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Drug Susceptibility of Uropathogens: Need for New Antimicrobial Guidelines

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ABSTRACT

Background: *There is increased prevalence of drug resistant strains responsible for community acquired urinary tract infection. To know the drug susceptibility of uropathogens to routinely used antibiotics.*

Material and Method*: The midstream urine specimen was collected from clinically suspected cases of urinary tract infection they were subjected for standard processing protocol to isolate the pathogen and study their susceptibility.*

Results: *Of the 1057 urine samples were studied over a period of one year out of which 234 samples (22%) yielded significant growth of single organism In the present study E.coli(55%),Klebsiella species (16%), Enterococcus fecalis,(09%) Staphylococcus aureus,(04%) , Nonfermenters excluding Pseudomonas spp (3%),Citrobacter spp(3%),Streptococcus spp(2%),Pseudomonas spp(2%),Proteus spp(2%)Enterobacter spp(2%),coagulase negative Staplococcus spp(01%) and Providentia spp(0.5%) Antibiotic sensitivity and resistance analysis was performed by the disc diffusion method employing multiple antibiotic discs. E.coli showed variable antimicrobial resistance to different antibiotics as*

85%,70%, 40%,and 28% of the isolates were found to be resistant to ampicillin ciprofloxacin , gentamicin, amikacin respectively. Klebsiella species showed resistance to various betalactam antibiotics 62%-100% ciprofloxacin 71%, gentamycin 67% and Nitrofurantoin 57%cefatoxime 80%

Staphylococcus isolates showed high resistance rate against ampicillin(82%), norfloxacin (82%),ciprofloxacin(70%) ,ceftriaxazone (73%), cefatoxime (67%) and cotrimaxazole (65%) all were sensitive to vancomycin and linozolid Non fermenters (excluding pseudomonas species) showed high degree of resistance to almost all the antibiotics used routinely.

Pseudomonas isolates were found to be resistant to gentamycin (80%) cotrimaxozole(100%),norfloxacin(100%),and amikacin(100%) Enterobacter spp were 100% sensitive to Nitrofurantoin and these isolates were almost resistant to other group of antibiotics.

Antibiotic susceptibility data revealed that majority of the isolates were resistant against 3 or more antibiotics that is 95% of the bacterial pathogens are multidrug resistant and the percentage of ESBL producers detected among E.Coli and Klebsiella species were 26%&40% respectively.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting. In almost all cases there is a need to start treatment before the final microbiological results are available. Accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture.

Women are significantly more likely to experience UTI than men. Nearly 1 in 3 women

will have had at least 1 episode of UTI requiring antimicrobial therapy by the age of 24 years. Almost half of all women will experience 1 UTI during their lifetime.[1] Specific subpopulations at increased risk of UTI include infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic abnormalities.

Catheter-associated UTI is the most common nosocomial infection, accounting for >1 million cases in hospitals and nursing homes. In noninstitutionalised elderly populations, UTIs are the second most common form of infection, accounting for nearly 25% of all infections[1]. When treated promptly and properly, lower urinary tract infections rarely lead to complications. But left untreated, a urinary tract infection can have serious consequences.

Complications of UTIs may include Recurrent infections, especially in women who experience three or more UTIs further there can be permanent kidney damage from an acute or chronic kidney infection (pyelonephritis) due to an untreated UTI, especially in young children .Also there is Increased risk of women delivering low birth weight or premature infants or even fetal mortality among pregnant women and there can be associated impaired renal function and end-stage renal disease among paediatric patients. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the right empirical treatment.

Many different antimicrobial agents are available in India on physician prescription, for the treatment of UTI like first and second-generation cephalosporins and semisynthetic penicillins with or without inhibitors azetronam, azthromycin, Co-trimoxazole, trimethoprim, ciprofloxacin, norfloxacin, nitrofurantoin, , tegicycline, tetracycline, meropenam, Nitlmycine, ticarcillin etc. The aim of this study was to obtain data on the prevalence of urinary pathogens and to study the antimicrobial susceptibility patterns of the commonly isolated pathogens from urine culture in the tertiary care hospital.

MATERIALS AND METHODS

The study was conducted on patients with clinical manifestation suggestive of urinary tract infection. Midstream urine specimens were collected in sterile wide mouthed containers for a period of one year. The samples collected were processed immediately within 30 minutes of collection for microscopy and culture. With standard calibrated loop urine was inoculated on Mac conkey and blood agar incubated aerobically at 37^0c for 18-24hrs. After the incubation, if the growth was single type and colony count was more than 10^5 it was further processed for identification and antibiotic susceptibility test. If the CFU was less than 10^5 and mixed growth of two or more organisms were observed it was considered to be contamination.

