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Antibiotic resistance patterns of pathogenic Gram negative bacteria isolated from UTI patients in Sirajganj district

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Urinary tract infection (UTI) is a common cause of illness in people of all age groups. The increasing trend of antibiotic resistance is now a concern worldwide. The present study was conducted to determine the antibiotic resistance pattern of Gram negative pathogenic isolated from UTI cases in Sirajganj district. A total of 297 uropathogens were included in this study of which 66 (22.2%) were isolated from male patients and 231 (77.8%) from female patients. *Escherichia coli* (52.9%) was found to be the predominant pathogen followed by *Klebsiella* spp. (22.9%), *Proteus* spp. (4.7%), *Psudomonas* spp. (4.4%), *Enterobacter* spp. (2.4%), *Citrobacter* spp. (3%) and *Morganella* spp. (5.7%). Most of the uropathogens were resistant against Ampicillin (AMP). Meropenem was the most effective antibiotic with resistance between 0 and 15.1% of the tested isolates. However, the trend of antibiotic resistance can vary with the change in the environmental and socioeconomic conditions. Accurate diagnosis of UTI and determination of antibiotic sensitivity pattern is important for selection of appropriate drug for effective treatment.

Key words: UTI; Antibiotic resistance; Pathogens; Gram negative bacteria

Urinary Tract Infection (UTI) is a broad term used to describe a group of diseases resulting from the microbial colonization of the urinary tract (1). It may affect the upper (pyelonephritis) or lower (cystitis) urinary tract. It is one of the most common bacterial illnesses which incur health care expenditures in the general population (1). Both Gram positive and negative bacteria can cause UTI upon entering the urinary tract at levels more than or equal to 10^5 colony forming units of bacteria/ml of urine. The predominant members of bacterial pathogens belong to the family *Enterobacteriaceae* (2). UTIs can occur in individual patient or may be acquired from hospitals (3).

According to previous reports, approximately 150 million individuals are affected annually worldwide (4). An increase in antibiotic resistance in pathogenic bacteria is now a prime concern worldwide. Likewise, increasing trend of antimicrobial resistance in uropathogens has complicated the empirical therapy leading to treatment failures (5-7). This study was aimed to determine the antibiotic resistance patterns of Gram-negative bacteria isolated form UTI patients in Sirajganj district

MATERIALS AND METHODS

Study area, sampling and sample processing. This study was carried out in the Microbiology laboratory of Avicenna Hospital Ltd., Sirajganj, Bangladesh. Urine samples of both hospitalized and outdoor patients from June 2012 to May 2013 were used for the investigation. Clean catch mid-stream urine samples were collected in sterile containers for microbiological analysis.

Urine culture. Urine samples (10 µl) were streaked on to MacConkey agar (Oxoid, UK) and Nutrient agar (Oxoid, UK) media and incubated aerobically at 37 °C for 24 hours for isolation and identification of pathogenic bacteria. Samples with colony counts on Nutrient agar of $\geq 1\times10^5$ cfu/ml were considered as positive for the presence of pathogens. Corresponding bacterial colonies on MacConkey agar were further identified to determine the type of pathogen.

Identification of bacterial isolates. Bacteria present in the positive urine samples were biochemically identified following standard methods. Clinical isolates were characterized by microscopic examination (Gram staining), oxidase test, motility, citrate utilization and Triple Sugar Iron utilization tests for species identification. The uropathogen *Pseudomonas aeruginosa* was also identified by production of diffusible pigments on Mueller-Hinton Agar.

Antibiotic susceptibility assay of bacterial isolates. Antimicrobial susceptibility testing of the bacterial isolates was performed by the disk diffusion method (8) in accordance with the Clinical & Laboratory Standards Institute guideline (9). Quality controls employed standard strains of *E. coli* ATCC 25922. Antibiotics included in this study include: Amoxy-Clavulanic acid (Aclav, 3µg), Amikacin (AK, 30µg), Ampicillin (Amp, 25µg), Ceftazidime (CAZ, 30µg), Cefaclor (CFC, 30µg), Cephalexin (CFX, 30µg), Cefixime (CFM, 5µg), Ciprofloxacin (CIP, 5µg), Ceftriaxone (CRO, 5µg), Cefuroxime (CXM, 5µg), Cloxacillin (CX, 5µg), Gentamicin (GM, 10µg) , Meropenem (MEM, 10µg), Azithromycin (ATH, 15 µg), Nitrofurantoin (NI, 50µg), Nalidixic acid (NA, 30µg) and Tobramycin (TOB, 30 µg).

