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Antimicrobial Resistance Pattern of Gram-negative Bacteria Causing Urinary Tract Infection.

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ABSTRACT

Urinary tract infection (UTI) is a common and occasional recurrent bacterial illness with an increasing resistance to antimicrobials. Antibiotic resistance in UTI is a growing public health problem in the world including Bangladesh. The study objective was to examine the present incidence of UTIs in Bangladesh in a point period of time from January to December 2007. A retrospective data analysis of culture results of urinary pathogens was performed. The data was collected from the Clinical Microbiology Laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The data included culture results of 9,854 urine samples and only Gram-negative isolates irrespective of age groups were analyzed. The prevalence of UTI was observed in 24.14%. It was observed that out of 2,379 uropathogens *Escherichia coli* (66.92%) was the most prevalent isolate followed by *Klebsiella spp*. (13.45%), *Proteus* spp. (6.77%) and *Pseudomonas* spp. (6.77%). The percentage of resistance to different antibiotics was higher in *E. coli*, *Klebsiella* spp. and *Pseudomonas* spp. isolates compared to that of others. Among the total number of isolates the resistant rate of *E. coli* and *Klebsiella spp.* to ampicillin was 86.09% and 83.33% and to cotrimoxazole was 67.61% and 59.81%, their resistance rate to nalidixic acid was 83.28% and 61.54%. All other isolates also showed above 80% resistance to ampicillin and above 50% to cotrimoxazole whereas imipenem was found to be the most effective against the uropathogens followed by amikacin.

Key Words: Urinary tract infection, Antimicrobial resistance, Uropathogens, Bacteriuria, Gram-negative bacteria

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial illnesses associated with leading cause of morbidity and health care expenditures in persons of all ages (Kunin CM, 1994). A variety of organisms entering the urinary tract establish bacteriuria often at levels more than or equal to 10^5 colony forming units of bacteria/ml of urine. It can affect both individual patients as well as in patients staying in hospital for long period especially in adult patients when they are some kind of incubations such as catherization and venous shunt. Several studies reported that hospital acquired UTI is about 33% (Gupta et al., 2001). Majority of pathogens are gram-negative species, with predominance of members of *Enterobacteriaceae* (Neu, 1992). Increasing antimicrobial resistance in bacterial pathogens is a worldwide concern. The prevalence of antimicrobial resistance among UTI agents is also increasing (Mathai et al., 2001; Karlowsky et al., 2001) and its treatment has become more complicated due to increasing resistance and empirical therapy leading to treatment failures mostly associated with gram-negative bacteria (Blondeau et al., 1999). The present study investigated the pattern of gram-negative uropathogens and their antimicrobial resistance pattern among the clinical isolates to thirteen commercially available antibiotics that are often prescribed in UTI cases.

METHODS AND MATERIALS

Study Samples and the site

The culture results of urine submitted to Microbiology Lab of Clinical Laboratory Services of ICDDR, B was retrospectively analyzed from January 2007 to December 2007. Culture results showing colony count of ≥1×10^4 cfu/ml of urine was considered for this analysis. However, patients with high number of pus cells in per high power field were also included in the study when associated with gram-negative bacteria for UTI.

Laboratory techniques

In brief, one calibrated loopful urine samples were streaked on to MacConkey agar (MAC) and Blood agar media and incubated aerobically at 37˚C for 24 hours for isolation and identification of urinary pathogens. The colony characteristics were then examined. The pink colonies on MAC were lactose-fermenters and most likely to be *E. coli, Klebsiella*, or other coliforms. If colonies were colorless, they were lactose-nonfermenters. The clinical isolates were characterized by gram-stain, oxidase test, motility, citrate utilization and biochemically for species identification (Cappuccino G. and Sherman N., 1996). The bacterial colony count was then determined following the defined formula.

