



# CHARACTERIZATION OF ANTIBIOTIC RESISTANCE PATTERN AMONG UROPATHOGENIC BACTERIAL ISOLATES

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**Abstract:** Urinary tract infection is a commonest infection among women. There is increased antibiotic resistant among most of strains of uropathogenic bacteria. Objective of this study is to evaluate antibiotic resistance among uropathogenic Bacteria. In this study total 237 urine samples were analyzed for occurrence of uropathogenic bacteria. From these urine samples, 180 strains of uropathogenic bacteria were isolated, 133 were gram negative and 47 were gram positive, *E.coli* (n=58), *Proteus spp.* (n=39), *Klebsiella spp.* (n=20), *Enterobacter spp.* (n=10), *Pseudomonas spp.* (n=6), *Enterococcus spp.* (n=19) and *S. aureus* (n=28). Out of total 180 isolates of uropathogenic bacteria, 96.67% (174) were found to be MDR. Out of 133 strains of gram-negative bacteria 127 (95.45%) were found to be MDR, all 47 strains of gram-positive bacteria were MDR. 168 (93.33%) uropathogenic bacteria were resistant to cefixime, 159 (88.33 %) resistant to cefepime and ceftazidime, 154 (85.56 %) resistant to erythromycin, tobramycin and ampicillin. Trimethoprim is found to be most effective antibiotic against uropathogenic bacteria, it is effective against 137 (76.11%) strains of uropathogenic bacteria. Gram negative bacteria were highly resistant to cefixime (90.98%), kanamycin (88.72%), ceftazidime (86.47%), ampicillin (80.45%), tobramycin (80.45 %) and nalidixic acid (80.45 %). Tri-methoprim is found to be most effective antibiotic against gram negative uropathogenic bacteria. Gram positive bacteria were highly resistant to tobramycin (100%), ampicillin (100%), cefixime (100%), cefepime (95.74%), erythromycin (95.74%) and ceftazidime (93.62 %). Tri-methoprim is found to be most effective antibiotic against gram positive uropathogenic bacteria. *S. aureus* isolates were highly resistant to cefepime (92.9%), vancomycin (89.5%), erythromycin (78.6%), ciprofloxacin (75.4%), nalidixic acid (64.3%). Tobramycin is most effective antibiotic against all (28) tested strains of uropathogenic *S. aureus*. Antibiotic resistance should be continuously evaluated in patients of all ages and in all hospitals where possible, so as to monitor the evolution of this major public health issue. This study reveals that antibiotic resistance is continuously increasing in all genera of uropathogenic bacteria. The resistance to all group of antibiotics observed in some strains is alarming and suggests the need for more judicious use of these antibiotics.

**Keywords:** UTI, Uropathogen, Uropathogenic Bacteria, Antibiotics , MDR

## Introduction:

UTI is a clinical condition in which pathogens are established and multiplied within and affecting the urinary tract of an individual. It is transmitted mainly by four routes -ascending route, lymphatogenous spread, hematogenous spread and direct extension from other organs. Urinary tract infection that occurs in a normal genitourinary tract with no prior instrumentation are considered as uncomplicated UTI, whereas complicated UTI are diagnosed in genitourinary tract that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters (Palupati S. et al, 2017). Urinary tract infection is a most common infection reported in primary care centers. Urinary tract infections (UTI) are a major burden of disease, causing around 150-250 million cases worldwide every year (Laupland K.B. et al, 2007).

These infections account for 75 percent of the community setting infections and 50 to 65 percent of the healthcare setting infections. (Laupland K.B. et al, 2007; Flores -Mireles A.L. et al, 2015). Prevalence of UTI is dependent on factors like-gender, age, disease, race, anatomy, ethnicity and nutritional status. Females are generally more susceptible to urinary tract infection compared to male. According to the analysis of

Millner & Becknell, 2019, different factors were associated with susceptibility of individuals for urinary tract infections. Unhealthy life style, kidney abnormalities, some foreign substances , use of catheters, menopause defective urinary tract by birth in children are the most common underlying risk factors of UTI. Less intake of water , fluids and healthy food and lack of physical activity can increase the risk of urinary tract infection. (Millner R. & Becknell B. , 2019)

Sexually active women , patients with suppressed or impaired immunity are vulnerable to UTI. Sexual intercourse, pregnancy and childbirth contribute to the increase in UTI in females. Pathogenic bacteria associated with urinary tract infection are *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter spp.* and fungi like *Candida albicans*. *Escherichia coli* is the most common pathogen that causes urinary tract infection. For treatment and management of urinary tract infection number of therapeutic practices are available, which include antibiotic therapy, estrogen treatment, cranberry juice treatment, forskolin and probiotics.

