ORIGINAL ARTICLE

A Study on Prevalence and Antimicrobial resistance pattern of Community acquired gram negative uropathogens at tertiary care teaching hospital, Ahmedabad.

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ABSTRACT

BACKGROUND AND OBJECTIVE: Urinary tract infections (UTI) are the common infections in outpatients as well as in hospitalized patients.Antimicrobial resistance remains the major threat due to broad spectrum usage of antimicrobials in community practice. The present study is aimed to determine the prevalence and antibiogramof gram negative uropathogensca using UTI in outpatients of tertiary care teaching hospital, Ahmedabad. **METHODS:**A total of 2354 urine samples from suspected UTI patients attending to the outpatient clinics and emergency rooms were collected and processed in bacteriology laboratory.Antimicrobial susceptibility testing was performed by modified kirby-bauer disk diffusion method from obtained isolates. Isolates which are resistant to third generation cephalosporins and aztreonamwere tested for extended spectrum beta lactamase (ESBL) production by double disk synergy test. **RESULTS:**Among 2354 Urine specimens, Significant bacteriuria was seen in 560 (23.79%) specimens. Majority of isolates were from females (59.82%). The most common organisms isolated were Escherichia coli (60.89%), klebsiella spp. (14.11%), pseudomonas spp.(12.68%), followed by Proteus spp.(3.04%).More than 60% of resistance was seen against ampicillin, cephalosporins, cotrimoxazole and ciprofloxacin. More than 97% of the isolates were sensitive to imipenem,while 79.25% were sensitive to amikacin, and 70.25% to nitrofurantoin. Majority of ESBL production was detected in 29.11 % of Klebsiella spp.and26.39% of Escherichia coli isolates. **CONCLUSION:** E. coli was the predominant isolateduropathogen. Nitrofurantoin should be used as empirical therapy for community acquired UTIs.This study could prove to be useful for clinician to improve the empirictreatment.

Keywords: urinary tract infection, uropathogens, antimicrobial resistance, ESBL.

INTRODUCTION

Urinary tract infections (UTIs) are the most common bacterial infections in the community and in the health care settings. Catheter associated urinary tract infection in about two percent of catheterised patients even with adequate precautions. The rate goes up to 50% or more in patients with indwelling catheters [1]. UTI is the second most common infectious presentation after respiratory tract infections in community practice[2].It represents a spectrum of clinical entities, ranging from asymptomatic infection to acute pyelonephritis with sepsis.

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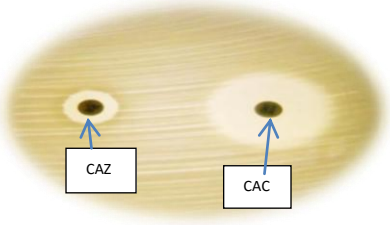
According to one estimation, more than 50% of women suffer with UTI once at some point in their lifetime and between 20% and 40% of women have recurrent episodes[3]. About 5 - 7% of pregnant women have been reported to have urinary infection without any symptoms. Uncomplicated UTI is defined as an infection in a structurally and neurologically normal urinary tract and Complicated UTI is defined as an infection in a urinary tract with structural or functional abnormalities, and also UTI in men and pregnant women[4]. Accurate and prompt diagnosis of UTI areimportant in shortening the disease course and so hospitalstay, cost and for preventing the ascent of the infection tothe upper urinary tract (pyelonephritis). The vast majority ofuncomplicated UTIs are caused by *Escherichia coli*, withother pathogens including *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus mirabilis*, Other*coliforms*,