Antibiotic susceptibility assay of bacterial isolates

The standard agar-disc-diffusion method (Kirby Bauer technique) was used to determine the bacterial in-vitro susceptibility to different antibiotics towards gram-negative bacteria. Antibiotic and their potencies included were ampicillin (10µg), cotrimoxazole(23.75µg), nalidixic acid (30µg), nitrofurantoin (300µg), ceftriaxone (30µg), ciprofloxacin (5µg), gentamicin (10µg), ceftazidime (30µg), imipenem (10µg), mecillinam (25µg), amikacin (30µg), cefixime (5µg), and netilmicin (30µg).

Bacterial inoculums

A suspension of test organisms was prepared using Muller-Hinton broth by adjusting the turbidity of the broth with normal saline to match the equivalent turbidity standard of McFarland (0.5 standards) and incubated for 2 hours. A sterile cotton swab was dipped into suspension and excess fluid was removed by pushing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was then lawned evenly over the entire surface of a Muller-Hinton agar plate to obtain uniform inoculums. Antibiotic discs were applied aseptically to the surface of the inoculated plates at appropriate spatial arrangement by means of sterile needle within a distance of 5mm. The plates were then inverted and incubated at 37˚ C for 24 hours. After incubation, the plates were examined and the diameters of the zones of complete inhibition were measured in mm. The zone diameters for individual anti-microbial agents were translated into susceptible, intermediate and resistant categories according to Clinical Laboratory Standard Institute (CLSI) guideline.

RESULTS

A total of 2,379 (24.14%) gram-negative isolates were yielded from 9,854 urine cultures. The detail background of the gram-negative isolates was included in Table-1. However,out of 2,379 isolates, the majority were *Escherichia coli* isolates (1592; 66.92%) followed *Klebsiella* spp. (320; 13.45%) and the equal numbers of *Proteus* spp. and *Pseudomonas* spp. (161; 6.77%). Less frequently isolated pathogens were *Acinetobacter, Enterobacter, Citrobacter* etc whose isolation rate in varied between 3.19 to 0.04%. Antibiotic susceptibility pattern of Gram-negative bacteria isolated from this study was shown in Table-2. Results showed that the majority (99%) of the isolates (*Escherichiae coli, Klebsiella, Proteus, Enterobacter, Pseudomonas, Acinetobacter and Enterobacter* spp.) were highly resistant to ampicillin, mecillinam, nalidixic acid, cotrimoxazole, nitrofurantoin, gentamicin, ciprofloxacin, cefixime, ceftazidime and ceftriaxone. Only a minor number of isolates (1%) were moderately resistance to imipenem, netilmicin and amikacin.

Table *1*. Frequency of bacteria causing urinary tract infection

Bacteria	No of isolates	Frequency (%)
Escherichia coli	1592	66.92
Klebsiella spp	320	13.45
Proteus spp	161	6.77
Pseudomonas spp	161	6.77
Acinetobacter spp	76	3.19
Enterobacter spp	49	2.06
Citrobacter spp	04	0.17
Moraxells spp	04	0.17
Salmonella typhi	03	0.13
Providencia spp	03	0.13
Serratia spp	01	0.04
Other minor pathogens	05	0.21

E. coli showed highest resistance to ampicillin (86.09%), nalidixic acid (83.28%), ceftazidime (73.04%) and cotrimoxazole (67.83%). *Klebsiella* showed resistance to ampicillin (83.33%), mecillinam 70.59%), nalidixic acid (61.54), cotrimoxazole (59.80%). The *Proteus* had mecillinam resistance (86.21%), ampicillin (84.17%), nitrofurantoin 79.35%); while *Pseudomonas* had mecillinam resistance (93.55%), nitrofurantoin (87.50%), ampicillin (82.86%), nalidixic acid (81.82%). The *Enterobacter* had ampicillin resistance (97.87%), ceftazidime (85.71%), while *Acinetobacter* had ceftazidime resistance (94.44%), nitrofurantoin (93.33%) and mecillinam (63.64%). *Citrobacter* had ceftazidime resistance (100%), and ampicillin (66.67%). The *Providencia* had 100% resistance to ampicillin, netilmicin, amikacin and imipenem and 66.77% resistance to cotrimoxazole, mecillinam and nitrofurantoin.