Out of this treatment with antibiotics is commonly recommended treatment practice. The phenomenal evolution and increase of multidrug-resistance of many bacterial pathogens is increasing and representing a growing public health problem in the world. Multidrug-resistance among uropathogenic bacteria calls for regular review of antimicrobial sensitivity pattern in order to institute appropriate antibiotic therapy. Thus, this study was conducted to evaluate the antibiotic sensitivities of uropathogenic bacterial strains isolated from patients diagnosed with UTIs at Hospital in Gondia city, Maharashtra, India.

### Materials and Methods:

- 1. Collection of urine sample:** For isolation of uropathogenic bacteria, 237 urine samples are collected from patients with symptoms of UTI from different hospitals of Gondia city, Maharashtra, India. Samples collected in sterile plastic universal containers and transported to laboratory in an ice-cold condition. Clean catch mid-stream urine (CC-MSU) were collected.
- 2. Study period and location:** This study was performed from May 2023 to December, 2024 and Gondia city, Maharashtra state of India.
- 3. Urine culture:** 0.1 ml the uncentrifuged urine sample was spread uniformly using sterile spreader on the surface of sterile CLED Agar plates and incubated for 24 hours at 37° C. All plates were observed for presence of typical colonies.

Bacteria	Colony Morphology
<i>Escherichia coli</i>	Large, elevated, yellow, opaque colonies with a centre more intense yellow; yellowish medium
<i>Klebsiella species</i>	extremely mucoid colonies varying in colour from yellow to whitish-blue. yellowish medium.
<i>Proteus species</i>	translucent blue colonies are usually smaller than <i>Escherichia coli</i> . blue-green to blue medium.
<i>Salmonella species</i>	Flat blue colonies
<i>Pseudomonas aeruginosa</i>	Green colonies with a typical matte surface and rough periphery. "Sweet" odour. Blue-green agar
<i>Enterococcus faecalis</i>	Yellow colonies about 0.5 mm in diameter. yellow medium
<i>Staphylococcus aureus</i>	Deep yellow colonies about 0.75 mm diameter, uniform in colour. yellow medium
<i>Corynebacteria</i>	Very small grey colonies
<i>Lactobacilli</i>	Similar to corynebacteria but with a rougher surface

- 4. Maintenance of isolates:** Typical colonies from CLED agar were subculture on nutrient agar slants and maintained as respective uropathogenic bacterial isolates.
- 5. Identification of isolates :** Identification of isolates carried out on the basis of morphological , cultural and biochemical characteristics. Each isolate subjected to morphological examinations by gram staining & motility test, cultural characteristics selective and differential media, biochemical test-sugar fermentation test, IMViC test, TSI test, urease test, catalase test, oxidase test and coagulase test.
- 6. Antibiotic sensitivity Test:** Antibiotic sensitivity test was done to identify the antibiotics for which the organism was susceptible and for which it was resistant. With the increase in the emergence of drug resistance of organism, it should be performed compulsorily before prescription of antibiotic

therapy. Antibiotic sensitivity test done Kirby Bauer disk diffusion method. Briefly, after bringing the bacteria at room temperature, they were cultured at 37°C for 6-8 hrs in sterile nutrient broth to obtain a concentration equivalent to 0.5 McFarland. Then, 100 µL of the culture was plated on Muller Hinton Agar plate and the antibiotic discs were placed aseptically. The following 12 antibiotics were used: ciprofloxacin, ampicillin, erythromycin, co-trimaxazole, nalidixic acid, tetracycline, kanamycin, tobramycin, trimethoprim, cefixime, cefepime and ceftazidime.

Interpretation of results and data analysis After 18-24 h of incubation at 37°C, the inhibition diameters were measured and interpreted in accordance with the NCCLS standards. **(Clinical and Laboratory Standards Institute, 2021)**

### Result and Discussion:

In this study total 237 urine samples from patients with early symptoms of urinary tract infection were collected from different hospitals of Gondia city, Maharashtra, India. Out of 237 collected samples, 64 from male patients and 173 from female patients. The median age was 26.05 years and the male to female ratio was 0.19 :1. To facilitate data analysis and interpretation of the results, the subjects were divided into nine groups based on age ranges: 1-10,11-20,21-30,31-40,41-50,51-60, 61-70, 71-80 and 81-90 years. Total 164 samples were found to be positive for uropathogenic bacteria.

