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Changing trends in resistance pattern as an alarm by bacteria before it's too late to treat

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Abstract ***Background****- Urinary Tract Infection is one of the most common site of bacterial infection particularly in female accounting for 2o – 3o % of women. UTI in men are less common and primary occur after 5o years of age. It is important to know the causative organisms in the hospital and community for optimum management of UTI.*

Objectives*-To investigate the prevalence and antimicrobial susceptibility of bacterial uropathogens from the patients attending S.N Medical College, Agra.*

Methods and Material*-The retrospective study involved 1022 UTI patient. Out of these 60% were females and 4o% males. Clean catch mid-stream urine from symptomatic UTI cases were analyzed in the microbiology laboratory, S.N Medical College, Agra. Bacterial isolate were identified using biochemical reaction. Antimicrobial susceptibility testing was performed by the Kirby- Bauer disc diffusion method as described in CLSI guidelines 2010.*

Results *- The overall prevalence of UTI was found to be 63.60 % (650/1022). Klebsiella pneumonia (38.46%) and Escherichia coli (42.76%) were most common organism isolates.*

Conclusion *- The most effective antibiotic for Escherichia coli and Klebsiella pneumonia were gentamycin, amikacin and cefoperzon- sulbactum. Regular monitoring is required to establish reliable information about susceptibility pattern of urinary pathogen for optimal empirical therapy of patient with UTI.*

Key-words: *Antimicrobial Susceptibility Pattern, Prevalence, Urinary Tract Infection.*

I. Introduction

Urinary Tract Infection (UTI) is one of the most important causes of long term morbidity and mortality with an estimated 150 million UTIs annually (Gobernado et al 2007). It is associated with renal damage reported in about 5% of affected children and is common both in the community and hospitalized patients [4]. Although UTIs are encountered in the both sexes and all ages but it is more common in females than the males. Uropathogenic *Escherichia coli* (UPEC) is the most common etiologic agent, responsible for 80 to 85% of community- acquired UTIs with other significant uropathogens; including *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, and *Proteus mirabilis* [9.10]. In present scenario, the essence of antimicrobial drug resistance of major uropathogens has posed a global threat. Updated knowledge of the prevailing etiological agent of UTI and their antimicrobial resistance patterns in specific geographical location may aid clinician in choosing the appropriate antimicrobial empirical treatment and development of appropriate drug policies. Therefore the present study was undertaken to find out the prevalence of UTI and to determine the antimicrobial susceptibility patterns of commonly used antibiotics among patient.

II. Material & Method

2.1 Sample collection and analysis-This study was conducted in the Department of Microbiology S.N Medical College, Agra located in the Northern region of India from 1st May 2012 to 1st Feb 2013 on in patients and patients attending outpatient clinics. A total of 1022 patient with sign and symptoms of UTI were included for this study. All study subjects were advised to collect the freshly voided mid-stream urine sample in wide mouthed leak proof sterile container to hold about 50 ml specimens. Samples from catheterization and suprapubic aspirate were also included in the study. The specimens were transported immediately within one hour to laboratory for further processing and to ensure that the pathogenic organisms present in the urine were isolated and also to avoid overgrowth of the pathogenic organism [2].

2.2 Exclusion criteria - Exclusion criteria for patients were antibiotic usage within week and large fluid intake (less than one hour) before clinic attendance. Only a single positive culture per patient was included in the analysis.

2.3 Direct microscopy: Well mixed, un-centrifuged 10 µl of urine were placed onto a glass slide and allow it to air dry without spreading and gram stain was performed. Each bacteria/oil immersion field indicate the count of 10^5/ml of urine and >5 pus cell/HPF were consider significant to indicate infection. [2]

2.4 Urine culture: Semi quantitative urine culture technique {using a calibrated loop (0.01 ml)} was used to inoculate blood agar and Cystein Lactose Electrolyte Deficient medium (CLED). The plates were incubated for 24 hour at 37^0C. The bacterial count was expressed as colony forming units (CFU) per milliliter (ml). Following the recommendations of Kass in distinguish in the infection from contamination, significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of $>10^5$ CFU/ml [11]. Further a single organism was identified by standard biochemical test [1].

