World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:9, No:9, 2015

Microbiological Profile of UTI along with Their Antibiotic Sensitivity Pattern with Special Reference to Nitrofurantoin

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Abstract**—**Urinary Tract Infections are considered as one of the most common bacterial infections with an estimated annual global incidence of 150 million. Antimicrobial drug resistance is one of the major threats due to wide spread usage of uncontrolled antibiotics. In this study, a total number of 9149 urine samples were collected from R.H Patiala and processed in the Department of Microbiology G. M. C Patiala (January 2013 to December 2013). Urine samples were inoculated on MacConkey's and blood agar plates and incubated at 37^0C for 24 hrs. The organisms were identified by colony characters, Gram's staining, and biochemical reactions. Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents (Hi – Media Mumbai India) by Kirby Bauer DISK diffusion method on Muller Hinton agar plates.

Maximum patients were in the age group of 21-30 yrs followed by 31-40 yrs. Males (34%) are less prone to urinary tract infections than females (66%). Culture was positive in 25% of the samples. *Escherichia coli* was the most common isolate 60.3% followed by *Klebsiella pneumoniae* 13.5%, *Proteus* spp. 9% and *Staphylococcus aureus* 7.6%. Most of the urinary isolates were sensitive to, carbepenems, Aztreonam, Amikacin, and Piperacillin + Tazobactum. All the isolates showed a good sensitivity towards Nitrofurantoin (82%). ESBL production was found to be 70.6% in *Escherichia coli* and 29.4% in *Klebsiella pneumonia.* Susceptibility of ESBL producers to Imipenem, Nitrofurantoin and Amikacin were found to be 100%, 76%, and 75% respectively. Uropathogens are increasingly showing resistance to many antibiotics making empiric management of outpatient UTIs challenging. Ampicillin, Cotrimoxazole and Ciprofloxacin should not be used in empiric treatment. Nitrofurantoin could be used in lower urinary tract infection. Knowledge of uropathogens and their antimicrobial susceptibility pattern in a geographical region will help in appropriate and judicious antibiotic usage in a health care setup.

Keywords**—**Urinary Tract Infection, UTI, antibiotic susceptibility pattern, ESBL.

I. Introduction

URINARY TRACT INFECTIONS (UTIs) account for a significant part of the workload in clinical microbiology laboratories [1]. UTI consists of microbial invasion in any structure of urinary system. The severity of infection ranges from asymptomatic colonization to symptomatic invasion of the tissues of any of the structures of the urinary system [2]. They have become the most common hospital acquired infections, accounting for as many as 35% of nosocomial infections [2], [5].

UTI is an extremely common condition that occurs in both male and female of all the age groups. The prevalence and incidence of UTI is higher in women than in men due to several clinical factors including anatomic differences, hormonal effects, and behavioral pattern. Low socioeconomic conditions, malnutrition, and poor hygiene are few of the main predisposing factors causing UTI [3].

The main causative agents of UTI are *Escherichia. coli*, *Klebsiella* spp., *Staphylococcus aureus, Staphylococcus saprophyticus, Proteus* spp., *Pseudomonas* spp., and *Citrobacter* [6]. The introduction of antimicrobial therapy has led to profound improvements in the management of urinary tract infections; however antimicrobial resistance is a growing problem and a cause of major concern in many countries. Over the past several decades, resistance too many of the commonly prescribed UTI antibiotics i.e. Ampicillin, Co-trimoxazole, Nitrofurantoin, and Fluoroquinolones - has emerged [4]. Inappropriate and empirical usage of wide spectrum of antibiotics, insufficient hygiene, immunosuppression, and prolonged hospitalization are some of the major etiological factors that increase the chance of UTIs [7], [9].