Table 2: Antibiotic resistance pattern of individual isolates*

Bacterial spp.	Antibiotic Used												
	Ak	Amp	Caz	Cip	Cot	Cro	Gen	Imp	Mec	Nal	Net	Nit	CFM
Escherichia coli	16.45	86.09	73.04	67.83	67.6	49.75	37.44	1.05	43.01	83.28	29.06	12.19	50.63
***Klebsiella* spp**	8.5	83.33	47.62	54.43	59.8	51.9	39.87	7.92	70.59	61.54	18.18	53.94	53.4
***Proteus* spp**	7.4	84.17	27.45	16.56	52.3	10.83	13.55	5.88	86.21	55	17.31	79.35	23.85
***Pseudomonas* spp**	30.66	82.86	46.66	49.68	52.3	53.85	48.72	18.88	93.55	81.82	30.03	87.5	69.34
***Enterobacter* spp**	25	97.87	85.71	26.53	31.3	31.25	18.75	0	40.91	48.94	33.33	56.25	29.55
***Acinetobacter* spp**	65.22	56.77	94.44	29.33	37.3	29.33	54.32	13.64	63.64	38.36	52.63	93.33	52.11
***Citrobacter* spp**	0	66.67	100	50	50	25	50	0	50	50	0	25	25
***Moraxella* spp**		0		0	50	0						0	0
Typhi gr Salm	0	50		0	67	0	0		33.33	100		0	33.33
***Serratia* spp**		100		0	0	0	0		100	0		100	0
***Providential* spp**	100	100	100	33.33	66.7	0	33.33	100	66.67	33.33	100	66.67	33.33
Non-typhi Sal		0		0	0	0	0		0	0		0	0

Abbreviations: Ak, amikacin, AMP, ampicillin, Caz, Ceftazidine, Cip, ciprofloxacin, Cot, cotrimoxazole, Cro, Ceftriaxone, Gen, gentamicin, Imp, imipenem, Mec, mecillinam, Nal, nalidixic acid, Net, netilmicin, Nit, nitrofurantoin, CFM, cefixime. * Data are given as percentage of resistance.

The urinary isolates did not show any distinct seasonality (Figure 1). However, majority (66.46%) of the isolates were identified in the hot, moist and rainy season (April through October) and the rest (33.54%) were isolated in the dry winter season (November through March).