From these samples total 180 strains of uropathogenic bacteria were isolated. Antibiotic resistance profile of uropathogenic bacterial isolates was determined using Kirby-Bauer disc diffusion method. Total 12 antibiotics of 7 different classes has been chosen for gram negative and gram positive bacterial additional 8 (total 20) antibiotics were chosen for gram positive *S. aureus*. Out of tested 180 strains of uropathogenic bacteria, 168 (93.33%) were resistant to cefixime, 159 (88.33%) to cefepime and ceftazidime, 154 (85.56%) to tetracyclines and trimethoprim, 145 (80.56%) to nalidixic acid, 133 (73.89%) to erythromycin, 121 (66.22%) to ciprofloxacin and 42 (23.33%) were resistant to kanamycin.

Tobramycin was found to be most effective antibiotic. This antibiotic is effective against 137 (76.11%) strains of uropathogenic bacteria. Out of 133 strains of gram negative uropathogenic bacteria, 121 (90.98%) were resistant to cefixime, 118 (88.72%) to nalidixic acid 115 (86.41%), to ceftazidime, 114 (85.71%) to cefepime, 109 (81.95%) to ampicillin and 107 (80.45%) to erythromycin, tetracycline and trimethoprim. Tobramycin was found to be most effective antibiotic against gram negative uropathogenic bacterial isolates. It is effective against 95 (71,45%) strains of gram negative uropathogenic bacteria.

**Table 1a: Antibiotic Resistance of uropathogenic Bacteria**

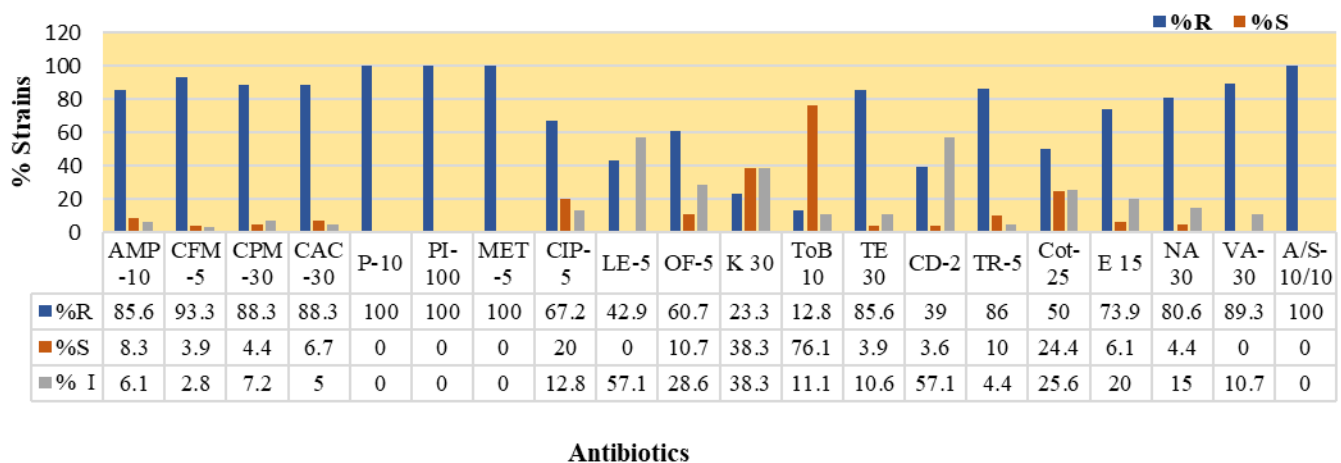
Antibiotic Class	FQ	BL	M	AM	Q	TC	AG	AG	AM	BL	BL	BL
Antibiotic	CIP	AMP	E	Cot	NA	TE	K	ToB	TR	CFM	CPM	CAC
Number of strains tested	180	180	180	180	180	180	180	180	180	180	180	180
	<b>Number of resistant strains</b>											
<i>E. coli</i>	46	49	56	25	52	48	23	5	48	53	42	44
<i>Proteus spp.</i>	30	33	37	20	39	32	0	10	32	39	39	39
<i>Klebsiella spp.</i>	12	12	9	2	19	14	6	8	12	15	19	18
<i>Enterobacter spp.</i>	10	10	3	4	6	10	1	0	10	10	10	10
<i>Pseudomonas aeruginosa</i>	1	5	2	5	2	3	1	0	5	4	4	4
<i>Enterococcus spp.</i>	1	17	4	19	9	19	2	0	19	19	19	16
<i>S. aureus</i>	21	28	22	15	18	28	9	0	28	28	26	28
Total strains	121	154	133	90	145	154	42	23	154	168	159	159

