Original Article

Antimicrobial Resistance Pattern of Uropathogenic *Escherichia coli* and *Klebsiella species* Isolated in a Tertiary Care Hospital of Sylhet

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Abstract

The rapid emergence of antimicrobial resistance in major uropathogens has created a global threat especially in the developing countries. In Bangladesh, prescribers of different regions generally diagnose microbial infection on clinical judgment and select antimicrobial on empirical basis, which unfavorably affects the sensitivity pattern of microbes. The present study was designed to determine the prevalence of antimicrobial resistance of Escherichia coli and Klebsiella species isolated from patients with UTI in a tertiary care hospital. This was a cross sectional study conducted at Sylhet MAG Osmani Medical College Hospital, Sylhet from 1st January to 31st December 2016. A total of 200 clinically suspected urinary tract infection patients aged 16-85 years were included in this study. The isolated Escherichia coli and Klebsiella species were tested for antimicrobial susceptibility according to the guideline of Clinical and Laboratory Standards Institute (CLSI) 2011 using the modified Kirby-Bauer disc diffusion technique. Out of 107 pus cell positive (≥05/HPF) urine samples, 75 (70.1%) yielded significant bacteriuria of which 49 (65.3%) were Escherichia coli and 9 (12%) were Klebsiella species. The isolated Escherichia coli showed absolutely high resistance to ampicillin and cefuroxime (100%), moderately high resistance to ceftazidime (81.6%), ciprofloxacin (77.6%), cotrimoxazole (75.5%), cefotaxime (67.4%) and ceftriaxone (59.2%), moderate rate of resistance to amikacin 48.9% and imipenem 46.9% and least rate of resistance to gentamicin (22.5%), nitrofurantion (22.5%) and netilmicin (6.1%). In addition, Klebsiella species revealed completely resistance to ampicillin, cotrimoxazole, cefuroxime, cefotaxime, ceftriaxone, ceftazidime (100%), moderately high resistance to imipenem (88.9%), nitrofurantoin (77.8%), moderate resistance to ciprofloxacin (44.4%), amikacin (33.3%) and gentamicin (33.3%) and least resistance to netilmicin (11.1%). Hence very high resistance rates of 1st line drugs found in uropathigenic Escherichia coli & Klebsiella species, it is necessary to diagnose clinically followed by culture and sensitivity testing. Regular antibiotic surveillance of a particular geographical area is required to establish reliable information regarding susceptibility pattern of uropathogens in order to preserve the continued usefulness of most antimicrobial agents.

Keywords: Antimicrobial resistance, UTI, Escherichia coli, Klebsiella species.

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Introduction

Urinary Tract Infections (UTIs) are the second most common bacterial infections after respiratory tract infections that lead patients to seek medical attention. Approximately 10% of humans will have a UTI at some time during their lives. UTIs are also the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections[1]. In Bangladesh, 20-35% females experience at least one episode of UTI in their lives. The prevalence increases among patients from lower socio-economic group[2,3].

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Acute infections of the lower urinary tract (urethritis and cystitis) are often considered superficial (or mucosal) infections, while upper tract (acute pyelonephritis, prostatitis, and intrarenal and perinephric abscesses) signify tissue invasion. In microbiological point of view, UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate. In most cases, growth of ≥105 organisms per milliliter from a properly collected midstream "clean-catch" urine sample indicates infection[4]. Typical organisms causing UTI in the community include Escherichia coli derived from the gastrointestinal tract (about 75% of infections), Proteus species, Pseudomonas species, Streptococci and Staphylococcus epidermidis. In hospital, Escherichia coli still predominates, but Klebsiella or Streptococci are more common[5].

In present scenario, the rapid emergence of antimicrobial resistance in major uropathogens has created a global threat especially in the developing countries. Moreover, Irrational and indiscriminate use of antibiotics as well as fake and substandard drugs is common in these countries[6,7,8]. The link between inappropriate use of antimicrobials and development of antimicrobial resistance has been acknowledged in different scientific studies and global proceedings. In Bangladesh, prescribers of different regions generally diagnose microbial infection on clinical judgment and select antimicrobial on empirical basis, which unfavorably affects the sensitivity pattern of microbes. In addition, reluctance of the law makers and regulators to enact law to overcome inadequacy in rules and regulation to control antimicrobial prescribing and dispensing led to worsening of the situation[9]. Thus, updated knowledge of antimicrobial resistance patterns in specific geographical location may aid clinician in choosing the appropriate antimicrobial empirical treatment and development of appropriate drug policies. Therefore, the present study was designed to determine the prevalence of antimicrobial resistance of Escherichia coli and Klebsiella species isolated from patients with UTI in a tertiary care hospital.

