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Original Research Article

Evaluation of uropathogens isolated in the outpatient department of a tertiary care hospital in south India

Farhat Subhaana Chilakapati[1], Sandeep Kumar Tipparthi[1], Ravi Shankar Reddy A[1], Raj Kumar H.R.V[1], Guru Prasad Manderwad[1,*], Pradeep Kumar Reddy Sripathi[1]

[1] *Dept. of Microbiology, Kamineni Academy of Medical Sciences and Research Center, Hyderabad, Telangana, India*

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ABSTRACT

Background: Urinary tract infections constitute a large percentage of cases encountered in outpatient departments at tertiary care hospitals. These infections have been associated with a rise in antimicrobial resistance, leading to high morbidity and mortality. This rise in drug resistance has been linked to an increase in unauthorized, unregularized use of higher generations of antibiotics, leading to the prevalence of multi drug resistant organisms in the community.
Objective: The aim of this study is to analyze the prevalence of various uropathogens in the general population, along with their antibiotic susceptibility patterns.
Materials and Methods: The study has been carried out for a period of six months, during which a total of 872 urine samples were collected from the outpatient department at Kamineni Academy of Medical Sciences and Research Center, Hyderabad. Urine samples were subjected to bacterial culture and significant bacterial growth was identified using the colony count method. Identification and antimicrobial susceptibility testing was done using an automated VITEK 2 compact system.
Results: A total of 236 cases (27.1%) showed significant growth of uropathogens. The common organisms isolated were *E.coli, K. pneumoniae, and Pseudomonas aeruginosa.* These organisms were seen more commonly in females. Multidrug resistance has been noted in multiple uropathogens, especially to fluoroquinolones and cephalosporins.
Conclusion: This study documented an increase in antimicrobial resistance of the uropathogens isolated at the outpatient department. Judicious use of antibiotics may aid in prevention of spread of antimicrobial drug resistance in the general population.

1. Introduction

Urinary tract infections (UTIs) are one of the most common infections occurring in the society, and they are especially prevalent in South Asian countries. A study conducted from Global Prevalence Study on Infections in Urology (GPIU) for a period of 10 years concluded that UTIs have been associated with high antimicrobial resistance to broad spectrum antibiotics and emphasized the need for proper management of UTIs, especially in Asian countries.[1] A study from African countries also projected the association of UTI isolates with high bacterial drug resistance. The nation of Ethiopia conducted a laboratory cross sectional study and found the presence of uropathogens susceptible only to Gentamicin and Chloramphenicol.[2]

India, being one of the most populous nations in the world, has been associated with a high percentage of UTIs being caused by drug resistant organisms. Among

* *Corresponding author*.
E-mail address: gurukmc@gmail.com (G. P. Manderwad).

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several population studies performed in different regions of India, isolation of various bacteria with completely different antibiotic susceptibility patterns has been shown. A study from Maharashtra revealed the isolation of *E.coli* associated with high drug resistance to fluoroquinolones, Amoxicillin and third generation cephalosporins, and emphasized the need for timely surveillance and monitoring.[3]

A study conducted by Jannifer, et al. in Chennai, which contradicted the study in Maharashtra, revealed uropathogens that were sensitive to third generation cephalosporins.[4] A study of comparative analysis of prevalence of antimicrobial resistance among community acquired UTIs from northern and southern states of India revealed the isolation of different bacteria as the primary pathogens of UTIs associated with different bacterial drug resistance patterns. The study authors also emphasized the need of development of regional surveillance programs for proper management and treatment of UTIs.[5]

In recent times, an increase in the number of patients with UTI-related complaints visiting outpatient departments (OPDs) has been noted. Studies found a substantial increase in the pattern of drug resistance in uropathogens in OPD cases due to the inappropriate use of empirical therapy in treating these infections, as well as occasional overtreatment.

A study done in Telangana, a state in southern India, found that UTIs have been associated with multidrug-resistant uropathogens, as well as with comorbidities such as diabetes, hypertension and chronic kidney disease.[6] The aim of the study is to evaluate the prevalence of bacterial pathogens associated with UTIs, along with the minimum inhibitory concentration (MIC) determining resistance to various antibiotics, especially in reference to the outpatient department.

