(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 4, Issue 3, Page No: 392-401
May-June 2021

Uropathogens and Their Antibiotic Sensitivity and Resistance Pattern in South India

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

ABSTRACT

Introduction: Urinary tract infection is one of the most common infection burden faced by a community. It is the need of the hour to study the changing antibiotic sensitivity and resistance patterns of the uropathogens to correctly diagnose and prescribe the empirical therapy and decrease this burden.

Methods: Urine samples were collected from 1072 symptomatic patients and analysed. The uropathogens isolated were subjected to antibiotic sensitivity and resistance to find out the community prevalence and distribution of different uropathogens and their antibiotic sensitivity pattern.

Results: E coli was the most common uropathogen isolated(62.46%) followed by klebsiella (22.54%). The other pathogens isolated were Proteus mirablis, Pseudomonas, and others, constituting less than 10% of the total isolates. E coli was highly sensitive to Tigecycline (96.51%) and Imipenem (92.63%) and showed maximum resistance to Colistin (100%) and Ciprofloxacin (92.6%). ESBL positivity was highest amongst Klebsiella species accounting to around 34.4% and In E.coli, ESBL positivity rate was 8.5%.

Conclusion: There is a high degree of antibiotic resistance of uropathogens to commonly used antibiotics making empirical therapy ineffective. There is a need to determine the local antimicrobial susceptibility pattern to decrease the therapeutic failures.

Keywords: Uropathogens, Antibiotic Sensitivity, empirical therapy, UTI

INTRODUCTION

Urinary tract Infection(UTI) is amongst the one of the most common infectious diseases seen both in the community and nosocomial settings.(1) As per the report of the National Ambulatory Medical Care Survey, Nearly 7 million patients visit Outpatient department due to UTI and is responsible for nearly about 1 million hospitalizations.(2) In majority of the cases, treatment begins empirically, before the laboratory results of urine culture are available. To ensure appropriate treatment, current knowledge of the common uropathogens and their susceptibility to commonly prescribed antimicrobial agents is mandatory (3,4) Most of the physicians prescribe broad spectrum antibiotics in view of probable resistance of the organism. This along with poor patient compliance and incomplete course have resulted in widespread evolution of resistance of organism to multiple antibiotics.(5) As per the report of the survey conducted by the European Survey of Antibiotic Consumption, nearly 25000 deaths of the Europeans per year were accounted to Multi Drug Resistant bacterial Strains causing UTI and its various complications(6). It is absolutely important to prescribe appropriate antibiotic as a first choice empirical treatment of UTI. It is also important to note that the causative organism and its antibiotic sensitivity and resistance pattern vary from region to region.(7) Numerous studies done worldwide has shown changing patterns in the etiology of UTI and its antibiotic resistance pattern.(8) However, there are

very few studies on UTI, their etiology and resistance patterns in India.(9,10,11,12) As per the recommendations of the Infectious Diseases Society of America, regional surveillance should be conducted to monitor the changes in the susceptibility of uropathogens in specific regions.(13)

The aim of the present study was to determine the prevalence of UTI among the patients attending OPD, their clinical presentation and to study the antibiotic sensitivity and resistance patterns of the uropathogens against the commonly used antibiotics.

Patients and Methods: The prospective study was conducted from January 2019 to December 2020. Institutional Ethics committee approved the study. All the adult patients visiting the OPD or IPD patients with symptoms of UTI were included in the study. UTI was confirmed by a positive urine culture reports. Patients who had symptoms of UTI but a negative culture report were excluded from the study. The patients who had prior antibiotic treatment, Gross hematuria or structural urinary tract anomaly were excluded from the study. Data was collected using a proforma documenting basic demographic and clinical data.

5-10 ml of single clean catch midstream urine was collected from symptomatic patients. A total of 1072 urine samples were collected. The container was labelled and immediately transported to the laboratory and processed without delay. All samples were processed for macroscopic appearance and then wet mount was prepared for cytological study. Each urine sample was subjected to culture by the standard loop method. The samples were inoculated on blood agar, Mac conkey agar and Urichrome (Himedia) agar. Colony counts of more than 10^5 CFU/ml were considered as significant bacteruria.