Identification of the isolated bacterial pathogens was done on the basis of gram staining, colony morphology and biochemical characteristics as per standard protocol. Antimicrobial sensitivity of the isolated pathogens was determined by using Kirby Bauer Disc Diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[2]. The antibiotics tested were ampicillin, amikacin, nitrofurantoin, co-trimoxazole, imipenam, ceftraxazone, cefotaxime, cefaxitine, ceftazidime, cefuroxime ,ciprofloxacin, gentamycin, netlimycin, and norfloxacin.

ESBL DETECTION BY NCCLS PHENOTYPIC METHOD:

Isolates with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to third generation cephalosporins were tested for ESBL by phenotypic confirmatory test as per the recommendations of CLSI. The ceftazidime (30µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10µg discs) were used. An increase of ≥5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

RESULTS

Out of 1057 sample processed 234 samples(22.13%) showed significant growth of pathogens including candida spp. The 138(58.97%) isolates were from female patients while the remaining were from male patients (Table1)

TABLE-1; Age And Sex Wise Distrubution Of Isolates:

Age group	Males	Females	Total
0-1year	06(37.5%)	10(62.5%)	16
1-10	21(60%)	14(40%)	35
11-20	11(36.6%)	19(63.3%)	30
21-30	11(31.4%)	24(68.6%)	35
31-40	14(45.1%)	17(54.8%)	31
41-50	11(31.4%)	24(68.6%)	35
51-60	12(48%)	13(52%)	25
61-70	0538.4%)	08(61.5%)	13
71-80	05(45.4%)	06(54.5%)	11
81-90	Nil	03(100%)	03
Total	96(41.02%)	138(58.97)	234

In females E.coli and Enterococcus faecalis isolated as common pathogens other pathogens isolated shown in table 2

Table 2- various pathogens isolated in male and female patients

Organism	Male no (%)	Female no(%)	Total (%)
Escherichia coli	41(33.3)	82(66.6%)	123(55)
Klebsiella	23(62.1)	14(37.8)	37(16)
Enterococcus faecalis	07(33.3)	14(66.6)	21(9)
Staphylococcus aureus	4 (40)	6(60)	10(4)
Candida	3(33.3)	6(66.6)	9(3)
Citrobacter	2(33.3)	4(66.6)	9(3)
Non fermenters(except pseudomonas)	4(577.1)	3(42.9)	7(2)
Streptococcus species	3(60)	2(40)	5(2)
Pseudomonas	4(80)	2(50)	4(2)
Proteus	2(50)	2(66.7)	4(2)
Enterobacter sp	1(33.3)	2(66.6)	3(1)
CONS	2(66.6)	1(33.3)	3(1)
Providencia sp	0	1(100)	1(0.5)
Total	96(41)	138(59)	234

The frequency and resistance pattern of urinary microbes to various antibiotics are shown in Table 3
E.coli isolates(table-4) showed resistance to most of the beta lactam antibiotics (40-70%) ,except for carbepenems like imepenem which showed sensitivity of 20%

TABLE-4

Antibiotic Susceptibility Pattren Of E.coli Isolates

Antibiotics	Resistance
Ampicillin	85
Amoxicillin	70
Amikacin	28
Nitrofurantoin	18
Co-trimoxazole	58
Imipenem	20
Ciprofloxacin	70
Ceftriaxazone	40
Cefotaxime	40
Cefoxitine	60
Cefuroxime	49
Gentamycin	40
Netlimycin	17
Norfloxacin	76