2. Materials and Methods

2.1. Study design and setting

A prospective study was conducted at the Department of Microbiology at Kamineni Academy of Medical Sciences and Research Center, Hyderabad. The study evaluated urine samples from the outpatient department for bacterial growth and minimum inhibitory concentration (MIC) over a period of 6 months from September 2022 to February 2023. A total of 872 urine samples were evaluated, and VITEK 2 compact systems were used for identification and evaluation of antimicrobial susceptibility of causative pathogens.

2.2. Measurements

A urinary tract infection is defined as detection of $\geq 10^5$ colony forming units (CFUs)/mL of bacteria in a mid-stream urine sample.[7] To differentiate true bacteriuria from bacterial contamination that arises due to a faulty collection technique of urine samples, a quantitative measurement method must be used. Viable bacterial colonies are numerically counted per milliliter of urine by a technique known as the colony count method.

2.3. Collection and processing of urine samples

For proper assessment of true bacteriuria, a mid stream urine sample is collected in a sterile disposable universal container. Significant or non-significant growth of urine culture was reported by the colony count method. Urine samples were inoculated on a culture media (urochrome agar) using a calibrated (1 μL) loop. Culture plates were incubated for 18 hours in ambient air at 35–37°C. Bacterial colonies were visualized in the culture plates. (Figures 1, 2 and 3)

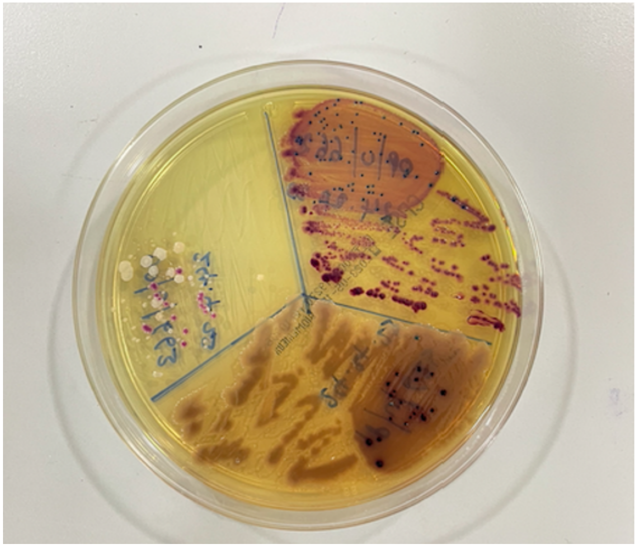

Fig. 1: Urochrome agar showing growth of *E. coli* (right) and *Pseudomonas* aeruginosa (bottom)

