ANTIBIOTIC SUSCEPTIBILITY PATTERN OF COMMUNITY ACQUIRED UROPATHOGENS AT M. B. GOVT. HOSPITAL IN UDAIPUR, RAJASTHAN

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Received: 14/12/2015 ***Revised: 06/08/2016*** ***Accepted: 18/08/2016***

ABSTRACT

Objectives: To determine prevalence of uropathogenic bacteria and their antibiotic susceptibility pattern along with detection of extended spectrum β-lactamase (ESBL) production in *Escherichia coli* and *Klebsiella spp.* and methicillin resistance in *Staphylococcus aureus* (MRSA). **Materials and Methods:** 250 urine specimens received in microbiology lab from suspected UTI patients attending to the outpatient department were processed by standard techniques. ESBL production was determined by double disc synergy test and phenotypic confirmatory method. **Results:** Of 250 samples 20.8% showed significant bacteriuria, higher in females (61.5%). *Escherichia coli* was the predominant uropathogen (35%), followed by coagulase negative Staphylococcus (10.6%), *Klebsiella spp.* (10.2%). Gram negative bacilli showed maximum sensitivity to nitrofurantoin (68.9%), amikacin (62.2%) and gram positive cocci showed maximum sensitivity for nitrofurantoin (83.5%) followed by vancomycin (79.7%), gentamicin (75.9%). High resistance was seen against ampicillin, nalidixic acid, co-trimoxazole, cephalexin and norfloxacin. Prevalence of ESBL in *Escherichia coli* and *Klebsiella spp.* and MRSA was found to be 45.3%, 40% and 70% respectively. **Conclusions:** *Escherichia coli* was the predominant uropathogen for community acquired UTIs in Udaipur, Rajasthan. Uropathogens showed resistance to commonly used antibiotics with increasing trend of ESBL production and methicillin resistance. Nitrofurantoin, vancomycin should be used as empirical therapy. The susceptibility and resistance patterns of uropathogens should be considered before starting empirical treatment.

KEYWORDS: Antibiotic Susceptibility, *Escherichia coli,* ESBL, MRSA, *Staphylococcus aureus* Urinary Ttract Infections (UTIs)

INTRODUCTION:

Infection of urinary tract (UTI) is one of the most common diseases next to respiratory tract in community affecting peoples of all age worldwide. It is the most common type of body infection clinicians encounter in developing countries causing serious health problems to millions and displays an overall estimated incidence of 150 million UTIs/annum worldwide. (1)

Globally, UTIs cause not only a significant amount of morbidity, but also a significant financial burden.(2) UTIs became quite alarming as isolated uropathogens exhibits high percentage of resistance to almost all antibiotics. Among several UTI implicated microorganisms, bacteria are the major causatives accounting for more than 95% cases.(1) Most infections are caused by retrograde ascent of bacteria from the

faecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra.(3) The most common pathogenic organisms of UTI are *Escherichia coli, Staphylococcus saprophyticus* and less common organisms are *Proteus,*, *Klebsiella*, *Pseudomonas*, *Enterococci* and *Candida albicans*.(4)

Although UTIs occurs in both males and females, clinical studies suggest higher prevalence of UTIs in females. Uncomplicated UTIs in healthy females have an incidence rate of 50/1000/year.(5) An estimated 50% of women experience at least one episode of UTI in their life time and between 20-40% women have recurrent episodes.(6,7) About 20% of all UTIs occur in males.(8)

In almost all cases, there is a need to start treatment before the final microbiological results are available. Area specific monitoring studies which are aimed to gain knowledge about the type of pathogens which are responsible for UTIs and their sensitivity patterns may help the clinicians to choose the right empirical treatment. Knowledge on the antibiotic susceptibility patterns of the pathogens is important not only to provide an appropriate therapy, but also for the prevention of resistance amongst the microbes. (9) This study was done to obtain data on the sensitivity patterns of the major uropathogens from patients with community acquired UTIs, along with detection of ESBL production in *Escherichia coli* and *Klebsiella spp.* and methicillin resistance in *Staphylococcus aureus*.

