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Research Article

BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF UROPATHOGENS AT TERTIARY CARE CENTRE

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Abstract- Background: Urinary tract Infections (UTIs) are a cause of significant morbidity and mortality requiring urgent antibiotic treatment. However, there is widespread antibiotic-resistance from the bacterial causes, necessitating regular surveillance for drug-resistant bacteria and their antibiograms. Objective: The aim of this study is to isolate and identify various bacterial causes of UTIs and determine their antibiotic susceptibility patterns. Methods: A total of 5017 urine samples were analyzed from January 2021 to December 2022. Out of these 1014 samples were positive. Identification of the pathogen and antimicrobial sensitivity were done using the Automated system, Vitek 2 compact (Biomereiux). Results: Culture positivity in this study was 20%. Gram-negative bacteria were predominantly isolated (79.7%), including *Escherichia coli* (52.8%) and *Klebsiella* aerogenes (14.2%). *Enterococcus faecalis* was the most common Gram-positive bacterium isolated. Antibiotic susceptibility pattern among gram negative bacilli has shown maximum sensitivity to Colistin, Fosfomycin, followed by Carbapenems (Imipenem, Meropenem & Ertapenem), Amikacin and Beta lactam- betalactam inhibitor (BL-BLI) combination drugs. Out- patient department (OPD) samples were found to be more susceptible to antimicrobials than In-patient department (IPD) samples. Maximum resistance was seen against Ciprofloxacin and Ceftriaxone. *Klebsiella* and *Pseudomonas* isolates were found to be more resistant when compared to *E.coli. E.coli* isolates were more sensitive to Nitrofurantoin (87%) than *Klebsiella* (22%). Carbapenem resistance was seen in 50% of the Inpatient isolates of *Klebsiella* and 36% of Inpatient isolates of *Pseudomonas*.Few isolates of *Klebsiella* and *Pseudomonas* were found to be resistant even to Colistin.

Keywords- *Urinary tract infections, Antibiogram, E.coli, K. pneumoniae, Enterococcus*

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Introduction

Urinary tract infections (UTIs) are one of the most common infections, both in the community and in health-care worldwide. The clinical spectrum of UTI can range from asymptomatic bacteriuria to severe forms like emphysematous pyelonephritis and sepsis [1]. It occurs more frequently in women than men due to the shortness of the female urethra. Symptomatic and asymptomatic UTI is common in pregnancy. Undetected, untreated, asymptomatic bacteriuria can lead to pyelonephritis later in pregnancy or during puerperium [2].

Escherichia coli is the commonest urinary pathogen causing 60-90% of infections. Some Strains are more invasive, *e.g.*, capsulated strains are able to resist phagocytosis, other strains are more adhesive. UTIs caused by *Pseudomonas*, *Proteus, Klebsiella* species and *Staphylococcus aureus*, are associated with hospital-acquired infections, often following catheterization or gynecological surgery [2]. And because of evolving antibiotic resistance phenomenon among uropathogens, regular monitoring is of utmost importance to provide guidelines for empirical antimicrobial therapy [3]. The aims of the present study were to isolate and identify the predominant bacteria causing UTI and to evaluate the susceptibility profiles of these pathogens.

Materials and Methods

This is an observational study carried out in the department of Microbiology in Krishna Institute of Medical Sciences, Kondapur, Hyderabad for a period of 2 years from January 2021 to December 2022. A total of 5017 urine samples were processed from patients of all age groups with suspected UTI symptoms. Urine culture was done by semi quantitative technique [2]. By means of a calibrated loop, 0.001ml of urine was cultured on CLED (Cystine lactose electrolyte deficient) agar medium.

CLED agar is used because it gives consistent results and allows the growth of both Gram negative and Gram positive pathogens and also prevents the swarming of Proteus species.

The plates were examined for growth after overnight aerobic incubation at 35-37°C. A growth of >=105 colony forming units (CFU)/ml is considered as active UTI with significant bacteriuria [4, 5]. Cultures having more than three types of colonies were considered as contaminants. Pathogenic organisms were identified using Vitek 2 compact (Biomerieux) GN & GP identification cards. The antimicrobial sensitivity testing was done by AST cards- N280 for Lactose fermenters & N281 for Non-Lactose fermenters.

