

Prevalence and Antibiotic Susceptibility Pattern of Bacterial Isolates from Urinary Tract Infections in a Tertiary Care Hospital in Tamilnadu

Dr. Mane Manisha S.¹, Dr. Sandhya Bhat K², R. Lakshmi Priya³,
Jesu Magdalene⁴

Associate Professor, Dept of Microbiology, ESIC Medical College & PGIMS, K K nagar, Chennai-78. (TN)

Associate Professor, Dept of Microbiology, Pondicherry Institute Medical Sciences, Pondicherry.

Tutor, Dept of Microbiology, ESIC Medical College & PGIMS, K K nagar, Chennai-78. (TN)

Tutor, Dept of Microbiology, ESIC Medical College & PGIMS, K K nagar, Chennai-78. (TN)

Abstract

Introduction: Urinary Tract Infections are one of the most common bacterial infections in developing countries, ranging from asymptomatic bacteriuria to severe urosepsis. It is a leading cause of hospital-acquired infections contributing approximately 35% of all nosocomial infections in many hospitals. Predominant uropathogens are gram negative bacteria and *Escherichia coli* is accounting for the highest prevalence in most instances. Widespread use of antimicrobial agents has led to the emergence of antibiotic resistant pathogens; also there is increase demand for new effective drugs.

Materials and Methods: This study was undertaken for a period of one and half years. Clean catch mid stream urine samples were collected from the all suspected UTI patients attending using sterile screw capped containers. The urine samples were processed for aerobic culture and susceptibility testing according to standard guidelines. Isolates were screened for ESBL production.

Results: A total of 7,868 samples were collected, of which 4,833 (61%) were from females and 3,035 (39%) were from males. Overall prevalence rate of UTI was 32%. The prevalence of UTI in females was 38% and 22.4% in males. 66% out of 71% of *E.coli* and 85% of 87% *Klebsiella* were confirmed to as ESBL producing strains by phenotypic confirmatory disc diffusion test.

Discussion and Conclusion: Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic. Hence it is now necessary to use these antibiotics with utmost care and also develop new antimicrobials having high effectiveness with minimal/ no side effects, freely available and less expensive.

Key words: Antibiotic resistance, Cephalosporins, UTI, ESBL, Uropathogens

I. Introduction

Urinary Tract Infections are one of the most common bacterial infections in many developing countries in routine clinical practice, ranging from asymptomatic to severe sepsis [1]. UTI is one of the most important causes of morbidity in general population, and is the second most important cause of hospital visits [2]. It also contributes as the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital-acquired infections[3,4]. This burden causes serious impact on the socioeconomic life of individuals and also leads to a large proportion of antibacterial drug consumption [5].

Generally, the predominant uropathogens for UTIs are gram negative bacteria and *Escherichia coli* accounting for the highest prevalence in most instances [6]. Other less commonly involved urinary pathogens are *Klebsiella* spp., *Proteus* spp., *Staphylococcus aureus*, *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterococcus* spp., *Candida albicans*[7]. UTI cases are treated with different broad spectrum antibiotics empirically and definitive therapy is based on information obtained from the antimicrobial susceptibility pattern of the urinary pathogens [2,8]. Widespread use of antimicrobial agents has led to the emergence of antibiotic resistant pathogens; also there is increase demand for new drugs [9].

Due to the high incidence of UTIs in general population, the potential for complications, especially in high risk groups, associated costs of treatment and rising antibiotic resistance among uropathogens, it is important to have local hospital based knowledge of the organisms causing UTI and their antibiotic sensitivity patterns. Hence this study was conducted to find out the common bacteria causing UTI and to determine the antibiotic susceptibility pattern of the urinary pathogens.