MATERIAL AND METHODS

It is a prospective study, which was conducted in Department of Microbiology, R.N.T. medical college, Udaipur, on all the pathogens isolated from 250 urine samples of suspected patients of UTIs who attend the outpatient departments (OPDs) of M.B. Govt. Hospital, Udaipur in between January 2014 to September 2014.

Urine sampling and processing:

Freshly voided mid-stream urine samples from the suspected UTI patients collected in sterilized containers were received in microbiology lab. Culture of un-centrifuged urine was done by semi-quantitative method. It was inoculated in glucose broth, on nutrient agar, MacConkey agar and also on 5% blood agar plates if required. The inoculated plates were incubated aerobically at 37°C for 18-24 hours. After incubation plates were examined for pure growth and colony counts for determination of significant and insignificant bacteriuria. Identification of growth was done by colony morphology, grams staining and standard biochemical tests. A growth of $\geq 10^5$ colony forming units/ml was considered as significant bacteriuria. (10)

Determination of antimicrobial susceptibility:

Antimicrobial susceptibility of isolates was done by Kirby-Bauer disc diffusion method on Mueller Hinton agar plates by following CLSI guidelines. (11) Separate set of antibiotics were used for gram positive and gram negative organisms. Following antibiotics were tested: ampicillin 10µg, amoxicillin 30µg, amoxiclav 30µg, oxacillin 1µg, carbenicillin 100µg, amikacin 30µg, gentamicin 30µg, cephalexin 30µg, cefotaxime 30µg, ceftazidime 30µg, ceftriaxone 30µg, nalidixic acid 30µg, ciprofloxacin 30µg, norfloxacin 10µg, vancomycin 30µg, novobiocin 5µg, nitrofurantoin 300µg, tetracycline 30µg, doxycycline 30µg, cefoxitin 30µg, co-trimoxazole 1.25/23.75µg.The standard antibiotic discs (Himedia laboratories, Mumbai, India) available were used for this study. Control strains

used were *E. coli* ATCC 25922 and *S. aureus* ATCC 25923.

Determination of ESBL production in E. coli and Klebsiella spp.:

Only those isolates which were resistant to one or more of the 3 third generation cephalosporins were selected for study and they were processed for ESBL production. ESBL detection was carried out by two procedures-

1.Screening for ESBL producers - Double disc synergy test (DDST)

DDST was performed as a standard disc diffusion assay on Mueller Hinton agar. Discs containing 30µg of ceftazidime, ceftriaxone and cefotaxime each were placed 15mm apart (centre to centre) around a disc containing amoxicillin plus clavulanic acid (augmentin 20µg + 10µg) and incubated. Enhancement of inhibition zone of any one of the test antibiotics towards augmentin disc was regarded as presumptive ESBL production and subjected to phenotypic confirmatory test. (12)

2. Phenotypic confirmatory test:

This test was performed on Mueller Hinton agar by disc diffusion test as recommended by CLSI. Ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic Acid, 30/10 µg) disc, were applied at a distance of 30mm from centre to centre onto a plate of Mueller Hinton agar which was inoculated with the test strain and incubated. A greater than or equal to five mm increase in zone diameter of ceftazidime tested in combination with clavulanate versus its zone diameter when tested alone confirmed an ESBL producing organism. The control strains used were *Escherichia coli* ATCC 25922 as a non-ESBL producer and *Klebsiella pneumoniae* ATCC 700603 as an ESBL producer**.** **(**11)

Detection of Methcillin Resistant Staphylococcus aureus (MRSA):

Detection of MRSA was done with Oxacillin (1µg) disc by disc diffusion test. Zone diameter of ≤10 mm was considered as resistant, ≥13 mm as susceptible whereas 11-12 mm was considered as intermediate. (13)

RESULTS

During the study period 250 consecutive urine samples were processed from patients who presented in the outpatient department of M.B. Govt. Hospital, Udaipur. Of these 52(20.8%) showed significant growth of pathogen. Remaining 198 samples had either non significant growth or were sterile or contaminated.