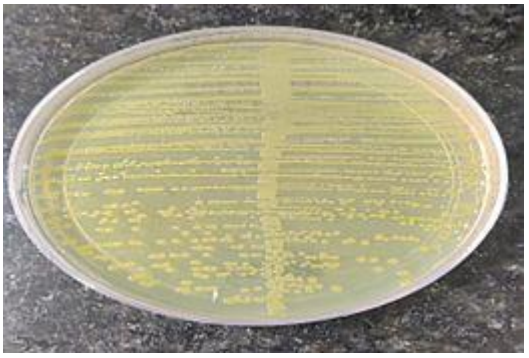


Fig-1 Showing *E.coli* on CLED agar with 105 CFU/ml

Procedure for Identification and Antimicrobial Sensitivity testing: Take 2 test tubes with 3ml of sterile saline for each isolate and place them on cassette. Mark tube 1 as ID tube (for identification) and tube 2 as AST tube (for sensitivity).

Isolated colonies from primary plate are selected and aseptically transferred into tube 1. Prepare the homogenous organism suspension with a density equivalent to a McFarland standard No. 0.50 to 0.63 using a calibrated Vitek 2 Densichek. If yeast is isolated, then adjust the turbidity to 1.8- 2.2 McFarland. Transfer 145µl (for Gram negative bacilli) & 280µl (for Gram positive cocci and yeast) from tube 1 to tube 2. Place ID card in tube 1 and AST card in tube 2. Complete the data entries and load the cassette into the instrument.

Quality control

Identification of organisms

Use of ATCC strains is done for GN & GP cards of Vitek 2 compact (Bio merieux). GN cards are used for identification of Lactose fermenter & Non Lactose fermenter.GP cards are used for identification of *Enterococcus*, *Staphylococcus* and *Streptococcus* and select group of Gram positive organisms. The following ATCC strains are used:

Table-1 ATCC strains used for Quality control of GN & GP Cards

SN	Name of the Card	ATCC number	Name of the Organism
1	GN	ATCC 700323	*Enterobacter hormaechei*
2	GN	ATCC 17666	*Stenotrophomonas maltophilia*
3	GP	ATCC 700327	*Enterococcus casseliflavus*
4	GP	ATCC BAA 750	*Staphylococcus saprophyticus*

Antimicrobial Susceptibility testing

Use of ATCC strains is done for AST N280, AST N281 & AST P628 cards of Vitek 2 compact (bio Merieux).

Table-2 ATCC strains used for quality control of AST 280, AST 281 & P628 cards

ATCC number	Name of the Organism	Name of the Card	Remarks
25922	*Escherichia coli*	AST N280	For all antibiotics
		AST N281	For all antibiotics
27853	*Pseudomonas aeruginosa*	AST N280	For all antibiotics
		AST N281	For all antibiotics
35218	*Escherichia coli*	AST N280	Beta lactam- Beta lactam inhibitor
		AST N281	Beta lactam- Beta lactam inhibitor
29213	*Staphylococcus aureus*	AST P628	For all antibiotics
51299	*Enterococcus fecalis*	AST P628	High level resistance for Gentamycin & Streptomycin
29212	*Enterococcus fecalis*	AST P628	For all antibiotics
BAA1026	*Staphylococcus aureus*	AST P628	Cefoxitin screen
BAA976	*Staphylococcus aureus*	AST P628	Inducible Clindamycin Resistance (ICR) Negative
BAA977	*Staphylococcus aureus*	AST P628	Inducible Clindamycin Resistance (ICR) Positive

Whenever a new lot of Antibiotic sensitivity cards is opened, the cards are validated by using the recommended strain. Results were analysed using MS EXCEL, 2013 version.