II. Materials And Methods

This study was undertaken for a period of one and half years from March 2013 to August 2014 at Department of Microbiology, ESIC Medical College- PGI MSR, KK Nagar, Chennai, Tamilnadu, India. Clean catch mid stream urine samples were collected from the all suspected UTI patients attending to OPD/IPD of various departments of ESIC Medical College- PGI MSR, KK Nagar, Chennai, using sterile screw capped containers. The name, age, sex, clinical history and treatment history were recorded. If there were two or more episodes of UTI for the same patient, either due to prolonged hospitalizations, each episode was considered as a separate case of UTI. The patients who had symptoms and/ or signs suggestive of UTI were included in the study.

Bacterial Isolates: The urine samples collected were examined microscopically for pus cells and casts and then were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar medium. Inoculated agar plates were incubated aerobically at 37 °C for 24 hours. The urine culture plates were examined for pure growth. Next day individual colonies were identified on the basis of colony morphology, gram staining and biochemical characteristics [10]. Culture results were interpreted as being significant and insignificant, according to the standard criteria. A growth of $\geq 10^5$ colony forming units/mL was considered as significant bacteriuria [11]. Patients with significant bacteriuria and symptomatic patients with lower colony counts were also considered. Cultures with more than two colonies were considered as contaminants and such samples were discarded.

Antimicrobial susceptibility testing: Antibiotic susceptibility tests and interpretations for the bacterial isolates were carried out by Kirby- Bauer disk diffusion technique on Mueller Hinton agar (Hi-Media), by following the zone size criteria as per standard guidelines. The diameters of the zones of inhibition were measured by measuring calipers [12,13]. The antimicrobial agents tested were gentamicin (30µg), amikacin (30 µg), piperacillin/tazobactam (100/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ceftazidime+ clavulanic acid (30/10 µg), amoxycylav (20/10 µg), co-trimoxazole (25 µg), norfloxacin (10 µg), ciprofloxacin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg) for all gram negative bacterial isolates and amoxycylav (20/10 µg), linezolid (30 µg), azithromycin (15 µg), cefotaxime (30 µg), norfloxacin (10 µg), ciprofloxacin (10 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), vancomycin (30 µg), cefoxitin (30 µg), piperacillin/tazobactam (100/10 µg), co-trimoxazole (25 µg) and nitrofurantoin (300 µg) for all gram positive isolates [13].

Criteria for the selection of the ESBL producing strains: The isolates were tested for their susceptibility to the third generation cephalosporins (3GCs) e.g. Ceftazidime (30 µg), Cefotaxime (30 µg) and Ceftriaxone (30 µg) by using the standard disc diffusion method, as was recommended by the CLSI. If a zone diameter of < 22 mm for Ceftazidime, < 27 mm for Cefotaxime and < 25 mm for ceftriaxone were recorded, the strain was considered to be “suspicious for ESBL production” [13].

The phenotypic confirmatory disc diffusion test (PCDDT): All the isolates were subjected to production of ESBL by using the PCDDT, as recommended by the CLSI. In this test, ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime +clavulanic Acid, 30/10 µg) discs, were applied onto a plate of Mueller Hinton Agar (MHA) which was inoculated with the test strain. An increase of ≥ 5 mm in the zone of inhibition of the combination discs in comparison to that of the ceftazidime disc alone was considered to be a marker for ESBL production [13].

Statistical analysis: The results were presented in terms of frequencies and percentages. The statistical analysis was performed by using the Chi-square test and a p value of less than 0.05 was considered as statistically significant.

III. Results

A total of 7,868 samples were collected in the study period of one and half year, of which 4,833 (61%) were from females and 3,035 (39%) were from males. Pathogenic bacteria were isolated from 2,518 samples with an overall prevalence rate of 32%. The prevalence in females was 38% (1,838/4,833) and the prevalence rate in males was 22.4% (680/3,035). Age and sex wise prevalence of UTI is displayed in Figure 1 and Table 1 & 2.

