ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Issue 3, 2013

ISSN - 0974-2441

Research Article

EXTENDED EXPECTRUM BETALACTAMASES IN UROPATHOGEN

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Received: 11 May 2013, Revised and Accepted: 4 June 2013

ABSTRACT

Background: Urinary tract is the second most common site of bacterial infections in humans. Gram-negative bacteria (GNB) that possess extended spectrum β-lactamases (ESBLs) genes have proven to be a concern to the medical community because of their high resistance rates to 3rd generation cephalosporins. ESBLs production has been associated with higher morbidity and mortality rates and has been reported in *Escherichia coli* and *Klebsiella pneumoniae*. ESBLs are emerging worldwide, making rapid and adequate ESBLs detection crucial for the choice of correct antimicrobial therapy. The aim of the study is to determine the profile of uropathogen, their antibiogram and detection of ESBLs producing strains. Materials and methods: Isolation, identification and antimicrobial susceptibility of organism was done by standard microbiological procedure. For Gram-negative bacilli ESBLs production was detected by DDST as per CLSI guidelines. Results: Three hundred urine specimens were studied. Significant bacteriuria was present in 35% of specimen. The most common pathogens isolated were *Escherichia coli* 52.4%. The resistance pattern of uropathogens was for amikacin (AK) 19.04%, nitrofurantoin (NIT) 40%. We found 55% Gram-negative uropathogen harbored the ESBLs. Majority of ESBLs seen in *Klebsiella pneumoniae* 60% and *Escherichia coli* 55%. The ESBLs producing *Escherichia coli* were highly susceptible to Imipenum, 90.90% and Meropenem, 94.45%. Conclusions: *Escherichia coli* are the commonest cause of UTI. Majority of UTI are mono-microbial. Screening of multidrug resistant bacteria especially GNB poses considerable therapeutic challenges in critical care patients because of the production of ESBLs. Amikacin and Nitrofurantoin are the most suitable antibiotics for treatment.

Keywords: UTI, Uropathogen, Antimicrobial Resistance, ESBL

INTRODUCTION

Urinary tract infection (UTI) is the most common infection experienced by humans after respiratory and gastro-intestinal infections, and also the most common cause of both community-acquired and nosocomial infections for patients admitted to hospitals. UTI may be defined as a condition in which bacteria are established and multiplying within the urinary tract[1]. UTI is the leading cause of morbidity and health care expenditures in persons of all the ages. The long range consequences of ignoring UTI can leads to kidney failure, septicaemia, bacterial endocarditis, prostatitis and infertility[2]. Approximately 1 in 3 women will require antimicrobial treatment for a Urinary Tract Infection (UTI) before age 24, and 40% to 50% of women will have a UTI during their lifetime. UTIs in male patients are considered complicated. Worldwide, about 150 million people are diagnosed with UTI each year. Uncomplicated UTIs occur in sexually active healthy female patients with structurally and functionally normal urinary tracts. More than 95% of urinary tract infections are caused by a single bacterial species. *Escherichia coli* is the most frequent infecting organism in acute infection[3].

Bacteriological examination of the urine is the major aid to the diagnosis of infection. Culture technique is employed to detect bacteria in urine. Many quantitative and semi-quantitative culturing methods are available. The organisms are identified and their susceptibility to antimicrobial agent is determined[4]. The uropathogen resistant to commonly used antimicrobials has long been recognised. It is also known that overuse and misuse of antimicrobial contributes to the emergence of resistance and its amplification. In Gram-negative pathogens, β-lactamase production remains the most important contributing factor for β-lactam resistance[5].

β-lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds. One group of β-lactamases, extended-spectrum β lactamases (ESBLs), have the ability to hydrolyse and cause resistance to various types of the newer β-lactam antibiotics, including the extended-spectrum (or third-generation) cephalosporins (cefotaxime, ceftriaxone and ceftazidime) and monobactams (aztreonam), but not the cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem, meropenem and ertapenem)[6]. The methods for detection of ESBLs can be done by phenotypic methods that use non-molecular techniques, which detect the ability of the ESBL enzymes to hydrolyse different cephalosporins; and genotypic methods which use molecular techniques to detect the gene responsible for the production of the ESBL[7]. Organisms that produce ESBLs remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control[8]. Resistance to older generations of antimicrobials is high in most areas and resistance to most new antimicrobials has appeared in community acquired uropathogens[5].

Inadequate data on local resistance patterns can lead to wrong choices of antimicrobial therapy and increase development of resistance. There is very little information on the rate of development of resistance in uropathogens in different areas and the groups of drugs that can be used for interventions. Therefore an attempt to establish a surveillance system for monitoring antimicrobial resistance (AMR) in uropathogens with ESBL production in order to compare it with antimicrobial used in Mysore, India is undertaken.

MATERIALS AND METHODS

This is prospective study conducted in Department of Microbiology, Yuvaraja's College (Autonomous), Mysore, India; from December 2011 to November 2012. Urine specimens obtained from patients attending to K. R. hospital Mysore, clinically diagnosed as UTI and submitted to Microbiology Department for bacteriological culture and sensitivity constitute the subject for study. Informed consent was taken from each subject included in the study. The study was ethically approved by Human Ethical Committee, University of Mysore.

