic disk was measured using engineer calipers. The result was interpreted as susceptible or resistance to the antibiotic agent used, depending on the length of zone of inhibition produced compared to reported standard length [23]. Multiple antibiotic resistance index (MAR) (number of antibiotics to which test isolate displayed resistance divided by total number of antibiotic to which the test organism has been evaluated for sensitivity) for each test isolate was calculated as recommended by Krumperman [24].

Results

Prevalence of Uropathogens:

In this study, 507 (25.91%) patients out of 1957 were showed to be urine culture positive (their colony counts were equal or more than 10^5). There were 327 female (64.5%) and 180 male (35.5%) patients with positive urine culture. The most common isolated uropathogens were *E. coli* (82.6%) and *Klebsiella pneumoniae* (14.6%). Other bacterial species named *M. morganii*, *Enterobacter spp*., *C. freundii*, *Pseudomonas spp*., *Yersinia enterocolitica* were also found in patients with UTI although they were least frequent (Table 1). In this study, the incidence of UTI was ranged in patients between 1-65 years old. The highest number of patients with UTI were found within the age range of 20- 40 followed by the age range 1-5 and then the age range 40-65 (Table 2).

Antibiotic Susceptibility:

The isolated bacteria showed wide range of differences in their susceptibility pattern to the tested antibiotics. As the result indicated, high proportions of the test organisms were sensitive to imipenem, nitrofurantoin, meropenem, ceftazidime, netilmicin, gentamicin, chloramphenicol and amikacin. Again, a significant percentage of the test organisms also showed resistance to amoxicillin, cefradine, ciprofloxacin, levofloxacin, ceftriaxone, co-trimoxazole and nalidixic acid (Table 3).

Table 1: Frequency of bacterial agents isolated from urine specimens and their relation to sex in this study

Bacterial Isolates	No. of Patients (%)	Male	Female
E. coli	419 (82.6)	141	278
K. pneaumoniae	74 (14.6)	30	44
Enterobacter spp.	1 (0.2)	0	1
Pseudomonas spp.	7 (1.4)	4	3
M. morganii	2 (0.4)	0	2
C. freundii	3 (0.6)	2	1
Y. enterocolitica	1 (0.2)	1	0
Total	**507**	**180 (35.5%)**	**327 (64.5%)**

Table 2: Distribution pattern of UTI patients in different age level

Age Level	Frequency	Percent
1-5	123	24.3
6-10	39	7.7
11-15	15	3.0
16-20	47	9.3
20-40	173	33.9
41-65	110	21.7
Total	**507**	**100**

Table 3: Susceptibility pattern (%) of bacterial agents isolated from urine specimens

Bacterial Isolates	Antibiotics														
	Amo	**Cepr**	**Cept**	**Cef**	**Imi**	**Mer**	**Cot**	**Gen**	**Net**	**Nal**	**Cip**	**Lev**	**Nit**	**Ami**	**Chl**
Escherichia coli	100	24.2	45.7	80.4	99.5	99.5	26.1	74.2	90.9	29.2	51.0	52.4	80.4	99.5	66.3
Klebsiella pneumoniae	1.4	17.6	45.9	81.1	100	100	20.3	79.7	93.2	36.5	54.1	55.4	70.3	98.6	66.2
Moganella morganii	0	0	100	100	100	100	0	100	100	0	0	0	100	100	100
Citrobacter freundii	0	66.7	66.7	100	100	100	66.7	100	100	33.3	66.7	66.7	66.7	100	66.7
Pseudomonas spp.	0	42.9	57.1	85.7	85.7	100	0	42.9	85.7	14.3	42.9	42.9	28.6	85.7	57.1
Yersinia enterocolitica	0	100	100	100	100	100	100	100	100	0	100	100	100	100	100
Enterobacter spp.	0	0	0	0	100	100	0	100	100	0	0	0	100	100	0

Amo = Amoxycillin; Cepr = Cephradine; Cept = Ceftriaxone; Cef = Ceftazidime; Imi = Imipenem; Mer = Meropenem; Cot = Sulphamethoxazole/Trimethoprim (Co-Trimoxazole); Gen = Gentamicin; Net = Netilmycin; Nal = Nalidixic Acid; Cip = Ciprofloxacin; Lev = Levofloxacin; Nit = Nitrofurantoin; Ami = Amikacin; Chl = Chloramphenicol.