ESBLs are strictly defined as β Lactamases capable of hydrolyzing Penicillins, broad and extended spectrum Cephalosporins. They have been isolated from wide variety of family *Enterobactreiacae* as well as from *Pseudomonas aeruginosa*. ESBLs are located on plasmids that are transferable from one strain to another bacterial strain and they are resistant to many other classes of antibiotics including Aminoglycosides and Fluoroquinolones; thus, treatment of these ESBLs is often a therapeutic challenge [10], [11]. ESBLs can confer resistance against all beta-lactam drugs except Carbapenems and Cephamycins. ESBL producing Gram negative organisms have inflicted a significant threat to hospitalized patients due to their hydrolyzing activity against extended spectrum Cephalosporins, which are mostly used in the treatment of hospital acquired infections. Use of broad-spectrum oral antibiotics and probably poor infection control practices may facilitate spread of this plasmid-mediated resistance. In addition to known populations at risk, ambulatory patients with chronic conditions represent another patient population that may harbor ESBL-producing organisms [16].

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II. Materials and Methods

The present study was conducted at Govt. Medical College and Rajindra Hospital Patiala a referral tertiary teaching hospital. A total of 9149 clean catch midstream urine samples were collected in a sterile container from both outpatient and inpatient attending Rajindra Hospital Patiala (January 2013-December 2013). Urine samples were transported immediately to Department of Microbiology Govt. Medical College Patiala for processing. Uncentrifuged urine samples were 1st examined under microscope for presence of pus cells, RBCs, epithelial cells and bacteria. Then the urine samples were inoculated on MacConkey's and Blood agar plates by using calibrated loop delivering 0.001 ml of sample and incubated at 37^0C for 24 hrs. For gram-negative bacilli more than 10^5 colonies per ml and for Gram positive cocci 10^3-10^5 colonies per ml of single organism were considered significant. The organisms were identified by colony characters, Gram's staining, and biochemical reactions.

Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents (Hi – Media Mumbai India) by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guidelines [7]. Antibiotics included for Gram negative bacilli were Ampicillin (10µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg) Ofloxacin (5µg), Norfloxacin (10µg), Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Piperacillin- Tazobactum (100/10µg), Nitrofurantoin (300µg), Cotrimoxazole (25µg), Imipenem (10µg), Meropenem (10µg), Aztreonam (30µg). Antibiotics included for Gram postivecocci were Ampicillin (10µg), Amoxycillin (30 µg), Amoxy-Clav (20/10µg), Erythromycin (15µg), Clindamycin (2µg), Netilmicin (30µg), Linezolid (30µg), Teicoplanin (30µg), Vancomycin (30 µg). The results were recorded and interpreted according to CLSI guidelines.

ESBL production was tested by double disk approximation test and combined disk method, which is recommended by CLSI:

1) **Doubledisk Approximation Test:** Double disk approximation test was performed by using Amoxy-Clav (20/10µg) + Ceftazidime (30µg). The disks were placed 15 mm apart.
2) **Combined Disk Method:** Combined disk method was performed using Ceftazidime (30µg) and Ceftazidime + Clavulanic acid (30/10µg). The disks were placed 20 mm apart.
3) **Quality Control:** *Escherichia. coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662 and *Staphylococcus aureus* NCTC 6571 strains were used as controls [1], [18].

III. Results

Out of 9149 clinically suspected cases of UTI, culture was positive in 2290 (25%) samples. Out of 2290 culture, positive cases 1390 samples were from indoor patients while 900 samples were from outpatient department. Out of 2290 maximum patients were in the age group of 21-30 yrs 55.4 % (n=1269) followed by 31-40 yrs 26% (n=596) (Table I). Among 2290 culture positive samples, 66 % (n=1512) were obtained from females and 34 % (n=778) were obtained from males. *Escherichia coli* was the most common isolate 60.3% (n=1378) followed by *Klebsiella pneumoniae* 13.5% (n=310), *Proteus* spp. 9% (n=209), *Staphylococcus aureus* 7.6% (n=173*), Pseudomonas aeruginosa 3.*7% (n=84), *Citrobacter* spp. 3.1% (70), *Staphylococcus saprophyticus* 1.8% (n=42), *Enterococcus faecalis* 0.8% (n=19) and *Acinetobacter* spp. 0.2% (n=5) (Table III).