	No. & percent (%)	Ampicillin	amo	AMK	NTF	CMX	IMP	cip	CI	CE	CX	CUF	G	NET	NX	Vacomycin	Linozolid
E. coli (123)	55%	85% (105)	70% (86)	28% (34)	18% (22)	58% (71)	20% (25)	70% (86)	40% (49)	40% (49)	60% (74)	49% (60)	40% (49)	17% (21)	76% (97)	ND	ND
K.pneumoniae (37)	16%	62% (23)	67% (25)	25% (09)	43% (16)	56% (21)	23% (08)	29% (11)	53% (20)	80% (30)	73% (27)	100% (37)	33% (12)	23% (08)	60% (22)	ND	ND
Ent.faecalis(21)	09%	74% (15)	80% (17)	05% (01)	18% (04)	59% (12)	29% (07)	89% (19)	59% (12)	69% (14)	63% (13)	73% (15)	84% (18)	76% (16)	94% (20)	08% (02)	07% (01)
Staph.aureus(10)	04%	82% (08)	82% (08)	09% (01)	ND	65% (06)	19% (02)	70% (07)	73% (07)	67% (06)	69% (06)	ND	35% (03)	ND	82% (08)	100% (10)	100% (10)
nonfermenters(excluding psuedomonas species) (07)	03%	100% (07)	ND	82% (06)	33% (02)	25% (01)	33% (02)	100 % (07)	100% (07)	100% (07)	100% (07)	ND	50% (03)	100 % (03)	75% (05)	ND	ND
Citrobacter (06)	03%	67% (04)	67% (04)	50% (03)	33% (02)	83% (05)	33% (02)	50% (03)	67% (04)	67% (04)	67% (04)	67% (04)	83% (05)	17% (01)	67% (04)	ND	ND
Streptococcus species (05)	02%	40% (02)	40% (02)	50% (02)	100 % (05)	100 % (05)	40% (02)	40% (02)	100 (05)	100 (05)	100% (05)	100% (05)	80% (04)	50% (02)	80% (04)	100% (05)	100% (05)
Pseudomonasspp (05)	02%	80% (04)	80% (04)	100 (05)	40 (02)	100 (05)	40 (02)	50 (02)	50 (02)	60 (03)	50 (02)	ND	80 (04)	00	100 (05)	ND	ND
Proteus species (04)	02%	50 (02)	50 (02)	25 (01)	50 (02)	75 (03)	25 (01)	25 (01)	00	75 (03)	75 (03)	ND	00	25 (01)	00	ND	ND
Enterobacter sp (03)	01%	100 (03)	100 (03)	100 (03)	00	67 (02)	33 (01)	67 (02)	100 (03)	100 (03)	100 (03)	ND	100 (03)	33 (01)	67 (02)	ND	ND
CONS(03)	01%	66 (02)	66 (02)	00	33 (01)	33 (01)	33 (01)	100 (03)	100 (03)	100 (03)	100 (03)	ND	00	33(01)	66 (02)	ND	ND
Providentia sp (01)	0.5%	00	00	00	00	100	00	00	100	100	100		00	00	00		

Amo-amoxicillin amk-amikacin NTF-nitrofurontoin CMX-cotrimaxaxole IMP-imipenem CIP-ciprofloxicin
CI-cefatriaxzone CE- cefataxim CX- cefoxitine CUF-cefuroxime G-gentamicin NET-netlimycin NX-norfloxcin] **(Table 5)**

DISCUSSION:

According to the study urine culture positivity was more among the female patients (58.97%). It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors [3,4].

Out of the 225 bacterial culture positive cases 186(83%) were gram negative organisms and remaining were gram positive isolates(17%).

According to our study E. coli was the commonest cause of urinary tract infection (55%), followed by Klebsiella pneumoniae (16%),Enterococcus fecalis(,09%)Staphylococcus aureus,(04%) , and others as shown in the (table -2).

So this study confirms that E. coli is still the most common uropathogen isolated from UTI patients. This study was similar to other studies[6,7,] where E.coli was the most frequent pathogen causing UTI fallowed Klebsiella species .Third prevalent

organism in our study was Streptococcus faecalis .this was similar to the study conducted by Tabish humayun etal.,[8] 2010 .

E.coli showed variable antimicrobial resistance to different antibiotics as 85%, 70%, 40%,and 28% , of the isolates were found to be resistant to ampicillin ciprofloxacin, gentamicin, amikacin respectively.

Eswarappa etal [9]has done the study on antibiotic resistance pattern of E.coli isolates where the isolates showed resistance percentage of 80%,74%49%and 28% to ampicillin,quinolones,gentamycin,and amikacin respectively.

In the present study drugs like netilmycin, and nitrofurontoin, showed resistance of 17%,and 18% for E.coli

The rate of resistance to nitrofurantoin remained low. Nitrofurantoin is bactericidal in urine at therapeutic doses, and its multiple mechanisms of action appear to have enabled it to retain potent activity against E. coli despite nearly 50 years of use [(10).] The consistent and high-level susceptibility of E. coli to nitrofurantoin may be influenced by nitrofurantoin's narrow spectrum of activity, limited indication (treatment of acute cystitis), narrow tissue distribution (low or undetectable serum concentrations), and limited contact with bacteria outside the urinary tract [(11).]