Table-1: Sex wise Distribution of Prevalence of Urinary Tract Infection

Sex	No. of Samples Tested	No. of Positive Samples
Female	4,833	1,838
Male	3,035	680

Table 2: Age and sex wise prevalence of UTI

Age (year)	Total No. of Samples with %		No. of Positive Samples with %		Prevalence	
	Male	Female	Male	Female	Male	Female
< 20	788	1466	140	411	17.77	28.04
21-40	978	1497	218	723	22.29	48.30
41-60	1002	1568	193	486	19.26	30.99
> 60	267	302	129	218	48.31	72.19
Total	3035	4833	680	1838	22.41	38.03

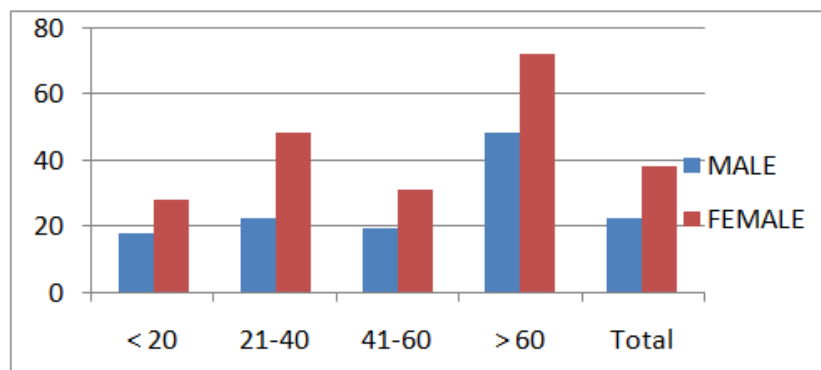


Figure1- Age and sex wise prevalence of UTI

Escherichia coli was the most frequently isolated as urinary pathogen (64%), followed by Klebsiella species (18%), Pseudomonas aeruginosa (3.5%), Proteus species (3.3%), Citrobacter species (2.5%), Enterococcus species (2.2%), Acinetobacter species (2.1%), Staphylococcus aureus(1.2%), Providencia species (1.1%), Morganella species (0.8%), Coagulase Negative Staphylococcus (CONS-0.8%)and Enterobacter species (13%) in decreasing order of frequency.

Frequency distribution of urinary isolates is shown in table No.3

Table 3: Frequency Distribution of Urinary Isolates

S.NO	Various Urinary pathogens isolated	Number	Percentage
1	Escherichia coli	1612	64%
2	Klebsiella species	453	18%
3	Pseudomonas aeruginosa	88	3.5%
4	Proteus species	83	3.3%
5	Citrobacter species	63	2.5%
6	Enterococcus species	55	2.2%
7	Acinetobacter species	53	2.1%
8	Staphylococcus aureus	30	1.2%
9	Providencia species	28	1.1%
10	Morganella species	20	0.8%
11	Coagulase Negative Staphylococcus	20	0.8%
12	Enterobacter species	13	0.5%
	TOTAL	2518	100%

The antibiogram of the frequently isolated gram negative uropathogens is shown in table 4.

Table 4: Antibiogram pattern of most frequently isolated gram negative urinary pathogens

Antimicrobial agents	E.coli (1612)			Klebsiella (453)			Pseudomonas (88)			Proteus (83)			Citrobacter (63)		
	S	IS	R	S	IS	R	S	IS	R	S	IS	R	S	IS	R
Gentamicin	452	53	1107	89	5	359	9	1	78	25	2	56	45	0	18
Amikacin	1002	52	558	398	0	55	80	2	6	72	0	11	55	0	8
Piperacillin/tazobactam	1469	9	134	403	3	47	82	0	6	83	0	0	63	0	0
Amoxycylav	369	0	1243	52	0	401	0	0	88	7	2	74	4	0	59
Co-trimoxazole	52	0	1560	45	0	408	5	0	83	24	0	59	8	0	55
Norfloxacin	1265	0	347	373	0	80	2	0	86	56	3	24	33	0	30
Ciprofloxacin	1268	2	342	375	3	75	8	0	80	56	5	22	35	0	28
Imipenem	1610	0	2	452	0	1	88	0	0	83	0	0	63	0	0
Nitrofurantoin	1358	0	254	407	0	46	0	0	88	75	0	8	56	0	7
Cefotaxime	467	0	1145	59	0	394	11	0	77	25	0	58	30	0	33
Ceftriaxone	468	0	1144	59	0	394	13	0	75	26	0	57	30	0	33
Ceftazidime	469	0	1143	60	0	393	15	0	73	25	0	58	30	0	33
Ceftazidime/clavulanic acid	1498	0	114	402	0	51	77	0	11	83	0	0	63	0	0