Urine culture of the un-centrifuged urine was done by semi-quantitative method using standard wire loop. Sterilized inoculation loop was dipped in urine pot at 90°, a loopful of urine holding 0.001 ml was taken. It was inoculated on cystein lactose electrolytes deficient agar (CLED) media, 5% sheep blood agar and MacConkey agar respectively. The inoculated plates were incubated aerobically

in bacteriological incubator set at 37°C for 24-48 h[9,10]. Identification was done on the basis of colony morphology, grams stain, catalase test, oxidase test and standard biochemical tests that include triple sugar iron agar (TSI) media, Simmons' citrate agar media, sulphide indole motility (SIM) media and Christensens' urease medium [11].

Antibiotic sensitivity test (AST)

It was done by Kirby-Bauer disk diffusion test method on Muller-Hinton agar (MHA) plate and interpreted according to CLSI guidelines, where the following antibiotics (from Himedia, Mumbai, India) were tested: Amikacin (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Cotrimoxazole (32.7 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Norfloxacin (10 µg) and Gentamicin (10 µg). *Escherichia coli* ATCC 25922 were used as control and tested along with the test strains daily as described for *Enterobacteriaceae*[12]. Imipenem (10 µg) and Meropenem (10 µg) disc were used for multiple drug resistance (MDR) ESBL producing isolates of *Escherichia coli* and *Klebsiella pneumoniae*.

Detection of ESBLs producing strains by the double disc synergy test (DDST)

All uropathogens showing resistance to one or more third generation cephalosporins (3GCs) were tested for ESBL production by the double disc synergy test (DDST) using cefotaxime (30µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg) and ceftazidime + clavulanic acid (30/10 µg). A ≥ 5mm increase in diameter of the inhibition zone of the cephalosporin+clavulanate disc when compared to the cephalosporin disc alone were interpreted as phenotypic evidence of ESBL production. *Klebsiella pneumoniae* ATCC 700603 was used as positive control and *E. coli* ATCC 25922 as negative control[13].

RESULTS

Three hundred urine specimens were studied. Significant bacteriuria was present in 35% of specimen (Table 1). Majority (95.24%) of UTI were due to Gram-negative bacilli and remaining (4.76%) Gram-positive cocci. The most common pathogens isolated were *Escherichia coli* (52.4%), followed by *Klebsiella pneumoneae* (14.3%), *Pseudomonas aeruginosa* (9.5 %), *Acinetobacter anitratus* (4.76%), *Proteus mirabilis* (4.76%), *and Enterobacter species* (4.76%), *Staphylococcus aureus* (1.9 %), and *Enterococcus faecalis* (2.85 %). The mean Resistance pattern of uropathogens was for amikacin (AK)19.04%, nitrofurantoin (NIT) 40%, cefotaxime (CTX) 45.71%, ceftriaxone (CTR) 49.52%, ceftazidime (CAZ) 48.57%, ciprofloxacin (CIP) 54.28%, cotrimoxazole (COT) 80.95%, nalidixic acid (NA) 20 %, norfloxacilin (NIT) 66.67% and gentamicin (GEN) 61.9% (Table 2). We found 55% Gram-negative uropathogen harbored the ESBLs. Majority of ESBLs seen in Klebsiella 60% and *Escherichia coli* 54.54% (Table 3). The sensitivity of ESBLs positive isolates of *Escherichia coli* were (90.90%) to Imipenem and 94.45% to Meropenem. Similarly the sensitivity of ESBLs positive isolates of *Klebsiella pneumonia* were to 86.66% to Imipenem and 93.33% to Meropenem.

Table 1: The age and sex distribution of patient of significant bacteriuria and insignificant growth or sterile.

Age in Years	Male					Female				
	Significant bacteriuria		Insignificant growth/sterile		Total No.	Significant bacteriuria		Insignificant growth/sterile		Total No.
	No.	(%)	No.	%	-	No.	%	No.	%	-
0-10	5	33.33	10	66.67	**15**	6	33.33	12	66.67	**18**
11-20	4	20.00	16	80.00	**20**	15	37.50	25	62.50	**40**
21-30	1	9.10	10	90.90	**11**	30	54.55	25	45.45	**55**
31-40	1	10.00	9	90.00	**10**	15	33.33	30	66.67	**45**
41-50	1	9.10	10	90.90	**11**	5	33.33	10	66.67	**15**
51-60	6	37.50	10	62.50	**16**	3	33.33	6	66.67	**09**
61-70	7	41.17	10	58.83	**17**	3	33.33	6	66.67	**09**
71-80	-	-	-	-	-	3	33.33	6	66.67	**09**
Total	**25**	**25**	**75**	**75**	**100**	**80**	**40**	**120**	**60**	**200**

Table 2: Antimicrobial resistance pattern of isolated uropathogens.