**Table 1b: Antibiotic Resistance of uropathogenic Bacteria**

Antibiotic Class	BL	BL	BL	FQ	FQ	TC	G	BI
Antibiotic	P	PI	MET	LE	OF	CD	VA	A/S
Number of strains tested	28	28	28	28	28	28	28	28
<b>Number of resistant strains</b>								
<i>E. coli</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>Proteus spp.</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>Klebsiella spp.</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>Enterobacter spp.</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>Pseudomonas aeruginosa</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>Enterococcus spp.</i>	NE	NE	NE	NE	NE	NE	NE	NE
<i>S. aureus</i>	28	28	28	12	17	11	25	28
Total strains	28	28	28	12	17	11	25	28

NE=Not evaluated, FQ=Fluroquinone, BL=Beta-lactam, M= macrolide, AM=Antimetabolite, AG=Aminoglycosides, TE= Tetracycline, G=Glycosides, BI=Beta lactamase inhibitor, CIP=Ciprofloxacin , AMP=Ampicillin , E= Erythromycin ,Cot= Co-trimaxazole , NA= Nalidixic acid, TE Tetracycline, K=Kanamycin, ToB=Tobramycin, TR=Trimethoprim , CFM=Cefixime , CPM=Cefepime, CAC= Ceftazidime, P=Penicillin, PI=Piperacillin, MET=Methicillin, LE=Levofloxacin, OF=Ofloxacin, CD=Clindamycin, VA=Vancomycin and A/S=Ampicillin/Sublactum.

**Figure 1: % Antibiotic resistance profile of uropathogenic bacteria**



**Table 2: % Antibiotic Resistance/ Sensitivity profile of gram negative uropathogenic Bacteria**

Antibiotic Class	FQ	BL	M	AM	Q	TC	AG	AG	AM	BL	BL	BL
Antibiotic	CIP	AMP	E	Cot	NA	TE	K	ToB	TR	CFM	CPM	CAC
% Strains of gram negative uropathogenic bacteria												
% Resistant	74.44	81.95	80.45	42.11	88.72	80.45	23.31	17.29	80.45	90.98	85.71	86.47
% Sensitive	12.78	9.77	6.77	30.08	0.75	5.26	36.84	71.43	13.53	5.26	6.02	7.52
% Intermediate	12.78	8.27	12.78	27.82	10.53	14.29	39.85	11.28	6.02	3.76	8.27	6.02

**Table 3a: % Antibiotic Resistance/ Sensitivity profile of gram positive uropathogenic Bacteria**

Antibiotic Class	FQ	BL	M	AM	Q	TC	AG	AG	AM	BL
Antibiotic	CIP	AMP	E	Cot	NA	TE	K	ToB	TR	CFM
% Strains of gram positive uropathogenic bacteria										
% Resistant	46.81	95.74	55.32	72.34	57.45	100	23.40	0.00	100	100
% Sensitive	40.43	4.26	4.26	8.51	14.89	0.00	42.55	89.36	0.0	0.00
% Intermediate	12.77	0.00	40.43	19.15	27.66	0.00	34.04	10.64	0.0	0.00