Results

Out of 5017 urine samples that were analyzed, 1014 samples (20%) were found to have significant bacteriuria and remaining 4003 samples were either non-significant bacteriuria or very low bacterial count or sterile urine. Prevalence was higher in females (61%) compared to males (39%). The overall infection rate was highest in the age group of 61-85 years (43%).

Table-3 Distribution based on Gender

Gender	Number of culture positive samples	% Culture positive
Male	395	39%
Female	619	61%
Total	1014	100%

Table-4 Distribution based on age

Age	No of culture positive samples	% Culture positive
<15 years	39	4%
16-40 years	283	28%
41-60 years	252	25%
61-85 years	440	43%
Total	1014	100%

Escherichia coli (52.8%) was the predominant uropathogen isolated followed by *Klebsiella species* (14.3%) and *Pseudomonas* (7.5%). Among Gram positive organisms, *Enterococcus faecalis* was most frequently isolated followed by *Enterococcus faecium*. Among yeasts isolated from Urine cultures, *Candida tropicalis* was found to be the most frequently isolated yeast.

Antibiotic susceptibility pattern among Gram negative bacilli has shown maximum sensitivity to Colistin, Fosfomycin, followed by Carbapenems (Imipenem, Meropenem& Ertapenem), Amikacin and BL-BLI inhibitors. Out- patient samples were found to be more susceptible to antimicrobials than In-patient samples. Maximum resistance was seen against Ciprofloxacin and Ceftriaxone. *Klebsiella* and *Pseudomonas* isolates were found to be more resistant when compared to *E.coli. E.coli* isolates were more sensitive to Nitrofurantoin (87%) than *Klebsiella* (22%). Carbapenem resistance was seen in 50% of the Inpatient isolates of *Klebsiella* and 36% of Inpatient isolates of *Pseudomonas*. Few isolates of *Klebsiella* and *Pseudomonas* were found to be resistant even to Colistin.

Among the *Enterococcus* isolates higher sensitivity was seen with Nitrofurantoin (88%) and 100% sensitivity was seen to Vancomycin, Teicoplanin, Linezolid and Fosfomycin [Fig-5]. Methicillin resistance was seen in 1 out of 9 Staphylococcus aureus isolates. No Vancomycin resistance was observed in any of the Gram positive isolates. Percentage sensitivity was not calculated for rest of the isolates as the number of isolates of these species were less than 30 (CLSI M39 document).

Discussion

The Antibiogram of uropathogens has been changing over the years. One of the important factors contributing to the high resistance rates may be due to the increasing use of antibiotics without knowing the causative organism and its susceptibility pattern towards the antibiotics.

A total of 1014 positive urine culture and sensitivity reports during a two year period were analysed in this study. In our study, the occurrence of UTI was found to be 20% which was comparable to the findings of 25% by Pramodh K *et al.,* [6] & Kaushik *et al.,* [7] but lesser when compared to the findings of 34.5% by Dash *et al.,* [8] and 36.6% by Mehta *et al.,* [9].

Prior antibiotic therapy before submitting the urine sample and clinical conditions like non-gonococcal urethritis or other conditions that mimic UTI could be responsible for such results [10]. In this study the prevalence of UTI was seen more in females (61%) as compared to males (39%). Females were more susceptible to UTI as already shown in studies done by Manjunath *et al.,* [11], Maheshwary *et al.,* [12], Prakash *et al.,* [13], Dash *et al.,* [8]. Females were more affected due to proximity of urethral meatus to the anus, shorter urethra, less acidic Ph of the vaginal fluid [14,15].

In our study, UTI was found to be more prevalent in the elderly age group (61-85 years) and does not correlate with other studies. This could be due to the more frequent geriatric population that attend to this hospital. The second most common age group to be affected is 16-40 years, which was the predominant age group to be affected in other studies [10,16].

Escherichia coli (52.8%) was the predominant uropathogen isolated followed by *Klebsiella* spp (14.3%) and *Pseudomonas* (7.5%). These results are consistent with studies done by Nzalie *et al.,* [17], Prakash *et al.,* [13], Mahajan *et al.,* [18] and Taye *et al.,* [19]. The most frequent Gram-positive pathogen is the Enterococcus group (6.5%) which is similar to other studies [3,10].