*S-Sensitive, IS- Intermediate Sensitive, R-Resistant

The antibiogram of the gram positive uropathogens is shown in table 5.

Table 5: Antibiogram pattern of gram positive isolates

Antibiotics	Enterococcus (55)			Staphylococcus aureus (30)			CONS (20)		
	S	IS	R	S	IS	R	S	IS	R
Gentamicin	1	0	54	17	3	10	2	0	18
Amikacin	2	0	53	25	1	4	12	0	8
Piperacillin/tazobactam	34	1	20	22	2	6	9	0	11
Amoxycylav	5	0	50	14	0	16	3	0	17
Co-trimoxazole	8	0	47	8	0	22	0	0	20
Cephalexin	0	0	55	5	0	25	4	0	16
Ciprofloxacin	3	0	52	14	0	16	2	0	18
Imipenem	32	0	23	24	0	6	13	0	7
Nitrofurantoin	4	0	51	15	0	15	3	0	17
Cefotaxime	12	0	43	19	0	11	6	0	14
Cefoxitin	5	0	50	16	0	14	8	0	12
Azithromycin	8	0	47	6	0	24	2	0	18
Linezolid	28	2	25	27	0	3	14	0	6
Vancomycin	49	1	5	30	0	0	15	0	5

Table 6: Antibiogram Pattern of Various Gram negative and Gram positive UTI isolates in Percentage:

Antibiotics	Gram Negative UTI Isolates (In Percentage) 2413			Gram Positive UTI Isolates (In Percentage) 105		
	S	IS	R	S	IS	R
Gentamicin	676 (28%)	63 (3%)	1674 (69%)	20 (19%)	3 (3%)	82 (78%)
Amikacin	1685(70%)	57 (2%)	671 (28%)	39 (37%)	1 (1%)	65 (62%)
Piperacillin/tazobactam	2200 (91%)	12 (0.4%)	201 (8.6%)	65 (62%)	3 (3%)	37 (35%)
Amoxycylav	467 (19%)	2 (0.001%)	1944 (81%)	22 (21%)	0	83 (79%)
Co-trimoxazole	188 (8%)	0	2225 (92%)	16 (15%)	0	89 (85%)
Norfloxacin	1803 (75%)	3 (0.1%)	607 (24.9%)	NT	NT	NT
Ciprofloxacin	1820 (75%)	12(0.4%)	581 (24.6%)	19 (18%)	0	86 (82%)
Imipenem	2410(99.9%)	0	3 (0.1%)	69(65%)	0	36(35%)
Nitrofurantoin	2010 (83%)	0	403 (17%)	22 (21%)	0	83 (79%)
Cefotaxime	668 (28%)	0	1745 (72%)	37 (35%)	0	68 (65%)
Ceftriaxone	672 (28%)	0	1741 (72%)	NT	NT	NT
Ceftazidime	674 (28%)	0	1739 (72%)	NT	NT	NT
Ceftazidime/clavulanic acid	2227 (92%)	0	186 (8%)	NT	NT	NT
Cefoxitin	NT	NT	NT	29 (28%)	0	76 (72%)
Azithromycin	NT	NT	NT	16 (15%)	0	89 (85%)
Linezolid	NT	NT	NT	69 (66%)	5 (4%)	31 (30%)
Vancomycin	NT	NT	NT	94 (90)	1 (1%)	10 (9)