For Gram negative isolates, a typical lactose fermenting colony was subcultured into peptone water and the following tests were performed: Oxidase test, Catalase test, Hanging drop (for motility) Indole test, Methyl red ,Voges Proskauer test, Simmons'Citrate utilization test, Christen's Urease test, Triple sugar iron test, Phenylalanine Deaminase Test, Amino acid decarboxylase test: Lysine, Arginine and Ornithine, Hugh Leifson's Oxidation/Fermentation test, Nitrate reduction test, Sugar fermentation tests for the following sugars: Glucose, Lactose, Sucrose, Maltose, Mannitol, and Xylose .

For Gram positive isolates: following tests were performed for identification and species differentiation of organisms: Catalase test, Coagulase test, OF sugars, Potassium tellurite agar, Bile esculin agar, Heat resistant test at 60^0C, Arabinose fermentation test (for Enterococci).

Antibiotic sensitivity was tested by Kirby- Bauer's disk diffusion method. Muller- Hinton agar plate was used Commercially obtained HiMedia discs were used. The strength of discs used and their zone size interpretative standards were according to guidelines by Clinical and Laboratory Standards Institute (CLSI)(14). The drugs used for Gram positive organisms were Ampicillin 10mcg, Gentamycin 10 mcg, Amikacin 30 mcg, Nitrofuarntoin 30 mcg, Ciprofloxacin 5 mcg, Linezolid 30 mcg. Amoxyclavunate 10 mcg, Cefoxitin 30 mcg, Vancomycin 30 mcg and Tigecyclin 15 mcg.

The drugs used for Gram negative organisms were Amikacin 30mcg, Genatmycin 10 mcg, Ciprofloxacin 5 mcg, Ceftazidime 30 mcg, Cefotaxime 30 mcg, Ceftriaxone 30 mcg, Imipenem 10 mcg, Colistin 10 mcg, Tigecyclin 15 mcg. Isolated Staphycococcal strains were subjected to further testing for Methicillin resistance. Potentiated disc diffusion tests were done for Gram negative bacilli to assess extended spectrum of beta lactamase resistance.

STATISTICAL ANALYSIS:

The data obtained was spread in Microsoft excel sheets and analysed using SSPE Software – version 20. Data was analysed with appropriate statistical methods. Age, gender, Symptomatic presentation, Organisms isolated and their antibiotic sensitivity and resistance patterns were evaluated.

A total of 1072 urine samples were screened for pathogenic organisms. 413 samples were culture positive indicating that only 38.52% of the urine samples collected were culture positive, 37.5% in females and 41.6% in males. Incidence of UTI was more common among females accounting to about 74.09% and males constituted only 25.9% of the cases of UTI. (Table 1). The incidence of UTI was found to be more between the ages 31-40 years accounting to more than 63% of the total UTI.

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Table 1: Age and gender wise distribution

Age(yrs)	Females(n=815)	Culture positive	Males (n=257)	Culture positive
18-25	93	22 (23.65%)	29	0 (0%)
26-30	112	19 (16.96%)	27	9(33.3%)
31-35	157	73 (46.49%)	62	33 (53.22%)
36-40	218	112 (51.37%)	57	45 (78.94%)
41-45	139	41 (29.49%)	45	20 (44.44%)
46-50	96	39 (40.62%)	37	00 (0%)
TOTAL	**815**	**306 (37.54%)**	**257**	**107(41.63%)**

Most of the organisms isolated were gram negative bacteria (92.4%) with E coli around 62.46% followed by K.pneumoniae (22.5%), Proteus mirabilis (7.5%), and 7.5% Gram positive bacteria i.e., Staphylococcus aureus (5.08%) and Enterococcus faecalis (2.42%). (Table 2)

Table 2: Organisms isolated (n=413)

Organism isolated	Total No	Percentage
Escherichia coli	258	62.46%
Klebsiella pneumonia	93	22.51%
Proteus mirabilis	13	7.50%
Pseudomonas	6	3.14%
Citrobacter	5	1.21%
Enterobacter	7	1.69%

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Staphylococcus aureus	21	5.08%
Enterococcus faecalis	7	1.69%
Streptococcus species	3	0.72%