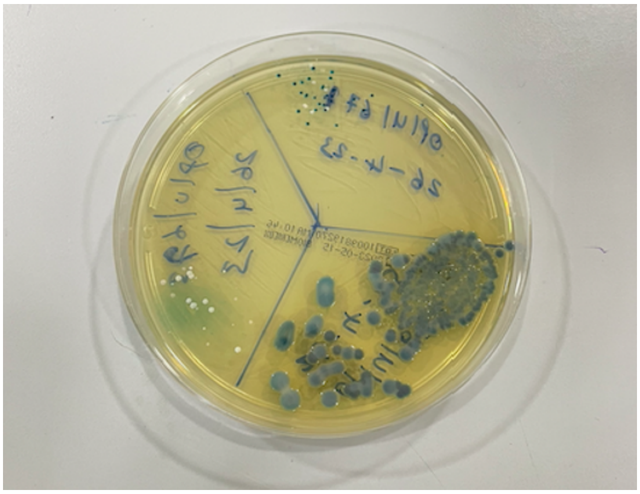

Fig. 2: Urochrome agar showing growth of *Klebsiella pneumonia*

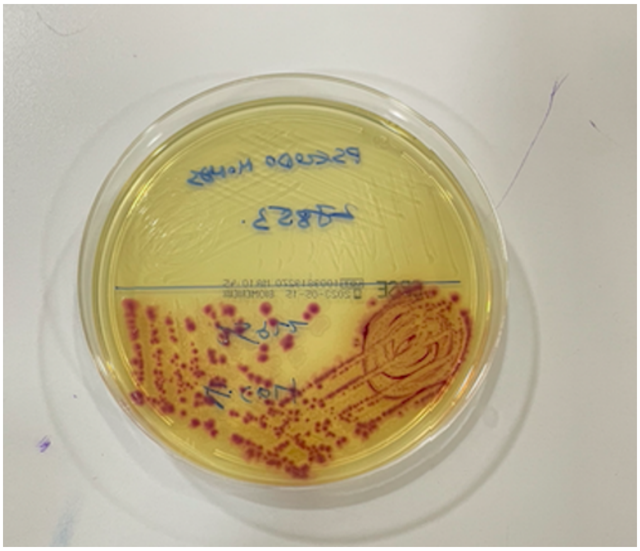

Fig. 3: Urochrome agar showing growth of *E. coli*

2.4. Bacterial identification and susceptibility testing

VITEK 2 compact system was used for the identification and antimicrobial susceptibility of bacteria isolated from urine samples. Bacterial growth was preliminary identified by colony morphology as well as Gram staining. Culture plates showing significant growth of bacterial colonies were used to formulate standardized saline inoculums recommended for VITEK identification.

The antimicrobial susceptibility tests (ASTs) and MICs were determined by special sensitivity (AST) cards. For gram negative bacteria we applied AST405 and AST406 antimicrobial susceptibility cards, whereas for gram positive we applied GP628 and ST03. Clinical and Laboratory Standards Institute (CLSI) criteria were used for the interpretation of AST results as per manufacturer's instructions (BioMérieux, France) and the Advanced Expert System. Antimicrobial susceptibility tested bacterial isolates are reported as Sensitive (S), Resistant (R) or Intrinsic Resistant (IR). Intrinsic resistance may be defined as a trait that is shared universally within a bacterial species, and is independent of previous antibiotic exposure.[8]

3. Results

Of a total of 872 urine samples received from 352 males and 520 females in the microbiology laboratory, 236 urine samples (27.1%) showed significant bacterial growth. Samples from the female gender were more commonly associated with significant growth. (Table 1) The average age of patients from whom samples were collected is 46.5 years, with a range of 5 years to 86 years.

Table 1: Gender distribution frequency of urine samples from the OPD of Kamineni Academy of Medical Sciences and Research Center, Hyderabad, India

Gender	No. of samples	Percentage
Male	85	36%
Female	151	64%
Total	236	

The most common urinary pathogens isolated were Gram negative bacteria including *Escherichia coli (E. coli), Klebsiellapneumoniae, Pseudomonasaeruginosa* and *Acinetobacterbaumannii*. *Enterococcus faecium* was the most commonly isolated Gram positive bacteria.(Table 2)

Antibiotic resistance to penicillins, fluoroquinolones and cephalosporins has been noticed in *E.coli*. *Klebsiella* isolates have been found to be resistant to the cephalosporin group. *Pseudomonasaeruginosa* has been noticed to have greater resistance to fluoroquinolones and cephalosporins. The detailed resistance pattern of each uropathogen isolated from urine specimens, as well as the individual antibiotic susceptibility patterns have been mentioned below. (Tables 3 and 4) Absence of drug resistance has been noted in Gram positive bacterial isolates. (Table 5)

Among the *E.coli* isolates, higher antibiotic resistance was observed towards Ampicillin (72%), Ticarcillin (70%), Norfloxacin (64%), Ciprofloxacin (62%), Ofloxacin (62%), Nalidixic acid (88%), Cefixime (75%), Ceftriaxone (75%), Cefotaxime (80%) and Ceftazidime (64%). *Klebsiella* showed high resistance to Ciprofloxacin (51%), Ceftriaxone (64%), Cefotaxime (79%), and Nitrofurantoin (65%). Higher resistance to Ciprofloxacin (62%), Ceftazidime (78%) was found in *Pseudomonas aeruginosa* isolates. *Enterococcus faecium* isolates showed high sensitivity to all antibiotics.