Bacteria	AK %	CTR %	CTX %	CAZ %	CIP %	COT %	NA %	NIT %	NX %	GEN %
Escherichia coli	16.37	50.91	49.1	49.10	58.18	78.81	80	38.82	60	50.91
Klebsiella pneumoniae	20	53.44	46.7	60	60	80	20	40	60	53.44
Pseudomonas aeruginosa	30	50	50	40	60	90	80	40	70	60
Enterobacter species	20	40	40	40	40	80	80	40	80	40
Proteus mirabilis	20	60	40	40	40	80	80	40	80	60
Citrobacter species	20	40	40	40	40	80	60	40	80	80
Acinetobacter anitratus	20	60	40	60	40	80	80	40	80	80
Staphylococcus aureus	-	-	-	-	50	-	-	50	-	50
Enterococcus faecalis	66.67	66.67	66.67	-	-	-	-	-	-	-
Total	**19.04**	**49.52**	**45.71**	**48.57**	**54.28**	**80.95**	**80**	**40**	**66.67**	**61.9**

Table 3: Prevalance of ESBL Production among Gram-negative Uropathogen

Bacteria	Total No.	ESBL Positive No.	%	ESBL Negative No.	%
Escherichia coli	55	30	54.54	25	45.46
Klebsiella pneumoniae	15	9	60	6	40
Pseudomonas aeruginosa	10	5	50	5	50
Enterobacter species	5	3	60	2	40
Proteus mirabilis	5	2	40	3	60
Citrobacter species	5	3	60	2	40
Acinetobacter anitratus	5	3	60	2	40
Total	**100**	**55**	**55**	**45**	**45**

DISCUSSION

In the present study, significant bacteriuria is seen in 35% of case, which is lower than 38%[14]. Members of the Enterobacteriaceae are the most common organisms isolated from uncomplicated UTIs[15]. Majority 95.24% of UTI was due to Gram-negative bacilli (GNB) and remaining 4.76% by Gram-positive cocci (GPC); the similar findings were reported of GNB 95.4% and GPC 4.6[16].

The most common pathogens isolated were *Escherichia coli* (52.4%), which is almost similar with study by Patel *et al* 53.38%[17] followed by *Klebsiella pneumoneae* (14.3%), which is in between the result of 14% and 14.9%[15,18]. *Pseudomonas aeruginosa* 9.5%, which is almost similar to 9.8%[19]. *Acinetobacter anitratus* 4.76%, almost similar to 4.2%[18]. *Proteus mirabilis* 4.76%, which is inbetween 5.5% and 4.2%[20,21]. *Enterobacter* species 4.76%, which is slightly lower than 5.5%[20]. *Citrobacter* species 4.76%, which is inbetween 4.5% and 5%[16,22]. *Staphylococcus aureus* 1.9 % and *Enterococcus faecalis* 2.85%. Almost similar finding reported were of 2.9%[23].

The relationship between antibiotic use and resistance is complex. The use of broad-spectrum antibiotic agents as a substitute for precise diagnostics or to enhance the likelihood of therapeutic success increases the rate of selection of resistant bacteria. Factors influencing antibiotic consumption include cultural conceptions, patient demands, diagnostic uncertainty, and the level of training among health care staff and pharmacists. High levels of antimicrobial resistance in uropathogens were also reported with similar rates in acute of resistance occurring to antibiotics commonly used in both out-patients and in-patients (a reflection of high community use of antibiotics). Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control, and information will directly affect selection of empiric therapy for UTI. However, the initial choice of empiric antimicrobial therapy should be based on Gram stain and urine culture and should integrate local sensitivity patterns of the infecting organism[15].

In the present study, the mean resistance pattern of uropathogens were like this; a low resistance of 19.04% to amikacin, and 40% to nitrofurantoin which is comparable to 24.4% and 34.3% respectively[20]. A moderate resistance to cefotaxime 45.71%, ceftriaxone 49.52%, ceftazidime 48.57% and ciprofloxacin 54.28% were seen in the present study. In present study 61.9% of uropathogen were resistant to gentamicin, which is higher than 57.1%[23]. A high resistance to cotrimoxazole 80.95% and nalidixic acid 80% in our study was comparable with 87.3%[24].

We found 55% Gram-negative uropathogen harbored the ESBLs. Majority of ESBLs seen in *Klebsiella* species 60% and *Escherichia coli* 54.54%. The sensitivity of ESBLs positive isolates of *Escherichia coli* and *Klebsiella pneumonia* were very high 86.66% - 94.45% to imipenem and meropenem, which is comparable with 94.5% and 95.7%[25,23].

CONCLUSION

Escherichia coli are the commonest cause of UTI. Majority of UTI are mono-microbial. We found 55% Gram-negative uropathogen harbored the ESBLs, where majority of ESBLs were seen in *Klebsiella pneumoniae* 60% and *Escherichia coli* 55%. Screening of multidrug resistant bacteria especially GNB possess considerable therapeutic challenges in critical care patients because of the production of ESBLs. Amikacin and nitrofurantoin are the most suitable antibiotics for treatment.

Acknowledgment: We thank the K.R. Hospital, Mysore for allowing us to collect the specimen for research work.

Conflict of interest: None

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