The most effective antimicrobial agents for Gram negative pathogens in our study were Colistin, Amikacin, Fosfomycin, Meropenem, Imipenem, Ertapenem & Piperacillin-tazobactam. *E.coli* isolates were more sensitive to Fosfomycin and Nitrofurantoin than *Klebsiella*. Higher resistance was observed among Fluoroquinolones, third generation Cephalosporins and Cotrimoxazole showing percentage susceptibility of 36%, 35.5% and 53.5% respectively. High sensitivity to carbapenem, aminoglycoside, nitrofurantoin and low sensitivity to 3rd generation cephalosporin, fluoroquinolones and cotrimoxazole was also reported in other studies [8, 12, 18, 20, 21].

Among the *Pseudomonas* isolates Colistin (90%) and Amikacin (65%) were sensitive respectively which corelates with the study of Deshpande *et al.,* [22]. Piperacillin-tazobactam was 63% sensitive which is less compared to studies by Baveja *et al.,* (76.4%) [23] & Mehrishi *et al.,* (70%) [24].

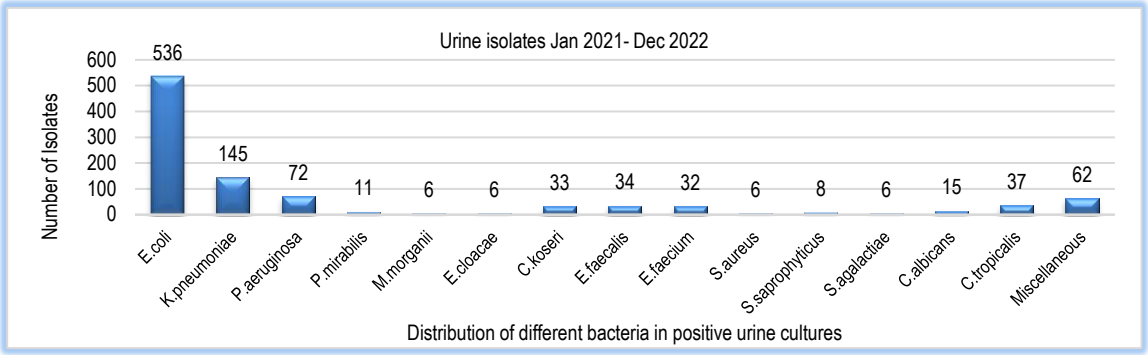
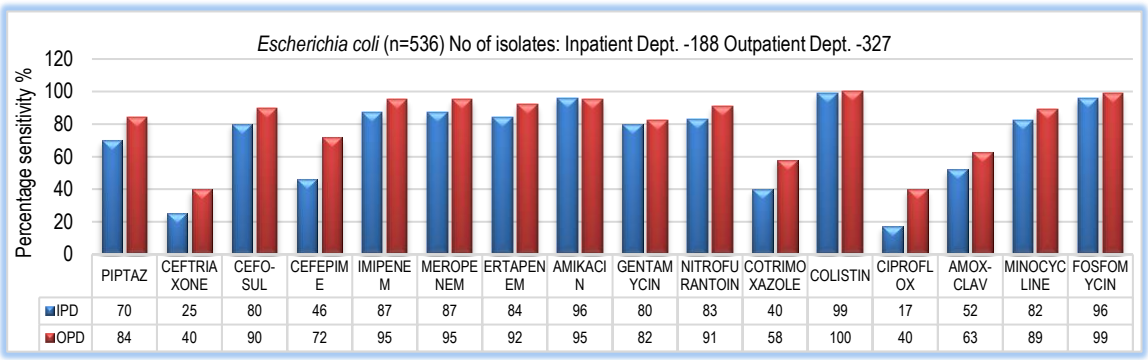
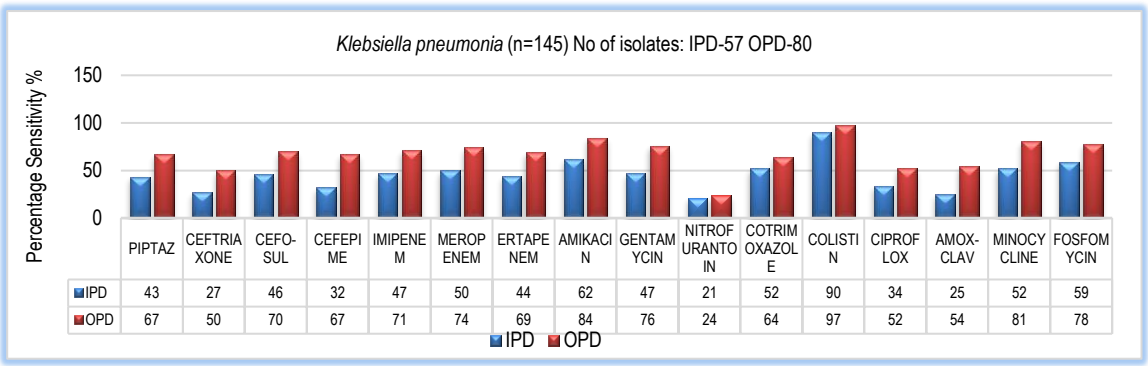