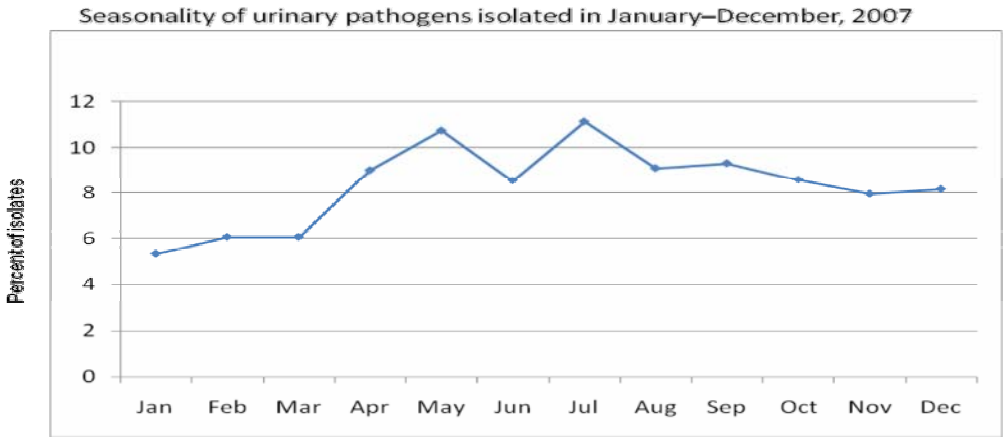

Figure-1: Seasonality of urinary pathogens in January-December, 2007

DISCUSSION

Urinary tract infection results from urethral entry of enteric group of organisms into the urethra, bladder and kidneys. Recurrent and complicated urinary tract infections occur in patients with functionally, metabolically, or anatomically abnormal urinary tract. They are often associated with significant mortality and morbidity (Jarvis, 1996). A higher prevalence of UTI was reported in women than men, principally owing to anatomic and physical factors (Kumar et al., 2006*;* Khan et al., 2004*).*Even in developed country the average stay in hospital with UTI is on average 2.4 days with an associated additional cost at least between $500 and $700 (Jarvis, 1996). However, such cost estimated has not been found in our region. The present study mainly focuses the gram-negative isolates associated UTI in a segment of time for the period between January and December, 2007 and its seasonal pattern.

Antibiotic resistance is a major clinical problem in treating infections caused by microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country (Gales et al., 2001*).* Overall, isolates from Latin American countries show the high resistance rates to all antimicrobial agents followed by Asian-Pacific isolates and European strains; while the strains from Canada exhibit the lowest resistance pattern (Gales et al., 2001*).* In this study, it was accounted that majority of the organisms isolated were the members of Enterobacteriaceae family. This is consistent with reports where *E. coli* was the predominant pathogen isolated from patients with community acquired UTIs (Manges et al., 2006; Philippon et al., 1996*). E. coli* is the most important pathogen for uncomplicated and complicated UTI and has shown a slow but steady increase in resistance to several antibiotics over the past decade (Kahlmeter, 2003; Gales et al., 2002*;* Turnidge et al., 2002*)***.** In this study, the range of resistance of *E. coli* to ampicillin was 86.09% and that for cotrimoxazole was 67.83%. This resistance rates were higher than those in studies from developed countries; in the US (Vromen et al., 1999) the rates are 39.1 and 18.6%, and in the EU (Kahlmeter, 2003) they are 29.8 and 14.1%, respectively. On the other hand, similar rates have been found in other countries, such as Senegal (77%, 55%) (Dromigny et. al., 2002); Spain (65%, 33%) (Daza et al., 2001); Taiwan (80%, 56%) (Lau et al., 2004); and Israel (66%, 33%) (Colodner et al., 2001). Therefore these drugs should no longer be prescribed as initial empirical therapy in our region. The susceptibility pattern of *E. coli* to imipenem and amikacin was very significant (98.95%, and 83.55%, respectively). There was also a significant susceptibility of *E. coli* to nitrofurantoin (87.81%), netilmicin (70.94%), and gentamicin (62.56%). The resistance rate of *E. coli* to extended spectrum cephalosporins (ceftazidime, ceftriaxone and cefixime) ranges from 60% to 75%, which is contrary to other community-acquired UTI studies in Europe, Israel and the US (Colodner et al., 2001 ; Mudur, 2000 ; Svetlansky et al., 2001 ; Hillier et al., 2002 ; Farrel et al., 2003). However, *Klebsiella pneumoniae* are rarely encountered in cases of community-acquired UTI (Kumar et al., 2006*;* Manges et al., 2006; Kunin, 1994) . However, the present study indicated a significant association of of *Klebsiella* spp. (13.45%) as UTI's pathogens, but due to data limitation it could not be ascertained to either community or hospital acquired infection. In this present study, *Klebsiella* spp. were shown high

sensitivity to amikacin (91.5%), imipenem (92.08%), and netilmicin (81.82%). The resistance rate to cephalosporins and fluoroquinolones (nalidixic acid, nitrofurantoin, ciprofloxacin) among *Klebsiella* spp. were moderate (40-60%). The isolates were highly resistant to ampicillin (83.33%) and mecillinam (70.59%). Isolates of *Pseudomonas* spp. were shown very high resistance to most antimicrobial agents. The rate of resistance to ampicillin, cotrimoxazole, mecillinam, nalidixic acid, nitrofurantoin and cefixime were 82.86%, 83.59%, 93.55%, 81.82%, 87.5%, and 84.38%, respectively. The resistance rate to ceftazidime, ciprofloxacin, ceftriaxone and gentamicin were moderate (49.46%, 49.68%. 53.85%, and 48.72%, respectively). Among antibiotics used in this investigation, *Pseudomonas* spp. isolates were very sensitive to imipenem (81.12%). Resistance rate to ampicillin, mecillinam and nitrofurantoin among *Proteus* spp. isolates was very high (84.17%, 86.21%, and 79.35%, respectively).These isolates were shown sensitivity to most of the antibiotics used in this study (ciprofloxacin, ceftriaxone, ceftazidime, gentamicin, netilmicin, and cefixime). They were shown high sensitivity rate to amikacin (92.96%) and imipenem (94.12%).