Enterococci spp., Staphylcoccus spp. Candida infection most commonly seen in diabetic and immunocompromised patients[5]. Theinappropriate use of broad spectrum antimicrobial agents hasresulted in the development of antibiotic resistance,which has becomea major problemworldwidein recent time[3].Now a days, a major cause of drug resistance even in uropathogens of Community acquired UTI is due to Extended-spectrum β-lactamases (ESBL) production.ESBL producing isolates are resistant to all penicillins, third generation cephalosporins(cefotaxime, ceftriaxone and ceftazidime) and monobactam such as aztreonam but not to the cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem, meropenem and ertapenem)[6].Besides this, ESBL production is associated with co-resistance to many other group of antibiotics such asco-trimoxazole, aminoglycosides, and quinolones which are common non beta-lactam antibiotics used for treatment of complicated and uncomplicated UTI and all these resistance makes empirical treatment of these infections difficult evenin outpatient based clinics in the developing countries where no strict regular monitoring regarding the usage of broad spectrum antibiotics[7]. Bacteriological examination of the urine is the major aid in the diagnosis of UTI. Many quantitative and semi-quantitative methods for urine culture are available[3]. Isolated organisms are then identified and their susceptibility to antimicrobial agent is determined. Also as part of stewardship programme (to avoid wrong choice of antibiotic therapy), there is need for monitoring of area or hospital wise antimicrobial resistance pattern (AMR) of isolated uropathogenswhich are causing urinary tract infection to start empirical treatment [3]. Therefore, the present study was aimed to determine the most common uropathogens and their antimicrobial resistance pattern with ESBL productionfrom outpatients referred to tertiary care hospital which will help the clinicians to start proper and timely empirical treatment before availability of laboratory results.

MATERIALS AND METHODS

Thisretrospective study was conducted during time period from Jan 2015 to September 2015 in Tertiary Care teaching hospital, Ahmedabad, India. In this study, we have included 2354patients suspected with community UTI from Outpatient clinicand emergency room. Patients with H/O previous or recent hospitalizations were excluded from study. Various Data such as age, sex, presence of diabetes mellitus and history of urinary instrumentationwere recorded for each patient in the study.Patients were divided into four different age groups as New born to 19 years, 20-49 years, 50-79 years and more than 80 years for analysis of study. Each patients included in the study were asked to collect clean voided midstream urine in sterile screw-cap container. Preventation of contamination by normal vaginal, perineal and anterior urethral flora is the most imp consideration for collection of a clinically relevant urine sample. Therefor Female patients were asked to clean area with soap and water then hold labia apart and begin to void, after several ml have passed then to collect midstream urine sample. Male patients were asked to clean glans with soap and water, retract foreskin, after several ml have passed then to collect midstream urine sample. Urine specimens were transported to the microbiology laboratory within half hour of collection and immediately processed, or refrigerated at 4 C° for only few hours before processing. Direct Microscopic examination of wet mount preparation from uncentrifuged urine observed for significant pyuria, yeast cells and bacteria. Bacterial culture and identification:Uncentrifuged urine was mixed thoroughly then culture was carried out by semi-quantitative method using standard wire loop holding 0.001 ml of urine. Semi-quantitative culture method was choosed to differentiate between contamination and infection. It was inoculated on 5% sheep blood agar and MacConkey agar. The inoculated plates were incubated aerobically in incubator at

37°C for 24-48 hours. Organisms isolated in pure culture were identified by characteristic colony morphology, motility, gram staining reaction, catalase test, oxidase test and by pattern of biochemical profile using standard procedures. For quality control, ATCC E. coli 25922, ATCC E. coli 35218, ATCC K. pneumoniae 700603, ATCC P. aeruginosa 27853 were used for comparison of identification and susceptibility testing. A diagnosis of symptomatic UTI was made when the patient had at least one of the following signs or symptoms with no other recognized cause for it: fever ≥ 38.8˚C, urgency and frequency of micturition with associated discomfort or pain, dysuria,suprapubic tenderness and patient had a positive urine culture with significant bacteriuria($\geq 10^5$organisms /ml) according to Kass criteria, from properly aseptically collected midstream clean-catch urine sample. However significant bacteriuria is lacking in some cases of true UTI especially in symptomatic patients who have already taken antibiotics, in urine samples obtained by suprapubicaspiration or catheterised sample (10^5 to 10^2 organisms / ml). Antimicrobial susceptibility testing of Isolated Organisms: Antimicrobial susceptibility testing (AST) on Muller Hinton agar was performed for isolated bacterial organism by Modified Kirby-Bauer disk diffusion method.The results were interpreted by measuring inhibition zone as sensitive (S), intermediate sensitive (I) or resistant (R) for each isolate identified according to Clinical Laboratory Standards Institute (CLSI), antibiotic susceptibility module, M100-S24, 2014. All isolated gram negative uropathogens showing resistance to one or more third generation cephalosporinswere tested for ESBL production by the double disk synergy test (DDST). An enhanced zone of inhibition with a difference of 5 mm around the ceftazidime - clavulanic acid disk (30/10 µg) as compared to the ceftazidime disk (30µg) alone was interpreted as presence of ESBL. Klebsiellapneumoniae ATCC700603 was used as a positive control and E. coli ATCC 25922 was used as negative control.