Antibiotic Resistance:

Calculated multiple antibiotic resistance indexes suggested that almost all the test organisms exhibited

multiple antibiotic resistances in the following order: *E. coli* > *Klebsiella pneumoniae* > *Pseudomonas spp.* > *C. freundii* > *M. morganii* > *Enterobacter spp.* > *Yersinia enterocolitica* (Table 4).

Table 4: Multiple Antibiotic Resistance (MAR) Indices of bacteria

MAR index	**Bacterial isolates**						
	E. coli	***K. pneumoniae***	***M. morganii***	***C. freundii***	***Pseudomonas spp.***	***Y. enterocolitica***	***Enterobacter spp.***
0	01	0					
0.07	19	3	0	1			
0.13	40	4	0	1		1	
0.20	45	10	0				
0.27	54	9	0		1		
0.30	1	0	0		1		
0.33	39	12	1		2		
0.40	57	6	1		1		1
0.47	70	18	0				
0.53	54	6	0				
0.60	31	5	0	1			
0.67	08	1	0		1		
0.73					1		

Discussions

Urinary tract infection is a common clinical condition worldwide, but the pattern of antimicrobial resistance varies in different regions. Here we described the relationships between sex, isolated

bacterial agents and antibiotic susceptibility and resistance of UTIs. The sex distribution of patients in our study is analogous with those of other reported studies, showing a predominance of females (64.5%) with UTI [25]. The elevated incidence of infection among females is related to the differences between male and female genitourinary systems in anatomy and host factors such as changes in normal vaginal flora [26]. The most common uropathogens in our study were *E. coli* (82.6%) and *Klebsiella pneumoniae* (14.6%). Although the percentage of *E. coli* is much higher in our study, it supports the previous findings indicating that *E. coli* is the principal etiological agent of UTI, accounting for 46.98% of the screened cases [27-29]. In another study, it was reported that predominant uropathogens are *E. coli* followed by *Klebsiella species* which also support our study [30]. Infection frequency of *M. morganii, Enterobacter spp., C. freundii, Pseudomonas spp., Yersinia enterocolitica* were found to be very few in this study (Table 2) which is also be affirmed by another work where the frequency of *Pseudomonas* and *Enterobacter spp*. is least [31]. Pathogens like *C. freundii, M. morganii* and *Yersinia enterocolitica* were not found in many other studies [32]. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and also from some practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each country. The most effective antimicrobial agents in our study were imipenem, nitrofurantoin, meropenem, ceftazidime, netilmicin, gentamicin, chloramphenicol and amikacin (Table 3). It has been reported that amikacin is the most effective antibiotic against *E. coli* [33]. Our result was further supported by another study where the susceptibility rate of *E. coli* to amikacin remained 93-100% [34]. Again, most of the pathogens showed considerable resistance to amoxicillin, cefradine, ciprofloxacin, levofloxacin, ceftriaxone, co-trimoxazole and nalidixic acid (Table 3). The widespread use, more often the misuse, of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria. It is reported that in United State resistant strains in USA to ampicillin and co-trimoxazole [35]. Multiple antibiotics resistance (MAR) index is a tool that reveals the spread of bacterial resistance in a given population [36]. Again, MAR index of a bacterial species greater than 0.2 implies that the strain originated from an environment where several antibiotics had been used [37]. In this study, we found strains which were resistant to most of the antimicrobial agents. The MAR indices obtained here (Table 4) is a possible indication that a very large proportion of the bacterial isolates have been exposed to several antibiotics. With this evidence from our study, we can suggest imipenem, nitrofurantoin, meropenem,

ceftazidime, netilmicin, gentamicin, chloramphenicol and amikacin to be prescribed as the empirical treatment for UTI. But by keeping the emerging antimicrobial resistance in mind, it is strongly recommended that the antibiotic therapy should only be commenced after the culture and sensitivity report from the microbiology laboratory. This would not only help in the sensible use of antibiotics but also would restrain the spreading of antimicrobial resistant strains in the community as well as in the hospital.

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