2.5 Antibiotic sensitivity testing: Antibiotic sensitivity testing was performed using the Kirby Bauer disc diffusion method as described in the CLSI guidelines 2010. The in vitro susceptibility to isolated urinary tract pathogen were tested against amoxicillin, co-trimoxazole, cefixime, oflaxacin, tetracycline, ciprofloxacin, norfloxacin, Cefotaxime, cefadroxil, gentamycin, amikacin, cefoperazone-sulbacta , imipenem, nitrofurantoin. Interpretation of result was done by measuring the zone of inhibition around the antibiotic discs in millimeters. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains for antimicrobial susceptibility. Statistical test: Microsoft office excel 2007 was used to analyze the data.

III. Result-

Of the 1022 urine specimens processed 650 (63.60%) showed significant growth of pathogens. The patients were between new born and 90 years of age. Remaining 372 samples had either contamination or a very low bacterial count / sterile.

3.1 Distribution Pattern of Isolates between the Sexes:

More cases of UTIs were recorded among females 390 (60%) than the males 260 (40%). The differences noted in the distribution of pathogens among the sexes were significant.

Table 1: Prevalence of urinary tract infection in relation to age and sex of patients.

Age	Male Examined	Male Positive (%)	Female Examined	Female Positive (%)	Total Number of Cases	Total Positivity %
0– 19	143	73 (51%)	186	115 (62%)	329	188(57.14%)
20 - 49	168	88 (52%)	288	197 (68%)	456	285(62.50%)
50 - 90	116	99 (85%)	121	78 (64%)	237	177(74.68%)
Total	427	260 (61%)	595	390 (66%)	1022	650(3.60%)

3.2 Bacterial Agents of Urinary Tract Infection:

Of the 650 significant isolates, gram-negative rods accounted for 94.31 % while gram-positive cocci accounted for the remaining 05.69 % of the total pathogens. The Gram Negative Rods isolated were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Serratia marcescens*. *Escherichia coli* alone accounted for 42.76% followed by *Klebsiella pneumoniae* 38.46% of the urinary isolates, *Serratia marcescens* was 7.23%, *Pseudomonas aeruginosa* 3.53%, with respectively *Proteus mirabilis* 2.31%. Among gram positive pathogens *S. saprophyticus* was the major pathogen accounted for 3.07 % followed by *Staphylococcus aureus* 1.69% and *Streptococcus pyogenes* 0.92%. The pattern of bacterial agents isolated is as shown in table 2.

Table 2 Pattern of etiological agents isolated from Patients ($N^+ = 650$)

Organism	No. of isolate % in male	No. of isolate % in Female	Total %
Escherichia coli	124(44.60%)	154(55.40%)	278(42.76%)
Klebsiella pneumoniae	98(39.20%)	152(60.80%)	250(38.46%)
Serratia marcescens	11(23.40%)	36(76.60%)	47(7.23%)
Pseudomonas aeruginosa	14(60.86%)	9(39.14%)	23(3.53%)
Proteus mirabilis	3(20.00%)	12(80.00%)	15(2.31%)
Streptococcus pyogenes	2(33.33%)	4(66.67%)	6(0.92%)
Staphylococcus aureus	5(45.45%)	6(54.55%)	11(1.69%)
Staphylococcus saprophyticus	7(35.00%)	13(65.00%)	20(3.07%)
Total Number N^+	264 (40.61%)	386 (59.39%)	650

3.3 Antibiotic Resistance pattern in Gram Negative Bacilli:

The antimicrobial Resistance pattern of Gram Negative Bacilli against selected antimicrobial agents is summarized in Figure 1.