Guptha etal[12], Eswarappa etal,[9] had done the study where nitrofurontoin resistance percentage varied from 12% to 38% respectively.

Kebira et al[13] in Kenya in 2009 revealed that E. coli isolated from urine specimens were 100% sensitive to amikacin and ofloxacin, and nitrofurantoin showed 77% sensitivity while norfloxacin, ciprofloxacin and ceftazidime were 95% sensitive. Uwaezuoke etal[14] in Nigeria in 2006, nitrofurantoin and gentamicin were found to be 80% effective against strains of E. coli.

Present study correlates with these,so it is observed that older antibiotics like nitrofurontoin,.gentamycin,are still effective for UTI treatment.

In our study, isolates of E.coli were resistant to Trimethoprim-sulfamethoxazole 60 %, ciprofloxacin(70%) and Norfloxacin(77%),which is of great concern because fluoroquinolones are the drugs of choice for first-line empiric treatment of both community and hospital acquired UTI. In settings where resistance to trimethoprim/sulfamethoxazole exceeds 20%, and they have become more commonly prescribed as first-line antibiotic .

The findings of this study indicate that betalactams, trimethoprim/sulfamethoxazole, and ciprofloxacin/norfloxacin should no longer be used as empirical treatments of UTI because of their high rate of resistance. Alternatives must be recommended, especially for empirical treatments of uncomplicated UTI (cystitis)

In the present study Imepenem showed resistence rate of 20% .while Mohamod akram etal[15], ,MF ,Bashir etal [16] and Eswarappa etal[9] studies showed resistence of 0% ,2% and 4% respectively for Imepenem. The high susceptibility to imepenem observed in our study is a clear indication that carbapenem resistance is still low in Enterobacteriaceae isolated from UTI in the region. This can be explained by the infrequent use of this antibiotic in the developing world because of its cost and limited availability.

The sensitivity of E.coli to ceftriaxazone,cefatoxime Cefaxitine and cefuraxime was found to be 40%, 40%, 60% and 49% respectively.

Presence of extended spectrum beta lactamases (ESBL) in these strains was the cause for the high resistence for the above mentioned cephelosporin group of drugs. Our Klebsiella isolates showed resistance to ciprofloxacin (71%) , gentamycin (67%), Nitrofurantoin(57%) and cefatoxime 80%.only imepenem,Netilmicin,and amikacin showed some degree of sensitivity Enterococcus faecalis was found to be susceptible to amikacin, linozolid and vancomycin .

Staphylococcus aureus isolates were found to be susceptible against vancomycin(100%), nozolid(100%) and imepenem(81%).

The high resistance rate against ampicillin(82%), norfloxacin (82%),ciprofloxacin(70%) ,ceftriaxazone (73%), cefatoxime (67%) and cotrimaxazole (65%)was also observed among these isolates.

Other non fermenters (excluding pseudomonas species) showed high degree of resistance to almost all the antibiotics used routinely necessitating its susceptibility testing for newer drugs In case of Citrobacter spp sensitivity to various antibiotic group ranged from 20%-50%. Pseudomonas isolates were found to be highly resistant to gentamycin

(80%)cotrimaxozole(100%),norfloxacin(100%),and amikacin(100%)

Proteus spp isolated were sensitive to gentamycin and norfloxacin(100% sensitive) where as nterobacter spp were 100% sensitive to Nitrofurantoin and these isolates were almost resistant to other group of antibiotics

Of the 123 E.coli isolates 40 pathogens showed resistance to cephotaxime in the present study. Of these, 32 isolates (26%)found to be ESBL producers. ESBL production in E. coli has been reported to vary from 21 to 34 per cent[17,18,19,20,21]. High prevalence rate of ESBL producing strains have been reported in Klebsiella species also[17,18,19,20,21]. In the present study, 15 (40.5%) Klebsiella isolates were ESBL producers

CONCLUSION

E. coli is still the most widely prevalent organism causing UTI in the community, and It is quite alarming to note that almost all of the isolates included in this study were found resistant to four or more antibiotics. The alarmingly high rate of resistant ESBL species should draw our attention. The resistance is ever increasing due to uncontrolled abuse of the available antibiotics. Therapy should only be advocated, as far as possible, after culture and sensitivity has been performed. This would not only help in the proper treatment of the patients but would also discourage the indiscriminate use of the antibiotics and prevent further development of bacterial drug resistance A strong decision has to be established regarding the antibiotic policies for UTI and stringent measures have to be taken to ensure the effectiveness of the same. Failing to do so, the time is not far where we would have to stand helplessly against these organisms.

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