Gram negative isolates showed higher sensitivity towards, Piperacillin+Tazobactum (67%), Amikacin (80%), Nitrofurantoin (82%), Aztreonam (100%), Imipenem (100 %) and Meropenem (100%) while they showed high degree resistance pattern against Penicillins, Cotrimoxazole, Ciprofloxacin, Norfloxacin and third generation Cephalosporin (Table IV). Gram-positive isolates showed good response towards Netilmicin (69%), Nitrofurantoin (79%), Linezolid (98%), Vancomycin (100%) and Teicoplanin (100%). The overall sensitivity of Nitrofurantoin is (81.5%). In case of individual sensitivity pattern of Nitrofurantoin, the Proteus group of organisms showed higher sensitivity i.e (88%) followed by *Escherichia. coli* (85%), *Staphylococcus aureus* (84%) and *Klebsiella pneumoniae* (69%), as shown in (Table V).

465 (23%) isolates were resistant to Penicillins, 1st generation and 2nd generation Cehalosporins, which were further tested by double disk and combined disk method for ESBL production. Out of 465 isolates, 375 were ESBL producers. Out of 375 ESBLs, 46% (n=172) were from ICU, 30% (n=113) were from Surgery Department, 12% (n=45) patients were from Gynae Department and 12 % (n=45) patients were from Medicine Department. Out of 375ESBLs isolates, there were 264 (70.6%). *Escherichia coli* and 111 (29.4%) *Klebsiellap neumoniae* (Table VI). Susceptibility of ESBL producers to Imipenem, Nitrofurantoin and Amikacin were found to be 100%, 76%, and 75% respectively (Table VII).

IV. Discussion

The changing trends in the aetiopatho genesis of urinary tract infections and increasing antimicrobial drug resistance are a matter of concern. Urethral catheterization and instrumentation related UTI is the most common nosocomial infection. The indiscriminate, inadequate usage of antibiotics has contributed to the emergence of resistance strains. Urine culture sensitivity is routinely done in suspected cases of UTI and empirical therapy should be started immediately and modified if required once the report of urine culture sensitivity is available [5].

The present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. *Escherichia. coli* 60.3% was the predominant pathogen followed by *Klebsiella pneumoniae* 13.5%, *Proteus* spp. 9%, *Staphylococcus aureus* 7.6%, *Pseudomonas aeruginosa* 3.7%, *Citrobacter* spp. 3.1%, *Staphylococcus saprophyticus* 1.8%, *Enterococcus faecalis*

0.8% and *Acinetobacter* spp. 0.2%. Results of this study are in concordance with the other studies [5], [8], [18]. Our study shows that females (66%) are more vulnerable to UTIs than males (34%) as they have shorter urethra and physiological changes [5], [7], [8].

The present study reveals that gram negative isolates showed good sensitivity to Piperacillin+Tazobactum (67%), Amikacin (80%), Nitrofurantoin (82%), Aztreonam (100%), Imipenem (100%) and Meropenem (100%); however, they showed heavy resistance to drugs like Penicillin, Co-trimoxazole, Ciprofloxacin, Norfloxacin and third generation Cephalospoirins. ESBL producers were highly sensitive to Carbapenems. Gram-positive cocci were resistance to Ampicillin and Amoxy – clav while they showed good susceptibility pattern towards Netilmicin (69%), Nitrofurantoin (79%), Linezolid (98%), Vancomycin (100%) and Teicoplanin (100%) [5], [8]. In present study, the overall sensitivity of Nitrofurantoin was 82%. In case of individual sensitivity pattern, the *Escherichia coli* showed highest sensitivity to Nitrofurantoin (85%) followed by, *Staphylococcus aureus* (84%) and *Klebsiella pneumoniae* (69%) [14], [15].