Materials and Methods

Study population

This prospective cross sectional study was carried out in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet from 1st January to 31st December, 2016. A total 200 consecutive urine samples were collected from clinically suspected patients of UTI of different age and sex attending either at the outpatient department (OPD) or admitted in this tertiary care hospital. An informed written consent was taken from patients before collection of their sample. Those with clinical manifestations of UTI but on antibiotics within seven days were excluded.

Collection of specimens

Patients were advised to collect clean-catch midstream urine (CCMU) into a sterile wide mouth container with all aseptic measures (information on how to collect proper sample in sterile container aseptically was given prior to collection). In case of catheterized patients, urine was collected from the catheter by sterile disposable syringe after proper cleaning of the catheter. After proper labeling with patient's name, ID number, collection date and time, the specimens were transported to the laboratory as soon as possible[10].

Direct microscopy

Urine was transferred into a clean and dry 15 ml centrifuge tube and was centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and one drop of sediment was taken into a clean glass slide, a cover slip was placed over it and then examined for pus cells under light microscope using 10x and 40x objectives. Microscopic demonstration of pus cells ≥5/HPF (high power field) was included as study cases[10,11].

Culture and identification of isolates

The well-mixed and noncentrifuged urine samples were inoculated by a wire loop that can deliver 0.004 mL of urine specimen into 5% sheep blood agar and MacConkey agar plates (Himedia, Mumbai, India) by using streak plate method following the standard microbiological procedures. Then, the plates were aerobically incubated at 37˚C for 24 hours and examined for the presence or absence of the bacterial growth. Colonies were counted and checked for significant bacteriuria on blood agar. A culture that grew ≥105 colony-forming unit (CFU/mL) was considered significant bacteriuria[11].

All positive urine cultures showing significant bacteriuria were further tested for their physical characteristics such as colony morphology, odor, swarming, and the presence of hemolysis on their respective media. Then, it was confirmed by the pattern of biochemical reactions using the standard procedures. Thus, Gram-negative rods were identified with the help of a series of biochemical tests such as triple sugar iron agar, indole, simmons citrate agar, oxidase, urease, and motility[10,11].

Antimicrobial susceptibility test

Samples which showed significant colony count were taken into consideration and antimicrobial susceptibility pattern of the isolated Escherichia coli and Klebsiella species was tested using Mueller Hinton agar media by the modified Kirby-Bauer disc diffusion technique[10] as described by Clinical and Laboratory Standards Institute 2011 (CLSI, former NCCLS). As per CLSI guidelines susceptibility was noted as sensitive (S), intermediate sensitive (I) and resistant (R) based on the diameter of

zone of inhibition. The following first and second line antibiotic discs (Himedia, Mumbai, India) were used in the testing: cotrimoxazole (25μg), ampicillin (10 μg), gentamicin (15μg), amikacin (30 μg), ceftazidime (30 μg), cefotaxime (30μg), ceftriaxone (30 μg), cefuroxime (30 μg), imipenem (10 μg), ciprofloxacin (5 μg), netilmicin (30 μg) and nitrofurantoin (300μg)[12].

Quality control

As per manufacturer's instruction, the discs were stored in refrigerator between temperatures 2-8^{0}C up to date of expiry. Discs from each batch was standardized first by testing against reference strain of Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 collected from BSMMU, Shahbag, Dhaka.

Data analysis

All Data were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences) Version 23.0. Qualitative data were analyzed by frequency and percentage and comparisons were performed by Pearson's Chi square (χ^2) test. A probability value ($p<0.05$) was considered statistically significant.

Results

A total 200 cases of clinically suspected UTI patients of different age and sex fulfilling the inclusion criteria either visited the outpatient department (OPD) or admitted in inpatient department (IPD) of Sylhet MAG Osmani Medical College Hospital, Sylhet were studied. Majority of patients belonged to the age group of 16-40 years and most of them were females (Table I).