4. Discussion

Urinary tract infections have been described since ancient times with the first documented description in the Ebers Papyrus dated to c. 1550 BC.[9] It is one of the most prevalent diseases in the community and is responsible for 7 million clinic visits annually.[10] There is an increasing trend in multidrug resistance of uropathogens and there are very few new weapons to fight the threat.[11] The possible explanations for antimicrobial resistance include bacterial factors, such as genetic mutations acquired by the uropathogens,[12] inappropriate use of broad spectrum antibiotics[13] without proper evaluation, lack of evidence based clinical management,[14] incomplete usage of prescribed antimicrobials, and easy access of various broad spectrum antibiotics in the community pharmacy.[15]

The current study focuses on UTI cases isolated from the OPD. Literature review initially revealed that OPD UTI bacterial isolates have been known to be associated with low level bacterial drug resistance. A study from the US from 2001-2010 found only a 2.1% rise in multidrug resistance to nitrofurantoin, and multidrug-resistant *E.coli* has been

Table 2: Frequency (%) and gender distribution of microorganisms isolated

Organism	Male (Percentage)	Female (Percentage)	Total
Escherichia coli	46 (32%)	96 (68%)	142
Klebsiella pneumoniae	10 (22%)	34 (78%)	44
Pseudomonas aeruginosa	6 (46%)	7 (54%)	13
Enterococcus faecium	7 (50%)	7 (50%)	14
Acinetobacter baumanii	3 (60%)	2 (40%)	5
Proteus mirabilis	2 (66%)	1 (34%)	3
Morganella morgani	3 (100%)		3
Citrobacter koseri	1 (34%)	2 (66%)	3
Serratia marcescens	1 (50%)	1 (50%)	2
Enterobacter cloacae	1 (50%)	1 (50%)	2
Providentia stuartii	-	1 (100%)	1
Streptococcus agalactiae	-	1 (100%)	1
Methicillin resistant staphylococcus aureus	1 (100%)		1
Aeromonas hydrophilia	1 (100%)		1
Stenotrophomonas maltophilia		1 (100%)	1
	85	151	236

found in only 1.4% of cases. Recently, there is a trending increase in bacterial drug resistance in the OPD.[16] A study from Nath, et al. found that there is an increase of antibiotic drug resistance associated with oral formulations, especially fluoroquinolones and Ampicillin, used in OPD cases.[17] Our results echo similar findings of Nath, et al. and found an increase in drug resistance, largely to fluoroquinolones and cephalosporins. Our results coincide with other studies, as we isolated similar common uropathogens including *E.coli* (60%), *Klebsiellaspp* (18.6%) and *Pseudomonasspp* (14%). The results were supported by a study conducted by Mohapatra, et al. which revealed that the high prevalence of *E.coli* is associated with an increase in drug resistance.[18]

In our study, isolates of *E. coli* showed higher resistance to fluoroquinolones and cephalosporins in coherence with a study by Prasad, et al. which showed increasing resistance to cephalosporins.[19] In developing countries, an increasing trend of fluoroquinolone resistance in *E.coli* isolates has been noticed.[20] European Association of Urology guidelines have postulated nitrofurantoin as first line empirical treatment of an uncomplicated UTI.[21] Nitrofurantoin is the drug of choice for treatment as many of the common uropathogens were found to be highly sensitive to its effects. A study from Parama, et al. found similar results and coincided with our drug sensitivity pattern, finding more drug resistance among various cephalosporins. Resistance pattern of *Klebsiellapneumoniae* isolates from the same study is similar to the findings of our study.[22] In contrast to our study, which showed higher resistance to fluoroquinolones and cephalosporins in *Pseudomonasaeruginosa* isolates, a study by Jombo, et al. showed higher sensitivity to Ciprofloxacin (92%) and Cefuroxime (86%),[23] stressing the need of local surveillance of antimicrobial resistance patterns. Fortunately, antimicrobial susceptibility patterns among Gram positive bacteria such as *Enterococcus faecium* were sensitive to different classes of antibiotics, and similar results were found in a study conducted by Rudy M, et al., emphasizing the importance of calculated selection of antibiotics in treating UTI cases to prevent the spread of bacterial drug resistance among drug susceptible organisms.[24]

The current study has certain limitations as it is confined to the people covered by a single tertiary care center in Hyderabad, India and doesn't necessarily reflect trends in the community. A meta analysis of antimicrobial susceptibility patterns would provide sufficient information to change and optimize the empirical management of UTIs. This study emphasizes the need for more antimicrobial surveillance at regional, national and international levels. Necessity has aroused the need to promote ideal use of antimicrobials.[25] A decrease in multidrug resistance thereby reduces prevalence of the disease in the community and subsequently improves quality of life.