**Table 3b: % Antibiotic Resistance/ Sensitivity profile of gram positive uropathogenic Bacteria**

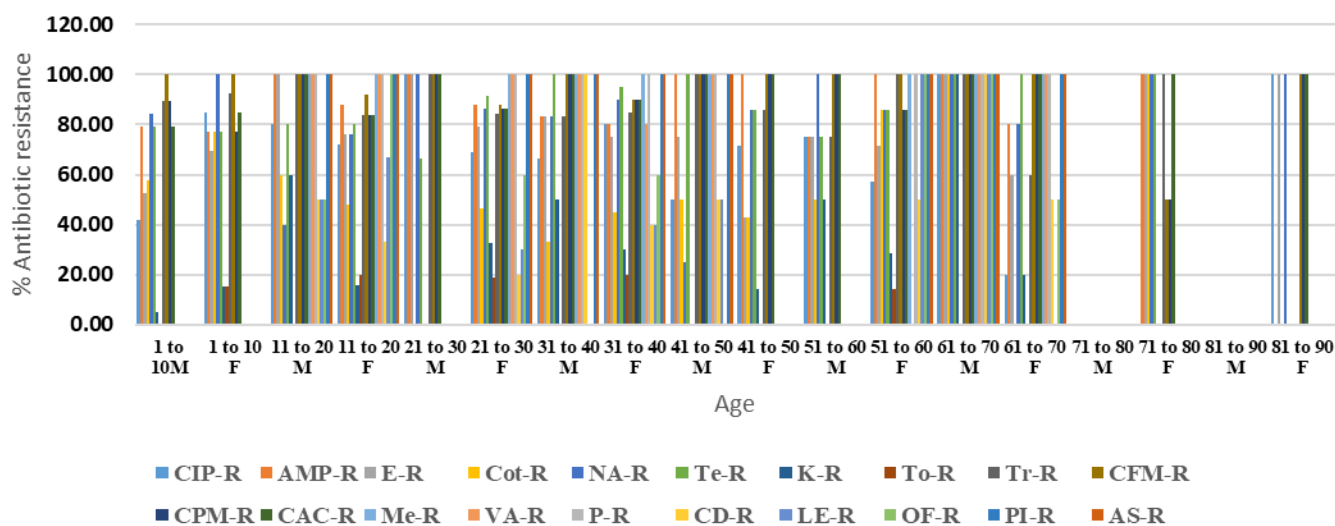
Antibiotic Class	BL	BL	BL	BL	BL	FQ	FQ	TC	G	BI
Antibiotic	CPM	CAC	P	PI	MET	LE	OF	CD	VA	A/S
% Strains of gram positive uropathogenic bacteria										
% Resistant	95.74	93.62	100	100	100	42.86	60.71	39.29	89.29	100
% Sensitive	0.00	4.26	0.00	0.00	0.00	0.00	10.71	3.57	0.00	0.00
% Intermediate	4.26	2.13	0.00	0.00	0.00	57.14	28.57	57.14	10.71	0.00

FQ=Fluroquinone, BL=Beta-lactam, M= macrolide, AM=Antimetabolite, AG=Aminoglycosides, TE= Tetracycline, G=Glycosides, BI=Beta lactamase inhibitor, CIP=Ciprofloxacin , AMP=Ampicillin , E= Erythromycin ,Cot= Co-trimaxazole , NA= Nalidixic acid, TE Tetracycline, K=Kanamycin, ToB=Tobramycin, TR=Trimethoprim , CFM=Cefixime , CPM=Cefepime, CAC= Ceftazidime