Table 1 outlines the demographic profile of patients with community acquired UTIs. Patients were between the age of 8 month to 86 year. Significant bacteriuria was reported in 32 (61.53%) females and 20 (38.46%) males. In females incidence was higher in the reproductive age group of 21-40 years, while in males in the age group of 61-80 years. (Table 1)

Pathogens were isolated from 194(77.6%) urine samples. Among these samples gram negative bacilli were isolated from 120 (61.9%), gram positive cocci from 53 (27.3%) and in 21 (10.8%) urine samples both gram negative and gram positive cocci were present.

Escherichia coli was the most frequently isolated urinary pathogen (35%), followed by Coagulase negative *Staphylococcus* (10.6%), *Klebsiella spp.* (10.2%) and *Enterococcus faecalis* (8.9%). The isolation rates of other organisms are shown in table 2.

Antibiotic sensitivity pattern of gram negative bacilli revealed that maximum sensitivity was seen for nitrofurantoin (68.9%),

followed by amikacin (62.2%), gentamycin (51.8%) and maximum resistance was seen against ampicillin (96.3%), cephalexin (92.1%), amoxicillin (90.2%), nalidixic acid (85.4%), ceftazidime (79.8%), cotrimoxazole (77.4%).(Table 3)

Antibiotic sensitivity pattern of gram positive cocci revealed maximum sensitivity to nitrofurantoin (83.5%) followed by vancomycin (79.7%), gentamicin (75.9%), amikacin (73.4%). Maximum resistance was seen against nalidixic acid (93.7%), cotrimoxazole (84.8%), ceftazidime (84.8%) and ampicillin (79.7%). (Table 4)

Out of 86 *E. coli* isolates, 39 were ESBL producers, while in 25 *Klebsiella* isolates 10 were ESBL producers. Prevalence of ESBL was higher in *E. coli* (45.3%), in comparison to *Klebsiella spp.* (40%). (Figure 1 & 2) Out of 20 *S. aureus* isolates, 14 (70%) were MRSA. (Figure 3)

DISCUSSION

Effective management of patients suffering from bacterial UTIs commonly relays on the identification of organism that caused the disease and the selection of an effective antibiotic agent to the organism. (14)This study provides current information regarding the etiologic agents that cause community-acquired UTIs in the outpatient setting and their antimicrobial susceptibility patterns.

The result showed 20.8% patients with significant bacteriuria. This was in consistence with the findings of Smita et al (12) and Kasi Murugan et al.(1) The incidence of UTI with significant bacteriuria was higher in females (61.5%) than in males (38.5%) with male to female ratio 1:1.6. This was in consonance with other studies. (1, 12, 15)

Among the females the prevalence of significant bacteriuria was higher in the reproductive age group (28.12% in 21-30 year age group, 25% in 31-40 year age group and 15.6% in 41-50 year age group). This higher incidence rate is due to short female urethra and its proximity to anus. (16) In males prevalence was higher in the elder age group (20% in 61-70 year age group and 25% in 71-80 year age group).This is probably because with the advancing age, the incidence of UTIs increases in men due to prostate enlargement and neurogenic bladder. (12) Similar results were obtained by Smita et al (12) and Rajak et al. (17)

The majority of community acquired UTIs in Udaipur division were due to gram negative bacilli. It was because gram negative bacilli have more virulence factors in comparison to gram positive cocci. These findings were consistent with findings of Smita et al, (12) Kasi Murugan et al (1) and Gaurav dalela et al. (18) *E. coli* was the predominant pathogen being responsible for 35% of community acquired UTIs. This was in consistent with other studies. (12, 17, 3)

Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. These bacteria colonize the urogenital mucosa with adhesins, pili, fimbriae and P1-blood group phenotypic receptor. (12) In the present study Enterobacteriaceae bacteria accounted for 57.31% of all isolates, followed by gram positive cocci (32.11%) and non-fermenter gram negative bacteria (9.34%). The frequency was slightly varied from studies of Smita et al (12) and Rajak et al. (17)

Generally, uncomplicated UTIs are treated in the community with short courses of empirical therapy. In many cases, urine samples are only sent for microbiological evaluation

following treatment failure, recurrent or relapsing infection. Although the levels of resistance we observed amongst community isolates may therefore overestimate the true rate of resistance in the community. (18)

Ampicillin, amoxicillin, amoxyclav and co-trimoxazole showed high levels of resistance in both gram negative bacilli and gram positive cocci. This could be attributed to their wide usage for a variety of other indications.(12) Our findings thus suggest that empirical treatment with these drugs should no longer appropriate in this region.

Majority of isolates were found to be resistant to nalidixic acid, norfloxacin and ciprofloxacin. Fluoroquinolones have a wide variety of indications, permeate most body compartments and are ubiquitously prescribed, accounting for the emergence of their resistance. Norfloxacin, as it is an oral drug which is cost effective and has an easy dosing schedule, is commonly prescribed for the treatment of UTIs, in India as well as in other countries. (12) It showed a high resistance rate (73.1% in GNB and 79.7% in GPC) in our study, which reflects an increased quinolone resistance in our area, which was showed by other studies also. All gram positive and gram negative isolates also showed high level of resistance to all the cephalosporin tested (cephalexin, ceftazidime, ceftriaxone and cefotaxime), which indicates towards their inappropriate use and increased trends of β-lactamases production in the community.

Aminoglycosides (amikacin and gentamicin) have shown very low resistance trends against both gram negative bacilli and gram positive cocci. Aminoglycosides being injectables are used restrictively in the community-care settings and hence have shown better sensitivity rates. (12)

Nitrofurantoin has shown the least resistance for both gram negative bacilli and gram positive cocci in our area. Our findings are in consonance with other studies which have also demonstrated nitrofurantoin as an appropriate agent for first line treatment of community acquired UTIs. (12, 17, 18) As nitrofurantoin has no role in the treatment of other infections, it can be administered orally and is highly concentrated in urine; it may therefore be the most appropriate drug for empirical treatment in uncomplicated UTI.(12) Majority of gram positive cocci have shown sensitivity to vancomycin (79.7%). Doxycycline and tetracycline can also be used as oral therapy for UTIs due to gram positive cocci.

In our study ESBL production was higher in *E. coli* in comparison to *Klebsiella spp.* The findings were in consonance with Babypadmini et al (19) and Akram et al. (15)

The prevalence of MRSA was 70% in our study, which was higher than that reported by K B Anand et al (56%)(20), Gaurav dalela et al (42.4%)(18) and B. Sasirekha (27.5%)(21). The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence. (22)

In the present study we observed a definitive increase in the antibiotic resistance in this region, which indicate that it is imperative to rationalize the use of antimicrobials and to use these conservatively. Our study confirms the global trend toward increased resistance to β-lactam antibiotics, co-trimoxazole and fluoroquinolones. Moreover, the prevalence and antibiotic susceptibility pattern of ESBL

producers and MRSA differs geographically. Hence, such institutional studies will help in the formulation of antibiotic policy for a particular geographical area.

CONCLUSION

Higher prevalence of community acquired UTIs was seen in adult females. *E. coli* was the predominant pathogen responsible for community acquired UTIs in this region. It is quite alarming that almost all isolates included in this study were found resistant to 3 or more antibiotics. Both gram negative bacilli and gram positive cocci showed high level of resistance against penicillins, quinolones and fluoroquinolones, co-trimoxazole and cephalosporins. So these drugs should no longer be used in the treatment of community acquired UTIs. Among the oral antibiotics, nitrofurantoin was found to be most effective showing least resistance, suggesting that it could be used as empiric monotherapy for uncomplicated UTIs in this region. Our findings suggest presence of ESBL and MRSA in the community; therefore, monitoring of antibiotic susceptibility of bacterial isolates in the community should be mandatory. The antibiotic susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for UTI.