Table -I: Prevalence of UTI in different age group and gender (n=200).

Age Group	Sex of the Patients			
	Male		Female	
	Number	Percentage	Number	Percentage
16yr-40yr	51	50.5	68	68.7
41yr-65yr	31	30.7	24	24.2
66yr-90yr	19	18.8	7	7.1
Total	101	50.5	99	49.5

In this study, samples with pus cells ≥05/HPF were included as study cases. Out of 107 pus cell positive (≥05/HPF) urine samples, 75 (70.1%) showed significant growth of bacteria of which 36 (78.3%) isolates were from IPD and 39 (63.9%) were from OPD. This result was statistically significant ($p<0.05$) as shown in the Table II.

Table-II: Distribution of urine samples with or without significant growth.

Urine Culture	IPD	OPD	Total	P-value*
Significant growth	36 (78.3%)	39 (63.9%)	75 (70.1%)	
No growth	10 (21.7%)	22 (36.1%)	32 (29.9%)	.022
Total	46 (100%)	61 (100%)	107 (100%)	

*Pearson's Chi-square test was applied to see the association. Here "P" value < 0.05 was taken as significant.

Among the isolated Gram-negative urinary pathogens Escherichia coli was the predominant (65.3%) followed by Klebsiella species (12%), Enterobacter species (9.3%), Pseudomonas species (9.3%) and Proteus species (4%) as depicted in the Table III.

Table-III: Frequency of Gram negative bacteria (GNB) isolated in UTI.

GNB isolated	Number of isolates	Percentage
Escherichia coli	49	65.3
Klebsiella species	9	12.0
Enterobacter species	7	9.3
Pseudomonas species	7	9.3
Proteus species	3	4.0
Total	75	100.0

Analysis of the antimicrobial susceptibility test in this study revealed that Escherichia coli showed very high rate of resistance to ampicillin and cefuroxime (100%), followed by high resistance to ceftazidime (81.6%), ciprofloxacin (77.6%), cotrimoxazole (75.5%), cefotaxime (67.4%) and ceftriaxone (59.2%). Moderate rate of resistance to amikacin and imipenem was found 48.9% and 46.9% respectively. Escherichia coli also showed least rate of resistance to gentamicin (22.5%), nitrofurantion (22.5%) and netilmicin (6.1%) as shown in Figure 1.

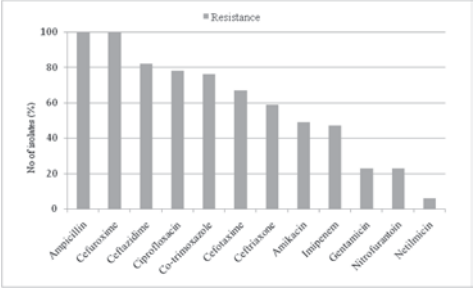

Figure-1: Antimicrobial resistance pattern of Escherichia coli.

In addition, the antimicrobial resistance patterns of Klebsiella species are shown in figure 2. Very high resistance to ampicillin, cotrimoxazole, cefuroxime, cefotaxime, ceftriaxone, ceftazidime (100%) followed by high resistance to imipenem (88.9%), nitrofurantoin (77.8%) were shown by Klebsiella species. Moderate resistance was found to ciprofloxacin (44.4%), amikacin (33.3%) and gentamicin (33.3%) and least resistance to netilmicin (11.1%).

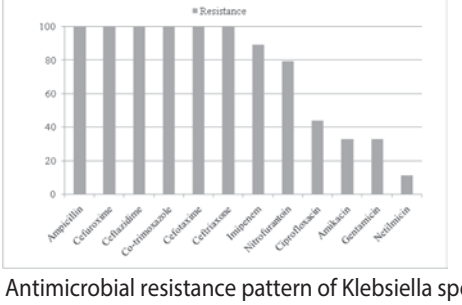

Figure-2: Antimicrobial resistance pattern of Klebsiella species.

Discussion

Adequate knowledge by continuous antibiotic surveillance on local antimicrobial resistance pattern of uropathogens is very important for clinicians to select and use the best effective antimicrobial agent for the treatment of UTI patients. The current study described the antimicrobial resistance rates of urinary pathogens especially Escherichia coli and Klebsiella species which are the predominant microbial agents in community and hospital acquired urinary tract infections respectively.