(CAZ:Ceftazidime, CAC: Ceftazidime + Clavulanic acid)

This Study is approved by Professor and Head, Department of Microbiology, B.J.Medical College,Ahmedabad.

RESULTS

A total of 2354 urine culture &sensitivity reports were analyzed in the present study between January 2015 to September 2015. The predominant growths of pure microorganisms wereseen in 560 (23.79%) samples. The most common organisms isolated were Escherichia coli, klebsiella spp., pseudomonas spp.followed by Proteus spp. (These represented 60.89%, 341; 14.11%, 79; 12.68%,71 and 3.04%, 17 of isolates respectively).

Table 1: Prevelance of isolated uropathogens in the present study

No.	Isolated Organisms	No. of Isolates	Percentage
1	Escherischia coli	341	60.89%
2	Klebsiella spp.	79	14.11%
3	Pseudomonas spp.	71	12.68%
4	Proteus mirabilis	14	2.5%
5	Enterococcus spp.	13	2.32%
6	Acinetobacter spp.	13	2.32%
7	Providentia spp.	07	1.25%
8	Staphylococcus spp.	07	1.25%
9	Candida spp.	06	1.07%
10	Morganella spp.	05	0.89%
11	Proteus vulgaris	03	0.54%
12	Citrobacterkoseri	01	0.18%
	Total	560	100

Table 2: Age distribution of UTI

ge group years)	lo. of uspected atients	olated ropathogens	ercentage from otal isolated tropathogens
– 19	01	47	6.25
0 – 49	053	18	8.93
0 – 79	66	79	1.63
80	4	6	.86

This shows UTI was more prevalent in the age group of 20 – 49 years followed by elder age group.

Table 3: Sex distribution of UTI

Gender	No. of isolated uropathogens
Male	225 (40.18%)
Female	335 (59.82%)

Above table shows, among 560 samples were analyzed, there is high vulnerability of female stewards UTI. About 12 species of microorganisms were identified that offered resistance to different antimicrobials in the following pattern.

Table 4: Antibiotic resistance patternof isolatedGram Negative uropathogens

Antibiotic	E.coli n=341(%)	Klebsiell n=79 (%)	Psedomonasspp, n=71 (%)	Other GNB n=43(%)
Ampicillin	74.78	*	ND	67.44
Cefuroxime	75.95	70.89	ND	60.47
Cefoperazone	63.99	67.09	65.05	25.58
Ceftazidime	61.58	62.03	59.15	30.23
Cefepime	25.22	32.91	35.21	2.33
Aztreonam	33.72	48.10	29.58	46.51
Ampicillin-sulbactam	25.22	34.18	ND	16.28
Piperacillin-tazobactam	9.68	29.11	18.31	13.95
Cotrimoxazole	61.87	62.03	ND	48.84
Nitrofurantoin	20.53	36.71	26.76	34.88
Gentamycin	37.83	45.57	52.11	30.23
Amikacin	11.14	32.91	15.49	13.95
Ciprofolxacin	64.52	59.49	69.01	41.86
Levofloxacin	33.14	45.57	61.97	25.58
Imipenem	0.88	15.19	2.82	0
Polymyxin B	0	0	0	32.56

(ND- testing not done, *- Inherently resistance) Above antimicrobial resistance pattern in most common uropathogens revealed that Enterobacteriaceae family showed predominant resistance against ampicillin, 2nd and 3rd generation of cephalosporins (cefuroxime, cefoperazone andceftazidime), ciprofloxacinand gentamicin. Pseudomonas spp, which was 3rd most commonly isolated organism in present study revealed more resistance to even Levofloxacin.