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TABLE 1: Age and sex wise distribution of patients with significant bacteriuria (≥10^5 CFU/ml)

Age group (in years)	Females No. (%)	Males No. (%)
0-10	1 (3.1)	1 (5)
11-20	3 (9.4)	2 (10)
21-30	9 (28.1)	1 (5)
31-40	8 (25)	1 (5)
41-50	5 (15.6)	3 (15)
51-60	4 (12.5)	3 (15)
61-70	2 (6.3)	4 (20)
71-80	0 (0)	5 (25)
80 above	0 (0)	0 (0)
Total	32 (61.5)	20 (38.5)

TABLE 2: Frequency of isolation of various urinary pathogens

Urinary pathogen isolated	Number (%)
Escherichia coli	86 (35)
Coagulase negative *Staphylococcus*	26 (10.6)
Klebsiella spp.	25 (10.2)
Enterococcus faecalis	22 (8.9)
Pseudomonas spp.	21 (8.5)
Staphylococcus aureus	20 (8.1)
Citrobacter diversus	12 (4.9)
Staphylococcus saprophyticus	11 (4.5)
Enterobacter spp.	10 (4.1)
Citrobacter frundii	6 (2.4)
Candida* other than *albicans	3 (1.2)
Proteus mirabilis	2 (0.8)
Acinetobacter spp.	2 (0.8)
Total	246 (100)

TABLE 3: Antibiotic susceptibility pattern of total recovered gram negative bacilli (164)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	102 (62.2)	7 (4.3)	55 (33.5)
Gentamicin	85 (51.8)	9 (5.5)	70 (42.7)
Cephalexin	12 (7.3)	1 (0.6)	151 (92.1)
Cefotaxime	32 (19.5)	29 (17.7)	103 (62.8)
Ceftazidime	27 (16.5)	6 (3.7)	131 (79.8)
Ceftriaxone	28 (17.1)	14 (8.5)	122 (74.4)
Amoxycillin	13 (7.9)	3 (1.8)	148 (90.2)
Ampicillin	4 (2.4)	2 (1.2)	158 (96.3)
Amoxycillin/ Clavulanate	15 (9.1)	4 (2.4)	145 (88.4)
Ciprofloxacin	49 (29.9)	7 (4.3)	108 (65.8)
Norfloxacin	38 (23.2)	6 (3.7)	120 (73.1)
Co- Trimoxazole	35 (21.3)	2 (1.2)	127 (77.4)
Doxycycline	37 (22.6)	9 (5.5)	118 (71.9)
Nalidixic acid	18 (10.9)	6 (3.7)	140 (85.4)
Nitrofurantoin	113 (68.9)	10 (6.1)	41 (25.0)
Tetracycline	40 (24.4)	15 (9.1)	109 (66.5)
Carbenicillin	47 (28.7)	9 (5.5)	108 (65.8)
Cefoxitin	81 (49.4)	8 (4.9)	75 (45.7)