Table 3: Antibiotic susceptibility pattern of Gram Negative bacteria

Sl No.	Antibiotic	No. of isolates tested	Sensitive		Resistance	
			Number	%	Number	%
1	Amikacin (AK)	382	227	59.46%	155	40.54%
2	Gentamycin (GEN)	382	62	16.22%	320	83.78%
3	Ciprofloxacin (CIP)	382	31	8.11%	351	91.89%
4	Ceftazidime (CAZ)	382	63	16.49%	319	83.5%
5	Cephatoxime (CTX)	382	93	24.32%	289	75.68%
6	Ceftrioxne (CTR)	382	145	37.95%	237	62.04%
7	Imipenem (IPM)	382	351	91.89%	31	8.11%
8	Colistin (CL)	382	124	32.46%	258	67.53%
9	Tigecycline (TGC)	382	299	78.27%	83	21.72%

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Most of the isolates were sensitive to Imipenem (91.89%), followed by Tigecycline (78.27%) and resistant to Ciprofloxacin (91.89%) followed by Gentamycin (83.78%). Resistance to Colistin was found to be 67.53%. (Table 3)

Table 4: Antibiotic sensitivity pattern from Gram positive bacteria

Sl. No	Antibiotic	No. of isolates tested	Sensitive		Resistance	
			Number	%	Number	%
1	Ampicillin (AM)	31	16	51.61%	15	48.38%
2	Gentamycin (GEN)	31	11	35.48%	20	64.51%
3	Amikacin (AK)	31	22	70.96%	09	29.03%
4	Nitrofuration (NIT)	31	19	61.29%	12	38.70%
5	Ciprofloxacin (CIP)	31	09	29.03%	22	70.96%
6	Linazolid (LEZ)	31	14	45.16%	17	54.83%
7	Amoxyclavunate (AMC)	31	23	74.19%	8	25.80%
8	Cefoxitin (CX)	3	29	93.54%	2	6.45%
9	Vancomycin (VA)	31	30	96.77%	1	3.23%
10	Tigecycline (TGC)	31	21	67.74%	10	32.25%

Tigecycline. (Table 4)

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Table 5: Prevalence of ESBL in Gram negative bacteria

Organisms	**Sensitive to 3rd generation cephalosporins**	**Resistance to 3rd generation cephalosporins**	**ESBL positive**
E. Coli (n=258)	184 (71.32%)	74 (28.68%)	22 (8.5%)
K. pneumoniae (n=93)	31 (33.33%)	60 (66.67%)	32 (34.40%)
Total : 351	**215 (61.25%)**	**134 (38.17%)**	**54 (15.38%)**

134 isolates were tested for ESBL producers. Out of this, 54 isolates (15.38%) were ESBL producers and remaining were non ESBL producers and they are not inhibited by clavulanic acid. K. pneumonia showed highest percentage (34.4%) of ESBLs. (Table 5)

DISCUSSION:

Urinary tract infection remains the most common bacterial infection even after the widespread use of antibiotics.(15) In the present study, 1072 subjects were included based on the clinical symptoms but only 413 samples i.e., 38.5% were culture positive, indicating that the diagnosis of UTI just on the basis of clinical signs and symptoms is not accurate and culture is an essential step in the definitive diagnosis of UTI. This is supported by a study by Ahmed SS et al (2019)(16) and Eshwarappa M et al (2011)(9) where majority of the urine samples collected on the basis of symptoms were culture negative. The low culture positivity rate might be because of the non specific symptoms of UTI like fever and abdominal pain. A combination of two or more symptoms might result in better estimation of the incidence of UTI. A point to be noted here is that even though the percentage of culture positive samples are less, still there is a high incidence of UTI, even after promoting hygienic habits, improving medical services and hospital care and better diagnostic and treatment options.

In the present study, 75% were females and 25% were males. Female to male ratio was 3:1. The result correlated well with the studies conducted by several other authors. The study conducted by Ahmed SS et al(16) in 2019 showed that the ratio of females patients with UTI were more than the males. Also ZR Khamenah(17) in 2009 demonstrated in his study that 81.6% of the patients affected by UTI were females. The higher prevalence of UTI on females might be due to numerous factors that predispose women to infection.(18) It includes the closeness between the female genital tract and the urethra(19) and the adherence of the mucopolysaccharide lining to the urothelial mucosa.(20) Not only the pregnancy and the sexual activity, but also menstrual unhygienic practices and birth control diaphragms also contribute to increased incidence of UTI in females(21,22,23)