*NT-Not Tested

Table 7:ESBL isolates among E.coli and Klebsiella species

Antibiotics	E.coli (1612)		Klebsiella (453)		P value
	Sensitive Isolates	Resistant isolates	Sensitive Isolates	Resistant isolates	
Cefotaxime	467 (29%)	1145 (71%)	59 (13%)	394(87%)	<0.001
Ceftriaxone	468 (29%)	1144 (71%)	59 (13%)	394(87%)	<0.001
Ceftazidime	469 (29%)	1143 (71%)	60 (13%)	393 (87%)	<0.001
Ceftazidime/clavulanic acid	1498 (93%)	114 (7%)	402 (88.7%)	51 (11.3%)	<0.05

The antimicrobial potency of 13 selected antimicrobial agents against most frequently isolated 5 gram negative uropathogens and 14 selected antimicrobial agents against gram positive uropathogens are summarized in table 4 and 5 respectively.

Nearly all the isolates (gram negative and gram positive) were found to be resistant against most of the antibiotics. Overall gram negative pathogens showed more resistance as compared to gram positive organisms.

Among gram negative bacteria *Pseudomonas aeruginosa*, *E.coli* and *Klebsiella* spp.were most resistant isolates against tested antibiotics. Among gram positive bacteria *Enterococcus* spp. showed highest resistance followed by *CONS*.

The resistance pattern among gram negative isolates was comparably high for antimicrobial agents like co-trimoxazole, amoxyclav, ceftriaxone, cefotaxime, ceftazidime and gentamicin. the resistance pattern of other antimicrobial agents like amikacin, norfloxacin, ciprofloxacin, nitrofurantoin, piperacillin/tazobactam, ceftazidime/clavulanic acid and imipenem were comparably low. Among all tested antibiotics imipenem showed lowest resistance (0.1%)

The resistance pattern among gram positive isolates was comparably high for antimicrobial agents like azithromycin,co-trimoxazole, ciprofloxacin, amoxyclav, cefoxitin, cefotaxime, gentamicin, amikacin and nitrofurantoin . The resistance pattern of other antimicrobial agents like vancomycin, linezolid, piperacillin/tazobactam, and imipenem were comparably low among all tested antibiotics vancomycin showed lowest resistance (9%).

Among most frequently isolated gram negative pathogens, 71% of *E.coli* isolates and 87% of *Klebsiella* isolates were resistant to all 3 third generation cephalosporins by ESBL screening test and all these isolates were 100% sensitive to Imipenem.66% out of 71% of *E.coli* and 85% of 87% *Klebsiella* were confirmed to as ESBL producing strains by phenotypic confirmatory disc diffusion test.

IV. Discussion

Bacterial uropathogens have the potentiality to change tissues of the urinary tract adjacent structures [14]. Early detection and selection of an appropriate effective antimicrobial agent is highly essential for effective management of patients suffering from UTIs to prevent any further complications. Diagnosis and adequate management is only possible by close association between the clinician and microbiologist [2].

In our study the prevalence rate of isolation of urinary pathogen was 32%, which is consistent with study by Bhowmick B.K. et al [7], when compared to Das RN et al, wherein isolation rate was 71.6% [15]. Female is more prone to UTI for anatomic reasons; short and straight urethra and short distance between the ostium of the urethra and the anus contribute to easy colonization of the peri-urethral region with enteric bacteria [16]. In the present study infection rate is also higher in females (38%) than male patients (22.4%), which is consistent with study by Razak SK et al [2].

In present study, among patients with UTI, both females (39%) and males (32%) were most commonly affected in the age group between 21-40 years followed by 41-60 years age group. This correlates with studies done by Anbumani N et al [17]. UTI is more common among females of reproductive age group, who are sexually active and in older males due to prostate enlargement and other age related problems [18].