Fig-2 Distribution of Uropathogens isolated from patients with UTI



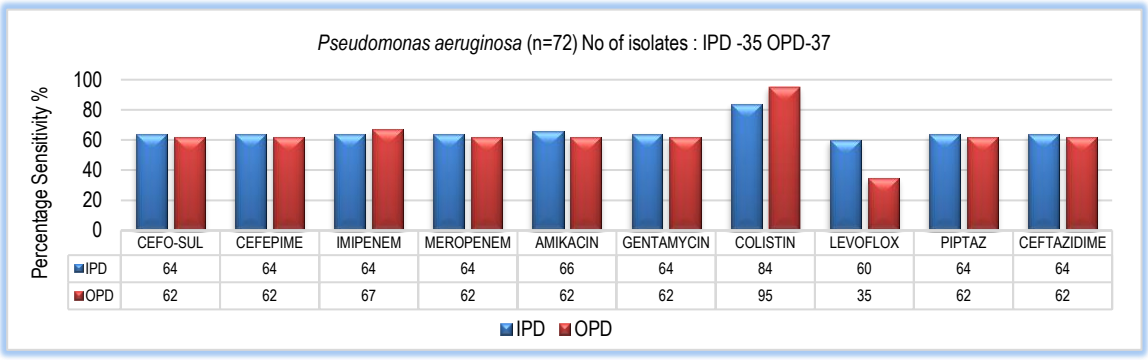
	PIPTAZ	CEFTRIAXONE	CEFO-SUL	CEFEPIME	IMIPENEM	MEROPENEM	ERTAPENEM	AMIKACIN	GENTAMYCIN	NITROFURANTOIN	COTRIMOXAZOLE	COLISTIN	CIPROFLOX	AMOX-CLAV	MINOCYCLINE	FOSFOMYCIN
IPD	70	25	80	46	87	87	84	96	80	83	40	99	17	52	82	96
OPD	84	40	90	72	95	95	92	95	82	91	58	100	40	63	89	99

Fig-3 Antibiogram of E.coli in Urine:



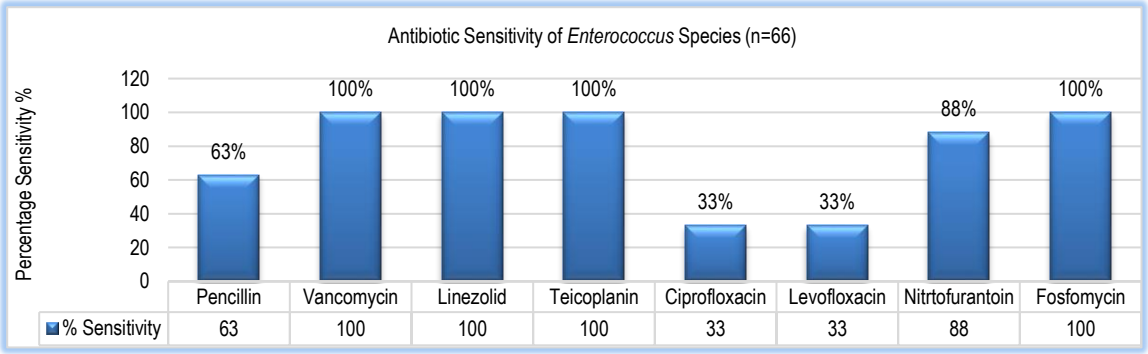
	PIPTAZ	CEFTRIAXONE	CEFO-SUL	CEFEPIME	IMIPENEM	MEROPENEM	ERTAPENEM	AMIKACIN	GENTAMYCIN	NITROFURANTOIN	COTRIMOXAZOLE	COLISTIN	CIPROFLOX	AMOX-CLAV	MINOCYCLINE	FOSFOMYCIN
IPD	43	27	46	32	47	50	44	62	47	21	52	90	34	25	52	59
OPD	67	50	70	67	71	74	69	84	76	24	64	97	52	54	81	78

Fig-4 Antibiogram of Klebsiella pneumoniae in Urine



	CEFO-SUL	CEFEPIME	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMYCIN	COLISTIN	LEVOFLOX	PIPTAZ	CEFTAZIDIME
IPD	64	64	64	64	66	64	84	60	64	64
OPD	62	62	67	62	62	62	95	35	62	62

Fig-5 Antibiogram of Pseudomonas aeruginosa in urine



	Pencillin	Vancomycin	Linezolid	Teicoplanin	Ciprofloxacin	Levofloxacin	Nitrtofurantoin	Fosfomycin
% Sensitivity	63	100	100	100	33	33	88	100

Fig-6 Antibiogram of Enterococcus isolates

In case of *Enterococci*, high sensitivity was seen in Linezolid, Vancomycin, Teicoplanin, Fosfomycin (100%) and Nitrofurantoin (88%). Low sensitivity was seen in Penicillin and Ciprofloxacin. This finding was similar to Dash *et al.,* [8], Mahajan *et al.,* [18], Prakash *et al.,* [21] and Khan *et al.,* [25].

Conclusion

This study concludes that *E.coli* is the principal pathogen of UTIs. It also indicates a high resistance to the most commonly used antibiotics due to indiscriminate use of antibiotics. There is an alarmingly increased resistance of *K.pneumoniae and P. aeruginosa* to carbapenems. Cotrimoxazole should not be used, and quinolones should be avoided for empirical treatment of UTI. Nitrofurantoin, Fosfomycin and aminoglycosides are suitable antibiotics for empirical treatment if suspicion for ESBL organisms is low. For sick patients requiring hospitalization, piperacillin-tazobactam, aminoglycosides, and carbapenems should be considered for empirical treatment. In order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications.

Application of research: Antimicrobial susceptibility of Urinary tract pathogens

Research Category: Antimicrobial susceptibility

Abbreviations:
UTI- Urinary Tract Infection
IPD- In-Patient Department
OPD- Out-Patient Department
BL-BLI - Beta lactam- Beta lactam inhibitor
CLED-Cystine lactose electrolyte deficient
CFU- Colony forming Units
GN- Gram negative
GP- Gram positive
AST- Antimicrobial Sensitivity Test
ATCC- American Type Culture Collection
QC- Quality Control
ICR- Inducible Clindamycin Resistance
CLSI- Clinical Laboratory Standards Institute

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University: KIMS Hospital Enterprises Pvt.Ltd., Kondapur, Hyderabad, 500084, Telangana, India
Research project name or number: Clinical Research study

Author Contributions: Sole Author

Author statement: Author read, reviewed, agreed and approved the final manuscript. Note-Author agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: KIMS Hospital, Kondapur, Hyderabad

Cultivar / Variety / Breed name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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