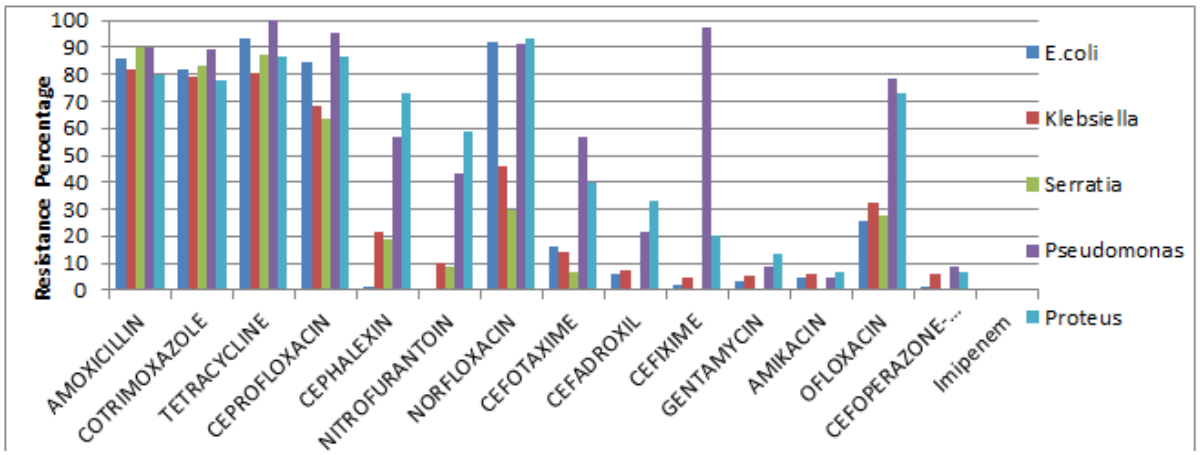

Fig 1 the antimicrobial Resistance pattern of Gram Negative Bacilli against selected antimicrobial agents.

3.4 Susceptibility pattern of most common isolated Gram-negative bacteria

E. coli and *K. pneumoniae* showed least resistance to the aminoglycosides - gentamicin (0.27% and 6.05%), carbapenems (0.31% and 0.46%) nitrofurantoin (0.36% and 9.90%), cephalexin (1.08% and 21.35%), cefoperazone-sulbactam (1.08% and 5.69%), cefixime (2.16% and 4.98%), cefdroxil (5.76% and 7.47 %), cefotaxime (16.19 %and 14.23%), ofloxacin (25.54% and 32.74%) respectively. *Serratia* was the third most common isolate in urinary tract pathogen which showed the similar resistance pattern like *E.coli* and *K. pneumonia*e in respective to amoxicillin , cotrimoxazole, tetracycline, ceprofloxacin, ofloxacin and it showed 100% susceptibility to cefixim, gentamycin, amikacin, and cefoperazone-sulbactam. While *P. aeruginosa* isolates showed the highest resistant towards routinely used antimicrobial and least resistance toward gentamycin 08.70%, amikacin 4.35%, cefoperazone-sulbactam 08.70%.The highest susceptibility to aminoglycosides tested was also obvious with *P. mirabilis* isolates as shown in Fig1.

3.5 Antibiotic susceptibility pattern of Gram Positive isolates:-

The Gram -positive cocci were tested against - amoxicillin (AX), cotrimoxazole (BA), tetracycline (TE), ceprofloxacin (RC), cephalexin (CN), erythromycin (EE), azithromycin (AZ), rotxithromycin (RM), norfloxacin (NX), ofloxacin (NZ), ampicillin (AM), cloxacillin (CX), cefotaxime (CF), cefuroxime (CB), cefoperazne-sulbactam (CS), vancomycin (VA). *S. pyogenes* isolate were 100% resistance against Aamoxicillin, cotrimoxazole, tetracycline and susceptibility of amoxicillin–clavulanic acid 100%, gentamicin 92.30%, and ceftriaxone 84.60% respectively.

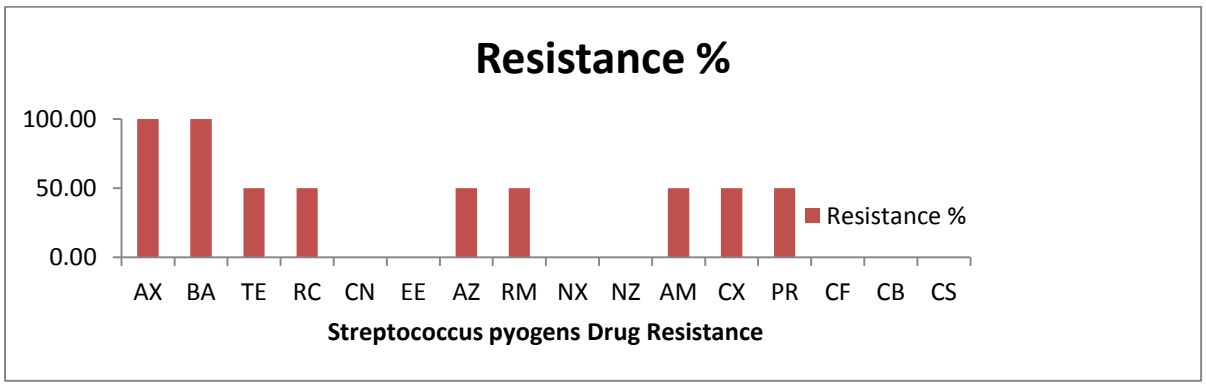

Fig 2 *Streptococcus pyogenes* drug resistance

S. aureus showed highest resistant 54.54% to gatifloxacin, 45.45% to erythromycin and least resistance towards Gentamycin 27.27%. *S. saprophyticus* isolate showed highest resistance towards erythromycin.