Most of the ESBLs were isolated from hospitalized patients. Out of 375 isolates, there were 264 (70.6%). *Escherichia coli* and 111 (29.4%) *Klebsiella pneumoniae* [17]. Susceptibility of ESBL producers to Imipenem, Nitrofurantoin and Amikacin were found to be 100%, 76% and 75%. Results of our study are in concordance with the other studies that showed similar type of sensitivity pattern in ESBLs [12], [13].

TABLE I
DISTRIBUTION OF CULTURE POSITIVE CASES ACCORDING TO AGE

Age group in Years	No. of cases
<20	164 (7.2%)
21-30	1269 (55.4%)
31-40	596 (26%)
41-50	100 (4.4%)
51-60	104 (4.5%)
>60	57 (2.5%)
Total	2290 (100%)

TABLE II
DISTRIBUTION OF CULTURE POSITIVE CASES ACCORDING TO SEX

Gender	No. of cases
Female	1512 (66%)
Male	778 (34%)
Total	2290 (100%)

TABLE III
DISTRIBUTION OF CULTURE POSITIVE ISOLATES

Name of the organism	No. of cases
Escherichia. coli **i**	1378 (60.3%)
Klebsiella pneumoniae	310 (13.5%)
***Proteus* spp.**	209 (9%)
Pseudomonas aeruginosa	84 (3.7%)
Citrobacter spp.	70 (3.1%)
***Acinetobacter* spp.**	5 (0.2%)
Staphylococcus aureus	173 (7.6%)
Staphylococcus saprophyticus	42 (1.8%)
Enterococcus faecalis	19 (0.8%)
Total	2290 (100%)

TABLE IV
ANTIMICROBIAL SENSITIVITY PATTERN (% AGE) OF 2056 GRAM NEGATIVE BACILLI ISOLATED FROM URINE SAMPLES

Name of antimicrobial agent	*E. coli*	*Klebsiella pneumoniae*	*Proteus* spp.	*Ps. aeruginosa*	*Citrobacter* spp.	*Acinetobacter* spp.	Total
Ampicillin	120 (8.7%)	15 (4.8%)	10 (4.7%)	NIL	10 (14.2%)	NIL	155 (7.5%)
Amoxy–clav	266(18.8%)	108 (35%)	20 (9.5%)	45 (54%)	40 (57%)	NIL	473 (23%)
Amikacin	1090 (79%)	252 (81%)	200 (96%)	45 (54%)	48 (69%)	3 (60%)	1638 (80%)
Gentamicin	659(48%)	152 (49%)	190 (91%)	20 (24%)	40 (57%)	NIL	1061 (52%)
Ciprofloxacin	600(44%)	130 (42%)	170 (81%)	32 (38%)	42 (60%)	NIL	974 (47%)
Levofloxacin	690(50%)	98 (32%)	90 (43%)	30 (36%)	46 (66%)	NIL	954 (46%)
Ofloxacin	810(59%)	97 (31%)	89 (43%)	31 (37%)	46 (66%)	NIL	1073 (52%)
Norfloxacin	663(48%)	152 (49%)	113 (54%)	44 (52%)	42 (60%)	NIL	1014 (49%)
Cefatzidime	790(57%)	168 (54%)	116 (56%)	46 (54%)	41 (58%)	NIL	1161 (56%)
Cefuroxime	690(50%)	152 (49%)	109 (52%)	43 (51%)	43 (61%)	2 (40%)	1039 (51%)
Cefotaxime	728(53%)	162 (52%)	106 (51%)	44 (53%)	42 (60%)	2 (40%)	1084 (53%)
Ceftrioxane	714(52%)	159 (51%)	111 (53%)	46 (54%)	48 (69%)	2 (40%)	1080 (53%)
Cefepime	ND	ND	ND	84 (100%)	ND	ND	%
Piperacillin+ Tazobactem	820(60%)	258 (83%)	172 (82%)	72 (86%)	60 (86%)	NIL	1382 (67%)
Nitrofurantoin	1173(85%)	214 (69%)	ND	ND	60 (86%)	NIL	1692 (82%)
Cotrimoxazole	312 (22%)	98 (32%)	20 (9.5%)	NIL	20 (29%)	NIL	450 (22%)
Imipenem	1378 (100%)	310 (100%)	209 (100%)	84 (100%)	70 (100%)	5(100%)	2056 (100%)
Meropenem	1378 (100%)	310 (100%)	209 (100%)	84 (100%)	70 (100%)	5 (100%)	2056 (100%)
Aztreonam	1378 (100%)	310 (100%)	209 (100%)	84 (100%)	70 (100%)	5(100%)	2056 (100%)