Table 5: ESBL production among isolated Gram Negative uropathogens

Isolated Uropathogens	Total	ESBL Positive	Percentage
E.coli	341	90	26.39%
Klebsiella spp.	79	23	29.11%
Pseudomonas spp.	71	11	15.49%
Proteus mirabilis	14	02	14.28%

Overall percentage of ESBL positivity from isolated gram negative uropathogens is 23.60 % (126/534) in the present study.

DISCUSSION

In the present study, significant bacteriuria is seen in 23.79 % of patients which is lower than some studies[3,8]and comparable with other studies [2,9]. The present study reveals the most common uropathogens and their resistance pattern from community acquired UTI. We found predominance of Gram-negative bacilli (95.36%) as the etiological agents of UTI,followed by Gram-positive cocci(3.57%) and the remaining 1.07% by candida spp which is comparable withstudy in maisur in which, 95.24% of UTI was due to Gram-negative bacilli (GNB) and remaining 4.76% by Gram-positive cocci (GPC)[3]. The most commonly identified uropathogens chiefly belonged to the family of Enterobacteriaceae (E.coli andKlebsiella spp.) followed by Pseudomonas spp. (Table- 1) which is comparable with other studies[10,11]. The age wise data was reviewed to determine the prevalence of UTI in different age groups. This study shows that age people 20-49 years was more prone to UTI than any other age groupsprobably because of sexually active people more in age group of 20-49 years. This result is in accordance with other studies [7,12,13]. Prevalence is also more in elderly 50-79 years age group same as in study of Rama biswas[14], due to advancing age, poor immune response, prostate enlargement and possibility of neurogenic bladder. Incidence is much less in age group of > 80 years due to only 34 patients of this age group were enrolled in the present study. *E.coli* was the most commonly isolated uropathogen irrespective of any age group. The study also revealed that higher percentage of females (59.82%) were suffered from UTI as compared to males (40.18%), which is also comparable with other studies [8,15,16,17]. well known fact is that UTI occurs more commonly in women due to their anatomy (short urethra and proximity to anal opening). Men are usually less prone to UTI as compared to females, due to the longer course of the urethra and bacteriostatic properties of prostate secretions [18]. Antimicrobial resistance offered by different isolated uropathogens is one of the barricades that might hinder a successful treatment. More than 60% of isolated GNB uropathogens were resistance to ampicillin, 2nd and 3rd generation of cephalosporins, and ciprofloxacin. This could be due to overuse of these drugs and the high prevalence of ESBL production in these

organisms. Rate of resistance to different antimicrobials was noted more in Klebsiella spp. than E.coli, this high resistance by Klebsiella spp. was also seen in many other studies[10,15] . More than 80% of the isolates of E.coli were sensitive to amikacin, nitrofurantoin, piperacillin-tazobactam. The Klebsiellaspp. showed highest sensitivity to amikacin 67.09%, nitrofurantoin 63.29%, and levofloxacin 54.43%.Pseudomonas spp. showed good sensitivity to aztreonam 70.42%,piperacillin-tazobactam 81.69%, amikacin 84.51%. All Gram negative isolates were found to be sensitive to polymyxin B (100%) and to imipenem (97.01%). Extended spectrum beta-lactamases are enzymes capable of inactivation of most beta-lactam drugs.Overall ESBL production was detected in 23.59% of isolates which is comparable with other studies [17,19,20,21]. They showed high resistance mainly against cephalosporins which were extensively used for treatment of these infections. These ESBL producing uropathogenswere difficult to treat and so costlier[18].

CONCLUSION

Escherichia coli were the commonest cause of community acquired UTI while the majority of ESBLs were seen in isolated *Klebsiella spp*. High rates of resistance to antibiotics in uropathogens of community acquired UTIseen as a reflection of much higher use of broad spectrum antibiotics in the community hence it is essential to diagnose clinically and followed by confirmation with culture and sensitivity testing. So screening of antimicrobial resistance pattern helps to clinicians in making of antibiotic prescription policy to start proper and timely empirical therapy.

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