The age and gender wise data was analyzed in order to assess the frequency of UTI in different age groups. This study showed that females in the reproductive age group of 16-40 years (68.7%) were more prone to UTI than any other groups. This result is in comprehension with other studies[13,14]. In this study, majority of isolated bacteria were Escherichia coli (65.3%) and the second most common was Klebsiella species (12%) among the Gram negative bacteria. Similar finding was reported by the other researchers[15,16].

The pattern of antimicrobial resistance to microorganism causing UTI varies with time and between geographical areas[17]. In the current study, the antimicrobial resistance rate of isolated Escherichia coli and Klebsiella species was very high to ampicillin, cefuroxime, cotrimoxazole and ciprofloxacin. High resistance to first line drugs found in this study is similar to other studies in developing countries[13,18]. The observation may be due to wide empirical use, excessive and injudicious use of these drugs because they are relatively cheap and being oral antibiotics easy to administer.

Surprisingly, it was observed that rate of resistance towards cefuroxime in both isolated Escherichia coli and Klebsiella species was absolutely high. In the year 2015, another study by Haque et al.[19] recorded 78.8% & 63.6% respectively of these urinary isolates resistant to cefuroxime whereas, present study yielded that value about 100% in both isolates. This finding is supported by the study of Davi and Rajkumar[20] who showed 94% and 100% of urinary isolates were resistant to cefuroxime respectively. Carelessness misuse of this drug for a long period in our country especially this region would have been thought as the cause of this rapid emergence of absolute cefuroxime resistance.

The prevalence of ciprofloxacin resistance in Bangladesh was 26 per cent[21]. In this study, Escherichia coli and Klebsiella species showed significantly high resistance to ciprofloxacin (77.6% & 44.4% respectively). These findings are similar to the result of Sarkar et al.[22], who found similar resistance pattern (78.9% & 64% respectively). Besides, there are more studies[23, 24] with similar results indicating high level resistance to ciprofloxacin. This increased resistance might be due to widespread indiscriminate use, their oral route of administration, easy availability and affordability of ciprofloxacin over the country. So, physicians should prescribe this drug with caution to preserve its effectiveness.

In our setting, we found imipenem had lost its sensitivity (40.7% & 14.3% respectively) in both Escherichia coli and Klebsiella species. Our result is similar with the works done in Saudi Arabia, India and Iran[25,26,27] where incidences of carbapenem-hydrolyzing enzymes amongst Enterobacteriaceae has been reported. Carbapenems are used to treat life threatening infections caused by MDR bacterial pathogens and antibiotics in this class represent the last line of therapy in treatment options against very serious infections such as those caused by extended spectrum beta-lactamases.

Urinary isolates of Escherichia coli of the present study showed low resistance (22.5%) against nitrofurantoin. Although the value was slightly higher compared to Haque et al.[19] (16.1%) but still it remains as most sensitive drug and similar results were also reported from others studies[28,29,30]. The reason behind this might be due to low use of this drug for long period considering its toxicity and side effects. With sensitivity rate of 74.1% for nitrofurantoin might be the only useful oral antibiotic in the treatment of uncomplicated UTI and prophylaxis in the context of gradually decreasing susceptibility of the most of the comparatively cheaper anti-UTI drugs.

In our setting, netilmicin was the most sensitive antibiotic (resistant rate 6.1% & 11.1% respectively) for both Escherichia coli and Klebsiella species to treat the UTI patients. As increased amikacin resistance to both the isolates showed in this study by 49% & 33.3% respectively, netlimicin may be an alternative parental drug for the treatment of complicated UTI in the local setting, at least in a tertiary health facility[31].

Conclusion

Hence very high resistance rates of 1st line drugs found in uropathigenic Escherichia coli & Klebsiella species, it is necessary to diagnose clinically followed by culture and sensitivity testing; otherwise direct empirical treatment might lead to treatment failure. Regular antibiotic surveillance of a particular geographical area is required to establish reliable information regarding susceptibility pattern of uropathogens in order to preserve the continued usefulness of most antimicrobial agents. However, nitrofurantoin and netilmicin are more sensitive compared to the other antibiotics tested in this study and therefore, these might be the drugs of choice for the treatment of uncomplicated community acquired and complicated hospital-acquired UTIs respectively after culture and sensitivity report available.

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