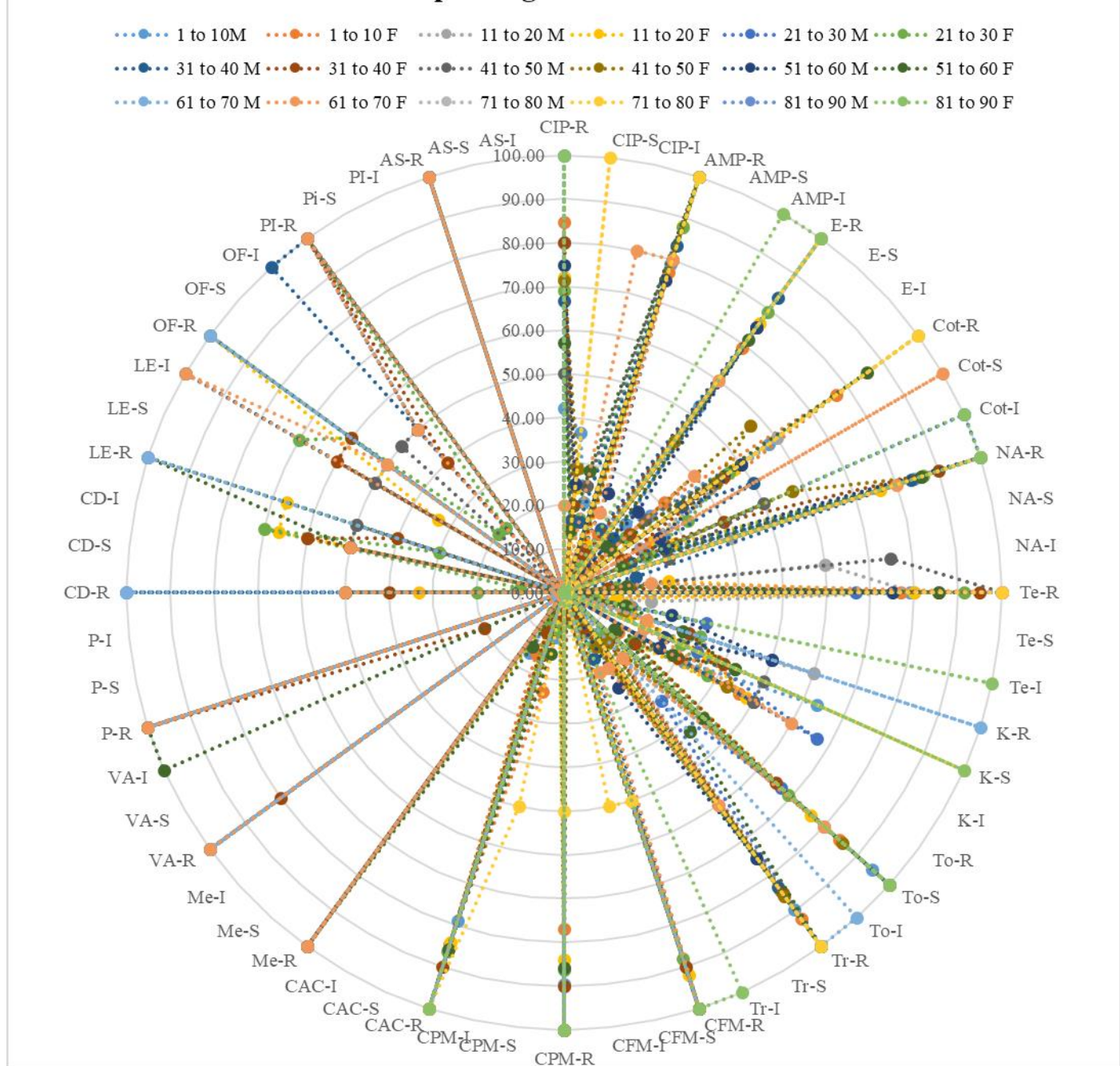
Tobramycin was found to be most effective antibiotic against uropathogenic *S. aureus* . It was effective against 23 (82.14%) strains of uropathogenic *S. aureus*. Isolates from patients with age 1-10 years shown highest resistance to cefixime (100%), from patients with age 11-20 years shown absolute resistance (100%) to ampicillin sulbactam, piperacillin, penicillin, vancomycin, methicillin and cefixime. Isolates from patients with age 21-30 years shown absolute resistance (100%) to ampicillin-sulbactam, piperacillin, penicillin, vancomycin and methicillin, from patients with age 31-40 years shown absolute resistance (100%) to ampicillin-sulbactam, piperacillin, penicillin and methicillin followed by 92.3 % strains were resistant to cefepime, ceftazidime and cefixime.

All 47 strains of gram positive uropathogenic bacteria were found to be resistant to trimethoprim, cefixime and tetracyclines, 45 (95.74%) to ampicillin and cefepime, 44 (93.62%) to ceftazidime and 34 (72.34%) to cotrimoxazole. Tobramycin is found to be most effective antibiotic against gram positive uropathogenic bacteria. It is effective against 42 (89.36%) strains of gram positive uropathogenic bacteria. All 28 strains of uropathogenic *S. aureus* found to be resistant to penicillin, piperacillin, methicillin and ampicillin-sulbactam, 25 (89.29%) to vancomycin, 17 (60.71%) to ofloxacin, 12 (42.86%) to levofloxacin and 11 (39.29%) to clindamycin.