5. Conclusion

The study concludes that judicial use of antibiotics, especially in OPD settings, will influence the overall drug resistance pattern in the community. This study emphasizes the need for antimicrobial surveillance at the local and regional areas.

Table 3: Count(number) and distribution (%) of sensitivity (S), resistance (R) and intrinsic resistance (IR) among common Gram-negative urinary pathogens

Antibiotic	***E. coli***		***Klebsiella***		***Pseudomonas***		***Acinetobacter***	
	S	R	S	R	S	R	S	R
Ampicillin	13 (28)	32 (72)		1 (100)				
Ticarcillin	11 (30)	25 (70%)	1 (100)	IR	1 (50)	1 (50)		
Amoxicillin/clavulanic acid	57 (54)	48 (46)	19 (57)	14 (43)		6 (100)	1 (33)	IR
Piperacillin/tazobactam	82 (71)	33 (29)	24 (63)	14 (37)	7 (63)	4 (37)	2 (50)	2 (50)
Gentamicin	98 (84)	18 (16)	37 (86)	6 (14)	9 (69)	4 (31)	4 (100)	
Amikacin	116 (95)	6 (5)	43 (98)	1 (2)	8 (61)	5 (39)	4 (100)	
Imipenem	51 (96)	2 (4)	29 (78)	8 (22)	5 (45)	6 (55)	3 (60)	2 (40)
Meropenem	51 (98)	1 (2)	31 (83)	6 (17)	6 (60)	4 (40)	3 (75)	1 (25)
Ertapenem	65 (94)	4 (6)	12 (75)	4 (25)				
Cotrimoxazole	72 (61)	46 (39)	24 (60)	16 (40)		3 (100)	4 (100)	
Ciprofloxacin	47 (38)	75 (62)	19 (49)	20 (51)	5 (38)	8 (62)	1 (33)	2 (67)
Norfloxacin	28 (46)	32 (64)	3 (75)	1 (25)	1 (50)	1 (50)		
Levofloxacin	1 (33)	2 (67)	1 (100)		1 (25)	3 (75)		
Ofloxacin	35 (38)	56 (62)	14 (58)	10 (42)	2 (28)	5 (72)		
Nalidixic acid	6 (12)	42 (88)	2 (67)	1 (33)				
Cephalexin		1 (100)						1 (100)
Cefototin	6 (37)	10 (63)						
Cefuroxime	4 (33)	8 (67)	6 (54)	5 (46)				
Cefuroxime axetil	1 (100)		1 (50)	1 (50)				
Cefoxitin	8 (50)	8 (50)						
Cefixime	7 (35)	20 (65)	1 (50)	1 (50)				
Ceftriaxone	34 (35)	63 (65)	12 (36)	21 (64)		3 (100)	2 (50)	2 (50)
Cefotaxime	4 (17)	20 (83)	3 (21)	11 (79)			1 (100)	
Ceftazidime	28 (46)	33 (64)	2 (28)	5 (72)	2 (22)	7 (78)		
Cefoperazone/sulbactam	30 (81)	7 (19)	20 (69)	9 (31)	3 (33)	6 (67)	3 (100)	
Cefipime	5 (38)	8 (62)	5 (62)	3 (38)	1 (50)	1 (50)	1 (33)	2 (67)
Aztreonam		1 (100)	1 (100)	IR				
Fosfomycin	51 (100)		9 (75)	3 (25)				
Colistin	48 (96)	2 (4)	14 (93)	1 (7)	5 83)	1 (17)	3 (100)	
Nitrofurantoin	17 (100)		8 (35)	15 (65)				
Minocycline	39 (97)	1 (3)	1 (100)					
Tigecycline	3 (100)		2 (67)	1 (33)				
Tobramycin						2 (100)		
Total number of isolates	142		44		13		5	

Table 4: Count (number) and distribution (%) of sensitivity (S) and resistance (R) among less common Gram-negative urinary pathogens