Escherichia coli is the most common isolated organism (64%) in our study followed by *Klebsiella* species (18%) among gram negative uropathogens, which is consistent with many other studies by Razak SK et al [2], Sibi et al [19]. Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. These gram-negative aerobic bacteria colonize the urogenital mucosa with adhesin, pili, fimbriae and PI-blood group phenotype receptor [15]. Among gram positive isolates *Enterococcus* is the most common isolated organism (2.2%), followed by *Staphylococcus aureus* (1.2%), which is consistent with study done by Das RN et al [15].

The antimicrobial sensitivity and resistance pattern varies from community to community and from hospital to hospital. This is because of emergence of resistant strains as a result of indiscriminate use of antibiotics. In our study gram-negative organisms showed following sensitivity pattern- co-trimoxazole (8%), amoxyclav (19%), cefotaxime (28%), ceftazidime (28%), ceftriaxone (28%), gentamicin (28%), amikacin (70%), norfloxacin (75%), ciprofloxacin (75%), nitrofurantoin (83%), piperacillin/tazobactam (91%), ceftazidime/clavulanic acid (92%), imipenem (100%).

According to Das RN et al [15], susceptibility pattern showed amikacin (87.2%), ciprofloxacin (74.8%), ceftazidime (71.5%), gentamicin (70.4%), nitrofurantoin (35%), and ampicillin (50.5%). according to supriya et al [20] susceptibility pattern showed, nitrofurantoin (62.5%), cefotaxime (58.7%), norfloxacin (44.9%), ampicillin (21.4%) and co-trimoxazole (18%).

In our study gram-positive organisms showed following sensitivity pattern- azithromycin (15%), co-trimoxazole (15%), ciprofloxacin (18%), gentamicin (19%), amoxyclav (21%), nitrofurantoin (21%), cefoxitin (28%), cefotaxime (35%), cefotaxime (35%), amikacin (37%), piperacillin/tazobactam (62%), imipenem (65%), linezolid (66%) and ceftazidime (85%), vancomycin (90%).

According to Gul N et al, susceptibility pattern of gram positive isolates was amoxicillin (53%), gentamicin (76%), norfloxacin (69%), ciprofloxacin (46%), co-trimoxazole (30%), lincomycin (15%) and amikacin (61%) [4].

In our study imipenem was found to be most sensitive followed by ceftazidime/clavulanic acid, piperacillin/tazobactam, nitrofurantoin, amikacin, norfloxacin and ciprofloxacin and ceftazidime, cefotaxime, ceftriaxone, co-trimoxazole, amoxyclav, gentamicin is found to be least sensitive. In Shobha KL et al [21] antibiotic sensitivity test performed for *Escherichia coli*, *Klebsiella* species showed lowest sensitivity to ciprofloxacin and nalidixic acid and highest sensitivity to imipenem 100%.

In our study 66% *Escherichia coli* and 85% *Klebsiella pneumoniae* isolates were found to be Extended Spectrum Beta Lactamases (ESBL) producers. This is much higher compared to studies done by Shobha KL et al [21] where 35% *Escherichia coli* and 41% *Klebsiella pneumoniae* were found to be ESBL producers and in Mohammed A et al [22] study 34.4% *Escherichia coli* and 27.3% *Klebsiella pneumoniae* were found to be ESBL producers.

ESBL production coexisted with resistance to several other antibiotics. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics. ESBL producers are multi drug resistant organisms [23]. Resistant organisms can pass their resistance genes to their offspring by replication or to related bacteria through conjugation. Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic [4]. Hence wide spread use of antibiotics should be monitored according to the real therapeutic need.

V. Conclusion

Present study showed that uropathogens have shown decreased susceptibility to most of the available antibiotics for treatment of UTI. Hence it is now necessary to use these antibiotics with utmost care and also develop new antimicrobials having high effectiveness with minimal/ no side effects, freely available and less expensive.

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Conflict of interest- Nil

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