RESULTS

A total of 297 uropathogens were investigated in this study. Of the 297 pathogens, 66 (22.2%) were isolated from male patients and 231 (77.8%) from female patients. The proportions of pathogenic bacteria isolated from UTI patients are shown in Table 1. *E. coli* was the predominant uropathogen (52.9%) causing UTI followed by *Klebsiella pneumonia* (22.9%) and *Proteus mirabilis* (4.7%).

Table 2 shows proportions of resistant bacteria against

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TABLE 1. Frequency of bacteria isolate for UTI patent

Bacteria	No. of isolates	Frequency (%)
Escherichia coli	159	52.9
Klebsiella spp	73	22.9
Proteus spp	14	4.7
Pseudomonas spp	17	4.4
Enterobacter spp	7	2.4
Citrobacter spp	9	3.0
Morganella morganelli	18	5.7

17 types of antibiotics. *E. coli* showed highest resistance Nalidixic acid (NA) (36.5%) and lowest against Meropenem. The majority of *Klebsiella* spp. was resistant against Cephalexin, CFX (4.7%) and less resistance against Nitrofurantoin, NI (4.1%). Proteus spp. were mostly resistant against Cloxacillin, CX (48.9%) followed by against Amoxycillin-clavulanic (Aclav) acid (35.7%) and cefixime, CFM (35.7%).They were sensitive against Meropenem (MEM) and Nitrofurientoin (NI). *Enterobacter* spp. was equally resistant (28.6%) against the antibiotics Aclav, CFX, CFM, Ciprofloxacin (CIP) and Tobramycin (TOB). They were completely sensitive to a number of antibiotics including AK, CAZ and MEM. Majority of the *Citrobacter* spp. showed resistance against Cefuroxime, CXM (55.6%) and were sensitive to MEM, NA and TBO. In contrast, significantly higher percentages of *Morganella morganelli* and *Pseudomonas* spp. showed resistance to the antibiotics investigated. A total of 72.2% *Morgenalla morganelli* showed resistance against Ampicillin followed by 66.66% of the isolates showing resistance against Aclav. In contrast, *Morganella* spp. was sensitive to AK. *Psedomonas* spp. demonstrated highest level of resistance against Aclav (71.42%) and showed sensitivity to CAZ and NI.

The majority of the uropathogens (*Morganella morganelli*, *Pseudomonas* spp., *Proteus* spp. and *Enterobacter* spp.) were resistant against Aclav. Two of the uropathogens (*Klebsiella* spp. and *Enterobacter* spp.) showed maximum resistance against CFX and two others, *E. coli* and *Klebsiella* spp., against NA. A maximum of four pathogenic genera (*E. coli*, *Proteus*, *Enterobacter* and *Citrobacter*) were fully sensitive to MEM, three genera (*Pseudomonas*, *Proteus* and *Citrobacter*) to NI, two genera (*Morganella morganelli* and *Enterobacter*) to AK and two genera (*Pseudomonas* and *Enterobacter*) to CAZ.

DISCUSSION

The prevalence of UTIs is high in Bangladesh and other developing countries, affecting individuals in the community of different age groups of both sexes (10). Urinary tract infection results as a consequence of entry and colonization of pathogenic bacteria in the urinary system. This study focuses on the antibiotic resistance patterns of the Gram negative bacteria isolated from UTI patients in Sirajganj district of Bangladesh. It may be worth to mention that most of the previous reports on UTI in Bangladesh are either from Dhaka or Chittagong city. This is possibly due to the limited laboratory facilities outside these areas. This study was conducted at the Avicenna Hospital Ltd., Sirajganj and to our knowledge constitutes the first report of its kind from this area.