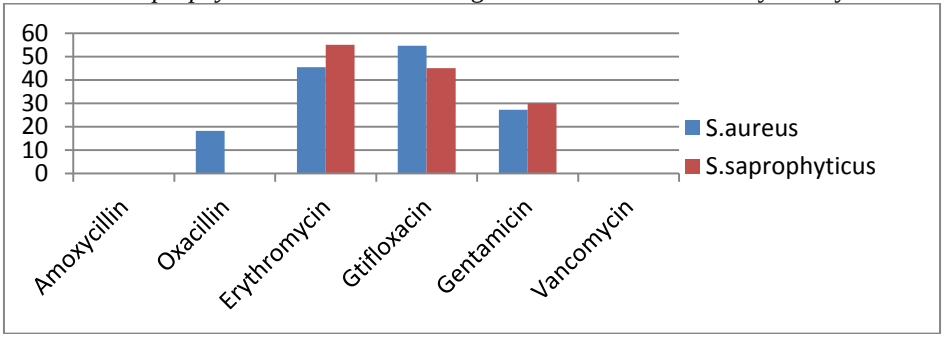

Figure 3 Drug resistance among other gram positives.

IV. Discussion-

Although UTI ranks among the most common infection in developing countries, in the present study a total of 650 (63.60%) out of 1022 patients had UTI. This indicates that urine culture and antimicrobial susceptibility is essential for a definitive diagnosis of UTI. Geographical location may be the reason for the difference in the percentage of population infected with UTI infection, the pattern of pathogenic organism and resistance pattern of different organism. According to other study prevalence rate of UTI was 24.94% Bharatpur [8]. The prevalence of 39.69% was reported in okada, a rural community in Nigeria in 2011[7].

In our study, UTI was more common in females. Male: female ratio was 1:1.46 as shown in table 2. Other studies also showed male: female ratio of 1:1.9 to 1:2[6, 12, and 15] in different regions. Our result corresponds to the result obtained in similar study conducted in India, Kuwait, Sharjah and Schaeffer et al. [2001] too with 60 % of infected population being women [13]. This is due to short urethra in females and its closeness to the anus. Sexual activity also appears to increase the chances of bacterial contamination of the female urethra.

The prevalence of antimicrobial resistance among micro-organism that causes UTI is increasing worldwide and is a major factor selecting antibiotics for treatment. In the present study, the most frequently isolate were *E.coli, K. pneumoniae, S. marcescens, P. aeruginosa, P. mirabilis, S. aureus, S. saprophyticus* and *S. pyogenes*. These were also the organism most commonly isolated India, Nepal, and Pakistan [9and 20]. In our study *E.coli* is the most common isolate in urine culture which was consistent with other study where *E. coli* is the most common isolate in urine culture [7, 8]. The highest percentage of *E.coli* obtained in this study is a cause of concern due to emerging drug resistance. More females (154) were infected with *E.coli* with only 124 males. While in case of *K. pneumoniae* the number of infected females and males were 98 & 152 respectively. Of the 47 isolates of *Serratia*, 11 were from males and 36 were from females which was significant (Table 2). Other organism isolated were *P. aeruginosa* (3.53%) and *P. mirabilis* (2.31%) as shown in Table 2. These organisms also have been reported as agents of UTIs and their presence in the sample population was not unusual as showed by S Shrestha et al in children at Nepal Medical College Teaching Hospital, Nepal Medical College, Kathmandu, Nepal. In this study infection caused by *P. aeruginosa* was very common in males 60.86% than females which correlate with study conducted by B.V Ramana and Abhijit Chaudhary in 2011 in Andhra Pradesh.