In our study, we have observed that UTI was most commonly found in the age group of 31-40 years. The observation correlated well with the study conducted by Akram M et al(12) in 2007 where most cases of UTI were recorded among young and middle aged patients (20-49 years). Similarly Dimitrov et al(24) in 2004 reported significant bacteruria among young and middle aged patients (20-40 years)

The most common organism isolated from the samples in the present study was E.coli (62.5%) followed by Klebsiella species (22.5%). Our results

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correlated with a number of studies conducted worldwide.(9,10,12,16,17,24,25) The other uropathogens isolated from the samples were Proteus mirabilis, Citrobacter, Staphylococcus aureus, Enterococcus faecalis, Streptococcus species and Enterobacter. The uropathogen profile is similar to that obtained by M sharifain(26) et al in his study conducted on 1177 patients in 2006. The uropathogenic profile also correlated with the study conducted in India on 1410 patients in 2002 by V Gupta et al(27)

Even though the uropathogen profile has remained similar, but the incidence and prevalence of the uropathogens varies not only from region to region but also from time to time. But E coli has remained first in the list of uropathogens for decades.

Table 6: Area wise prevalence of E coli and Klebsiella in UTI

Year	E.Coli %	Klebsiella%	Region
2021	36.11	18.06	Gujrat(28)
2018	40	17	West Bengal(15)
2015	52.4	12.3	Uttar Pradesh(29)
2011	67	15.5	Karnataka(9)
2009	71.3	13.5	Tamil Nadu(10)
2008	68	16.9	Delhi(30)

It is also observed that even though the uropathogenic profile is almost similar in all the studies, there is a drastic change in the antibiotic sensitivity and resistance patterns. In the present study, we have observed a high degree of resistance to Ciprofloxacins, cephalosporins and gentamycin, which remained the first choice of drugs in UTI for several decades. The susceptibility of E coli to Ciprofloxacin has decreased from 28% in 2008(10) to 7.36% in our present study. Gentamycin resistance has increased from 49.2% in 2011(9) to 83.72% in the present study. E coli demonstrated maximum resistance to colistin, which was a highly effective drug back in time. As per the present study, highly effective drugs were Tigecyclin, Imipenem and Amikacin. From the above result, it is quite evident that uropathogens are becoming more and more resistant to oral antibiotics making the treatment of UTI more and more difficult.

Klebsiella was the second most common uropathogen isolated which showed higher degree of resistance to commonly used antibiotics like ciprofloxacin gentamycin and cephalosporins. It was highly sensitive to Colistin demonstrating significant difference in the antibiotic sensitivity and resistance patterns amongst the two main uropathogenic organisms. It was also sensitive to Imipenem, Tigecyclin and Amikacin.

Prevalence of ESBL in gram negative bacteria was also tested which showed that 54% of the isolates tested were ESBl positive with 34% positivity rate amongst the Klebsiella species and 8% in E coli. This indicates that ESBL prevalence is more common in Klebsiella species. Our result doesnot correlate with the study conducted by Eshwarappa et al where 42.2% of the E coli were ESBL positive and only 9.6% of Klebsiella species were ESBL positive. This discrepancy in the result might be because we had excluded samples from complicated UTI cases from our study group, indicating that ESBL posivity rate may vary amongst the uropathogens causing complicated and uncomplicated UTI.

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Based on our results, empirical therapy with quinolones and cephalosporins may not result in treatment of UTI, rather increases the development of resistant strains. The most beneficial drug, active against most uropathogens is Amikacin. Still, waiting for a urine culture and sensitivity report and later prescribing an antibiotic based on it would definitely be a better option in treating UTI rather than starting an empirical therapy.

CONCLUSION:

Diagnosis of UTI only on the basis of clinical signs and symptoms is not adequate and requires Urine culture report for confirmation of the diagnosis. E.coli is still the most common uropathogen isolated from UTI patients in a community, followed by Klebsiella. There is also an increase in the rate of resistant ESBL species. The trend of empirically treating UTI may not be affective in South India due to varying degrees of resistance of common uropathogens. There is a need for larger studies to frame specific region based guidelines for treatment of UTI, failing which there is high chance of development of multidrug resistant uropathogens, posing a serious threat to the community.

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