TABLE 2. Proportions of pathogenic bacteria exhibited resistance to 21 selected antibiotics

Antibiotic Disc	*E. coli* (%)	*Klebsiella* spp. (%)	*Morganella morganelli* (%)	*Pseudomonas* spp. (%)	*Proteus* spp. (%)	*Enterobacter* spp. (%)	*Citrobacter* spp. (%)
Aclav	24.5	16.4	66.7	58.8	35.7	28.6	22.2
AK	3.1	8.2	0.0	27.8	7.1	0.0	11.1
AmP	28.3	21.9	72.2	52.9	50.0	21.4	44.4
CAZ	7.6	8.2	22.2	0.0	7.1	0.0	22.2
CFC	27.0	16.4	44.4	23.5	7.1	21.4	33.3
CFX	33.3	24.7	50.0	47.1	21.4	28.6	44.4
CFM	23.3	17.8	33.3	23.5	35.7	28.6	22.2
CIP	20.8	9.9	33.3	35.3	21.4	28.6	33.3
CRO	17.2	19.2	38.9	17.6	28.6	21.4	11.1
CXM	23.9	16.4	55.6	47.1	21.4	21.4	55.6
CX	18.9	20.5	27.8	35.3	48.9	14.3	44.4
GM	6.9	13.7	44.4	5.9	14.3	14.3	11.1
MEM	0.0	15.1	1.4	11.8	0.0	0.0	0.0
ATH	16.9	13.7	61.1	11.8	14.3	0.0	11.1
NI	3.1	4.1	5.6	0.0	0.0	14.3	0.0
NA	36.5	24.7	61.1	52.9	28.6	21.4	22.2
TOB	4.4	6.8	11.1	17.6	7.1	28.6	0.0

Aclav = Amoxy-Clavulanic acid (3µg); AK = Amikacin (30 µg); Amp = Ampicillin (25 µg); CAZ = Ceftazidime (30 µg); CFC = Cefaclor (30 µg); CFX = Cephalexin (30 µg); CFM = Cefixime (5 µg); CIP = Ciprofloxacin (5 µg); CRO= Ceftriaxone (5 µg); CXM= Cefuroxime (5 µg); CX = Cloxacillin (5 µg); GM = Gentamicin (10 µg); MEM = Meropenem (10 µg); ATH = Azithromycin (15 µg); NI = Nitrofurantoin (50 µg); NA = Nalidixic acid (30 µg); TOB = Tobramycin (30).

The antibiotic susceptibility pattern of the major uropathogens in all age groups from Sirajganj district demonstrates that a higher proportion of females (n=231, 77.8%) were infected than males (n=66, 22.2%). Similar observations were made by other workers in studies with uropathogens from Bangladesh (10, 11). Several factors are reported to be associated with the development of UTIs viz. poor personal hygiene, pregnancy, urinary tract obstruction, urethral reflux, long term catheterization, sexual intercourse, spermicidal contraception and a history of UTIs (11, 12). It has been observed that a large portion of all women, up to one-third, experience UTI at some point during their lifetime which may be related to their anatomical structure of genitourinary system (13,14). The notable features which are found to be associated with UTI are short urethra, proximity of the urethra to the anus and colonization of the vagina by members of the faecal flora that facilitate ascending infection into bladder (14).

Until recently, fluoroquinolones were preferred antibiotics prescribed for UTI patients because of their low rates of resistance, among most common uropathogens and high efficiency in curing patients (15, 16). They are available in both oral and intravenous formulations and easy for administration in different medical conditions. Fluoroquinolones are easily absorbed and rapidly excreted from the body under normal conditions (15, 17, 18). However, the wide spread use of this broad spectrum antibiotics has provoked pathogens to acquire drug resistance in UTI patients (19-23). Continuous evolution of antibiotic resistance has emerged due to the indiscriminate use of antibiotics and not completing the antibiotics course properly. It is now necessary to reassess the antibiotic sensitivity patterns to determine the drug of choice for managing UTI (20, 24).

E. coli was the predominant organism causing UTIs in the majority of the screened patients. This is in concordance with other findings both from Bangladesh (10, 11) and overseas (25, 26). It has been observed in previous study that bacterial resistance emerges subsequent to the clinical use any antibiotic (27). Antibiotic resistance by pathogenic microorganisms was recognized as an increasing global problem and had become an important factor to be considered in the treatment of infections (25). In the present investigation, most of the predominant uropathogens were sensitive to Meropenem. Sensitivity of *Morganella morganelli* and *Pseudomonas* spp. was observed maximally to NI. This suggests that at the current antimicrobial sensitivity status of the uropathogens, Meropenem or NI is a better option for treatment over other antibiotics. As antibiotic resistance is an important factor, urine culture and antibiotic sensitivity tests should be routinely performed in all UTI cases. Appropriate antibiotics need to be prescribed based on the antibiotic susceptibility test which will be narrow spectrum, effective and less expensive with least side effects.

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