V. CONCLUSION

A large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections. As a result, the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide.

The current study elaborates different antimicrobial susceptibility pattern among uropathogens. High degree of resistance was found for organisms belonging to family *Enterobacteriacae* towards Cotrimoxazole, Fluroquiolones and Cephaolsporins. However, these organisms showed good

response to antibiotics like Amikacin, Nitrofurantoin, Piperacillin + Tazobactam and Carbapenems. Nitrofurantoin, which is an under used antimicrobial agent for empiric therapy of acute lower UTI is a very cheap and effective drug. ESBL producing organisms pose a major problem in treatment so misuse of extended spectrum Cephalosporins should be avoided. Therefore, it can be concluded from the present study that the drug resistance among pathogens is an evolving process, therefore routine surveillance and clinical trials should be done regularly with the assistance of treating physicians to reach the most effective empirical treatment.

TABLE V
ANTIMICROBIAL SENSITIVITY PATTERN (% AGE) OF 234 GRAM POSITIVE COCCI ISOLATED FROM URINE SAMPLES

Name of antimicrobial Agent	*Staph. aureus*	*Staph. saprophyticus*	*Enterococcus faecalis*	Total
Ampicillin	60 (35%)	20 (48%)	5 (26%)	85 (36%)
Amoxy – clav	102 (59%)	26 (62%)	13 (68%)	141 (60%)
Amikacin	120 (69%)	28 (67%)	10 (53%)	158 (68%)
Gentamicin	115 (66%)	25 (60%)	9 (47%)	149 (64%)
Netilmicin	130 (75%)	32 (76%)	ND	162 (69%)
Erythromycin	95 (55%)	19 (45%)	9 (47%)	123 (53%)
Clindamycin	120 (69%)	ND	ND	120 (51%)
Nitrofurantoin	145 (84%)	31 (74%)	10 (53%)	186 (79%)
Linzeolid	168 (97%)	42 (100%)	19 (100%)	229 (98%)
Vancomycin	173(100%)	42 (100%)	19 (100%)	234 (100%)
Teicoplanin	173 (100%)	42 (100%)	19 (100%)	234 (100%)

TABLE VI
DISTRIBUTION OF 375 ESBLS

Name of the organism	No. of ESBL detected
E. coli	264 (70.6%)
Klebsiella pneumonia	111 (29.4%)
Total	375 (100%)

TABLE VII
ANTIMICROBIAL SENSITIVITY PATTERN (% AGE) OF 375 ESBLS

Name of the Antibiotic	*E. coli*	*Klebsiella pneumoniae*	Total
Amikacin	205 (79%)	75 (68%)	280 (75%)
Gentamicin	160 (61%)	43 (39%)	203 (54%)
Ciprofloxacin	138 (52.2%)	43 (39%)	181 (50%)
Piperacillin + Tazobactum	191 (72.3%)	49 (44%)	240 (64%)
Nitrofurantoin	210 (80%)	76 (68.4%)	286 (76%)
Imipenem	264 (100%)	111 (100%)	375 (100%)
Meropenem	264 (100%)	111 (100%)	375 (100%)
Aztreonam	264 (100%)	111 (100%)	375 (100%)

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