This study also showed that *Enterobacter* spp. isolates were very resistant to ampicillin and ceftazidime (97.87%, and 85.71%, respectively). The rate of sensitivity to imipenem among these isolates was 100% and above 65% isolates were sensitive to ciprofloxacin, ceftriaxone, cotrimoxazole, gentamicin, netilmicin, cefixime and amikacin (73.47%, 68.75%, 68.75%, 81.25%, 75%, and 70.45%, respectively). The resistance rate to mecillinam, nalidixic acid and nitrofurantoin were moderate (40-56%).

All the *Acinetobacter* spp. Isolates were shown high sensitivity to imipenem, ciprofloxacin, and ceftriaxone (86.66%, 70.67%, and 70.675%, respectively).The resistance rate of these isolates to ceftazidime and nitrofurantoin were high (94.44%, and 93.33%, respectively).

In this study, all the isolates were found to be sensitive to imipenem, netilmicin and amikacin. Imipenem exerts a bactericidal action by inhibiting cell wall synthesis in aerobic and anaerobic gram-negative bacteria, including most strains which are beta-lactamase producer. Inhibition of cell-wall synthesis is achieved in gram-negative bacteria by the binding of imipenem to penicillin binding proteins (PBPs). In the case of *E. coli* and selected strains of *Pseudomonas aeruginosa*, imipenem has been shown to have highest affinity for PBP-2, PBP-1a and PBP-1b, with lower activity against PBP-3. The preferential binding of imipenem on PBP-2 and PBP-1b leads to direct conversion of the individual cell to a spheroplast resulting in rapid lysis and cell death without filament formation. Imipenem is highly stable against β-lactamase and has an unusual property of causing a post antibiotic effect on gram-negative bacteria (Neu, 1992). Due to its small molecular size it can over come the poor permeability of β-lactams for Pseudomonas by efficient penetration through the porin, OMPD (El Amin et al., 2005).

According to this study, all the isolates were shown differences in resistance between ampicillin, ceftazidime, ciprofloxacin, ceftriaxone, ceftazidime, cotrimoxazole, nitrofurantoin, nalidixic acid and gentamicin. Increasing resistance rate to all antibiotics used in this study with the exception of imipenem and amikacin may be explained as uncontrolled consumption of these antibiotics during the past decade in our region (Mudur et al., 2000; Svetlansky et al., 2001; Hillier et al., 2002).

Finally it can be concluded that the present study demonstrated that the uropathogens isolated have shown various trends in isolation rates and resistances to various classes of antimicrobial agents. In addition, multidrug-resistant *E. coli, Klebsiella* spp., *Pseudomonas* spp. can be expected to cause serious treatment problems. Therefore, it is essential to use the most appropriate antimicrobial management based on this study, in order to accurately assess the severity of patient's UTI, and to recognize the limits of conservative treatments.

CONCLUSION

It is quite alarming to note that almost all of the isolates included in this study were found resistant to multiple drugs (four or more antibiotics). Antibiotic resistance has been emerged as a major problem in the management of hospitalized patients as well as those with chronic conditions and adds considerably to health care cost. It has been considered a threat to the public health problem worldwide. This important issue is to be addressed by the policy makers to formulate a strict antibiotics prescription policy in our country, which would aware the practioners and care giver to make a prudent use antibiotics.

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