Resistance rates vary from country to country [15] this is probably due to the fact that the antibiotic has been widely used in treating UTIs over the past decade in the region. The antimicrobial sensitivity findings in our study was similar to a study conducted by Das et al. 2006, Tiwan, Senegal and India [8, 12 and 18] that showed the greatest sensitivity to all the isolated organism against amoxacillin, cotrimoxazole, tetracycline, ciprofloxacin, ofloxacin and norfloxacin in comparison, low resistance rates were found against cephalexin, nitrofurantoin, cecfotaxime, cefadroxil, cefixime, gentamycin, amikacin and cefoperazone-sulbactamin Fig 1. The highest efficacy of gentamicin in the treatment of UTIs has also been reported by Al Sweih et al [14] and the antimicrobial that showed the greatest resistance to the isolate organism were amoxicillin, cotrimoxazole, tetracycline and ciprofloxacin. The resistance pattern of *K. pneumonia* and *E.coli* was similar and higher for amoxicillin (82.02% & 85.56%), tetracycline (80.78% & 93.17), co-trimoxazole (78.95% & 81.76%), and ciprofloxacin (84.57% & 68.29%). Our isolates showed higher resistance against ampicillin and co-trimoxazole than the isolates from USA (39.1% and 18.6 % respectively) [16] and Europe (29.8% and 14.1% respectively) [17]. Resistance rate for nitrofurantoin and carbapenems was very low (showed only 9.90% & 0.36% resistance for nitrofurantoin and 0.46% and 0.31% for resistance for carbapenems) in *K. pneumoniae* and *E.coli* isolates respectively which was similar to major part of world [20]. But *P. mirabilis* showed highest resistance towards nitrofurantoin 59%. On the other hand rate of resistance against these antibiotics is comparable in countries like Senegal, Taiwan and India. [12, 18] Resistance pattern of *S. marcescens* isolates was similar to *E.coli* and *K.*

pneumoniae isolates, where resistance percentage of ceprofloxacin was 64%, cotrimoxazole 83%, tetracycline 87% and for amoxacillin 90%. These isolates were highly susceptible to other antimicrobials (Susceptibility varies from 70 % to 100%). Fig 1

P. aeruginosa was resistant against Tetracycline 100%, amoxicillin 90%, cefixime 97%, norfloxacin 91%, ciprofloxacin 96%and co-trimoxazole 89% respectively. Resistance pattern in case of *Proteus* was also not unusual with highest resistance to, amoxicillin, tetracycline, norfloxacin, ciprofloxacin, and co-trimoxazole. imipenem had wide coverage for all isolates with almost 100% susceptibility among the all beta-lactam.

Percentage of Gram Positive isolates were very less in our study accounting for 05.69% of all the isolates Which was also similar to the studies conducted by Manjunath GN, Prakash R et al. from 2005 to 2010 and Zakieh Rostamzadeh Khameneh, Ali Taghizadeh *et* al. in 2006 Iran. However antibiotic resistance in Gram Positive cocci was observed from 0.00% to 100% as indicated on Fig 2 & Fig 3. *S. pyogenes* isolates were 100% resistance when they were tested against amoxacillin and cotrimoxazole. Isolates also showed 50% resistance towards erythromycin, azithromycin, tetracyclin, ciprofloxacin, rotxithromycin, cloxacillin, cefotaxime, cefuroxime and cefoperazone-sulbactam antibiotics showed good efficacy against gram positive isolates being 100% sensitive. Fig 2 Other gram positive isolates were *S. aureus* and *S. saprophyticus* which were tested against selective antibiotics and showed less resistance. *S. aureus* showed low level resistance towards gentamycin and erythromycin 27.27% and 45.45 %. Among 11 *S. aureus* isolates 2 were Methicillin Resistant. *S. saprophyticus* showed more resistance in comparison to *S. aureus* while tested against eythromycin and gentamycin. None of the Gram positive isolate showed resistance against vancomycin.

Most of isolated uropathogens showed multiple antibiotics resistance in this area. It may be due to large portion of the bacterial isolate being previously exposed to several antibiotics. The present study data gives idea about the common trend of increased antibiotics resistance of uropathogens in this region, which may be due to geographic variation or indiscriminate or sub lethal use of antibiotic.

The emergence and spread of resistance can be reduced through appropriate or careful use of antimicrobial drugs and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaign.

V. Conclusion–

An overall prevalence 63.79% of UTI was observed in this study. This information will directly affect selection of empiric therapy for UTI. Regular monitoring is required to establish reliable information about susceptibility pattern of urinary pathogen for optimal empirical therapy of patient with UTI. We suggest that empirical antibiotic selection should be based on the knowledge of local prevalence of bacterial organism and antibiotic sensitivities rather than on universal guidelines.

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