TABLE 4: Antibiotic susceptibility pattern of total recovered gram positive cocci (79)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	58 (73.4)	4 (5.1)	17 (21.5)
Gentamicin	60 (75.9)	3 (3.8)	16 (20.3)
Cephalexin	15 (18.9)	5 (6.3)	59 (74.7)
Cefotaxime	28 (35.4)	24 (30.4)	27 (34.2)
Ceftazidime	10 (12.7)	2 (2.5)	67 (84.8)
Ceftriaxone	25 (31.6)	20 (25.3)	34 (43.0)
Amoxycillin	32 (40.5)	3 (3.8)	44 (55.7)
Ampicillin	16 (20.3)	0 (0)	63 (79.7)
Amoxycillin/ Clavulanate	30 (37.9)	2 (2.5)	47 (59.5)
Ciprofloxacin	26 (32.9)	8 (10.1)	45 (56.9)
Norfloxacin	11 (13.9)	5 (6.3)	63 (79.7)
Co- Trimoxazole	11 (13.9)	1 (1.3)	67 (84.8)
Doxycycline	42 (53.2)	8 (10.1)	29 (36.7)
Nalidixic acid	4 (5.0)	1 (1.3)	74 (93.7)
Nitrofurantoin	66 (83.5)	1 (1.3)	12 (15.2)
Tetracycline	50 (63.3)	3 (3.8)	26 (32.9)
Oxacillin	18 (22.8)	2 (2.5)	59 (74.7)
Vancomycin	63 (79.7)	3 (3.8)	13 (16.5)

Figure 1: Prevalence of ESBL production in *E. coli*

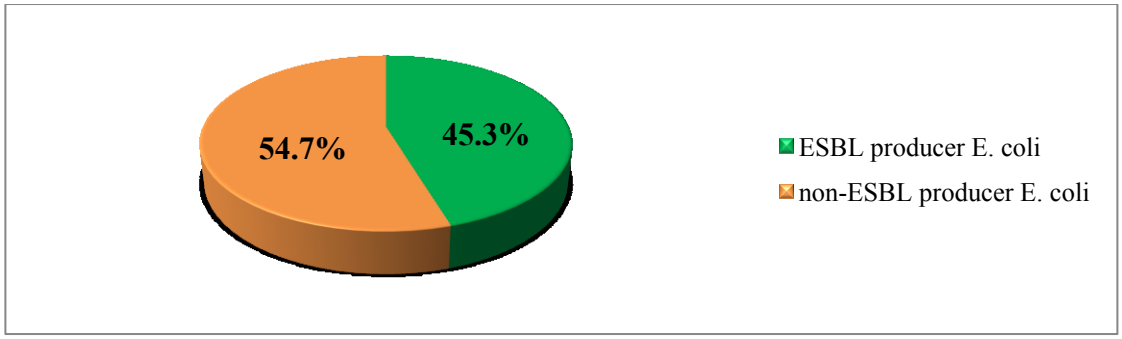

Figure 2: Prevalence of ESBL production in *Klebsiella spp.*

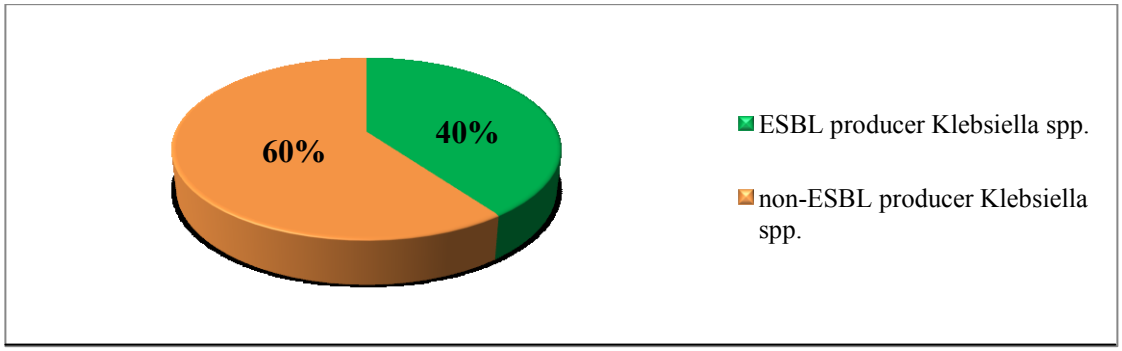

Figure 3: Prevalence of methicillin resistant *S. aureus* (MRSA)