Antibiotic	Proteus		Morganella		Citrobacter		Serratia		Enterobacter	
	S	R	S	R	S	R	S	R	S	R
Amoxicillin/clavulanic acid	3 (100)			2 (100)	3 (100)					2 (100)
Piperacillin/tazobactam	3 (100)		2 (67)	1 (33)	2 (100)				1 (50)	1 (50)
Gentamicin	3 (100)		2 (67)	1 (33)	3 (100)		2 (100)		1 (50)	1 (50)
Amikacin	3 (100)		3 (100)		3 (100)		2 (100)		2 (100)	
Imipenem	3 (100)		1 (33)	2 (67)	2 (100)		2 (100)		1 (50)	1 (50)
Meropenem	3 (100)		3 (100)		2 (100)		2 (100)		1 (50)	1 (50)
Ertapenem		1 (100)	1 (100)		3 (100)		1 (100)		1 (100)	
Cotrimoxazole	2 (67)	1 (33)	1 (33)	2 (67)	3 (100)		2 (100)		1 (50)	1 (50)
Ciprofloxacin	1 (100)		1 (50)	1 (50)	3 (100)		2 (100)		1 (50)	1 (50)
Norfloxacin					1 (100)					
Levofloxacin			1 (100)		1 (100)					
Ofloxacin				2 (100)	1 (100)					
Nalidixic acid					1 (100)					
Cefuroxime	1 (50)	1 (50)			2 (100)			1 (100)		
Cefuroxime axetil					2 (100)					
Cefixime					1 (100)					
Ceftriaxone	2 (67)	1 (33)		2 (100)	5 (100)		2 (100)			
Ceftazidime				1 (100)	1 (100)					
Cefoperazone/sulbactam	2 (100)		1 (100)		2 (100)		2 (100)		1 (50)	1 (50)
Cefipime	1 (100)				2 (100)		2 (100)		1 (100)	
Fosfomycin			1 (50)	1 (50)				1 (100)		1 (100)
Colistin				1 (100)					2 (100)	
Nitrofurantoin			1 (100)							
Tigecycline									1 (100)	
Total number of isolates	3		3		3		2		2	

Table 5: Count (number) and distribution (%) of sensitivity (S) and resistance (R) among common Gram-positive urinary pathogens

Antibiotic	*Enterococcus faecium*		*Streptococcus agalactiae*		MR *Staph aureus*	
	S	R	S	R	S	R
Benzylpenicillin	6 (54)	5 (46)	1 (100)			1 (100)
Amoxicillin	2 (67)	1 (33)				
Ampicillin	3 (100)					
Oxacillin					1 (100)	
Amoxicillin/clavulanic acid	4 (80)	1 (20)				
Piperacillin/tazobactam		1 (100)				
Gentamicin	1 (20)	4(80)			1 (100)	
Levofloxacin	5 (62)	3 (38)	1 (100)			1 (100)
Ciprofloxacin	8 (58)	6(42)				1 (100)
Ofloxacin	1 (25)	3 (75)				
Linezolid	6 (100)				1 (100)	
Teicoplanin	5 (71)	2 (29)		1 (50)	1 (50)	
Vancomycin	6 (67)	3(33)		1 (50)	1 (50)	
Fosfomycin	1 (25)	1 (8)				
Nitrofurantoin	12 (92)	1 (25)	1 (100)		1 (100)	
Daptomycin	3 (75)			1 (50)	1 (50)	
Tigecycline	4 (100)				1 (100)	
Erythromycin	5 (71)	2 (29)		1 (100)		1 (100)
Cotrimoxazole		1 (100)			1 (100)	
Clindamycin					1 (100)	
Total number of isolates	14		1		1	

6. Source of Funding

None.

7. Conflict of Interest

There are no conflicts of interest to declare.

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Author biography

Farhat Subhaana Chilakapati, Research Scholar https://orcid.org/0009-0007-7472-9733

Sandeep Kumar Tipparthi, Senior Resident https://orcid.org/0000-0002-4843-0048

Ravi Shankar Reddy A, Professor

Raj Kumar H.R.V, Professor & HOD

Guru Prasad Manderwad, Associate Professor https://orcid.org/0000-0003-4576-9465

Pradeep Kumar Reddy Sripathi, Senior Lab Technician

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