**Figure 2 Antibiotic resistance profile of uropathogenic bacteria**



**Figure 3: Prevalence of antibiotic resistance among uropathogenic bacteria**



Isolates from patients with age 41-50 years shown absolute resistance (100%) to ampicillin-sulbactam, piperacillin, penicillin, vancomycin, methicillin, cefepime, ceftazidime ampicillin and cefixime, from patients with age 51-60 years shown absolute resistance (100%) to ampicillin-sulbactam, piperacillin, ofloxacin, levofloxacin, penicillin, methicillin and cefixime, from patients with age 61-70 years shown absolute resistance (100%) to ampicillin-sulbactam, piperacillin, penicillin, vancomycin, methicillin, ceftazidime, cefepime, tetracyclines and cefixime. Isolates from patients with age 71-80 years shown absolute resistance (100%) to ceftazidime, trimethoprim, tetracyclines, nalidixic acid, co-trimoxazole, erythromycin and ampicillin, from patients with age 81-90 years shown absolute resistance (100%) to ceftazidime, cefepime, cefixime, nalidixic acid erythromycin and ampicillin.

Antibiotic resistance among bacteria is the health threat affecting human wellbeing. Problem of emergence of antibiotic-resistant bacteria become severe with every year. Some pathogens now a days shown resistance to multiple classes of antibiotics. The widespread non-regulated use of antibiotics playing a significant role in the emergence of resistant bacteria. The present study showed that 176 out of 180 isolates were resistant more than 3 antibiotics. The higher resistance against antibiotics can be as a result of repeated and prolonged use or exposure of uropathogenic bacteria to antibiotics. (Pitout J.D. , 2012)

Our findings showed that most of the prescribed antibiotics were frequently ineffective against all tested genera of uropathogenic bacteria. Similar findings reported by **Elizabeth F. et al, 2018, Prakash D. et al, 2013, Wasamwar M. et al, 2014 and Jha T. et al, 2024.**

This high rate can be explained by the fact that these antibiotics are regularly used as first-line treatments as well as self-medication in the treatment of common bacterial infections. These practices lead to the recurrent exposure of bacteria to these antibiotics, which may prompt adaptation of the strains and thus antibiotic resistance. (**Pandey J.K. & Tyagi A.K.S., 2013; Bahashwan S.S. & Shafey H.M., 2013**)

In this study highest resistance observed against ampicillin was in accordance with previously reported results. (**Mukharjee M. et al, 2013, Okesola A.O & Aroundegbe T.I., 2011; Shirani K. et al, 2019**)

This high rate of antibiotic resistance against selected ampicillin antibiotics shown by uropathogenic bacteria because this antibiotic is regularly used as first treatment against common bacterial infection. (**Salama B. et al, 2020, Widayati A. et al, 2011**)

Regular exposure of bacteria with antibiotics lead to development of antibiotic resistance among uropathogenic bacteria due to adaption. (**Arsene M.M.J. et al, 2021, Paseshke et al, 2010; Widayati A. et al, 2011**)

Uropathogenic bacteria also shown resistance to trimethoprim and co-trimoxazole. Trimethoprim and co-trimoxazole are the most recommended antibiotics for treating acute uncomplicated urinary tract infection. (**Mbarga M.J.A. et al, 2021**)

Bacterial resistance to these antibiotics is mediated by following mechanisms-1) Permeability barrier and/or efflux pumps, 2) Naturally insensitive target enzymes 3) Regulatory changes in the target enzymes 4) Mutational or recombinational changes in the target enzymes 5) The acquisition of resistance by drug resistant target enzymes.

In this study higher resistance observed against nalidixic acid was in accordance with previously reported results. (**Devanad P. & Saxena R.S, 2013, Patil P.S. et al, 2018, Sarkar A. et al, 2023**). Uropathogenic bacterial isolates also shown resistance to Erythromycin and ciprofloxacin. Our findings were consistent with findings of **Devanand P. et al. 2013, Sarkar A. 2023, Patil P.S. et al, 2018, Bazira J. et al, 2024, Agrawal A., et al, 2021, Karyakarte A. et al, 2022, Sharif A. et al, 1999 ; Wasamwar M. et al, 2014**)

**Conclusion:** This study conducted at Gondia city of Maharashtra state, India, providing information on the prevalence and diversity of uropathogenic bacteria in urine samples of patients with UTI and their resistance to antibiotics. Women are most affected by urinary tract Infection. Antibiotic resistance should be continuously evaluated in patients of all ages and in all hospitals where possible, so as to monitor the evolution of this major public health issue. In this investigation performed on uropathogenic bacterial strains isolated from urine samples of patients diagnosed with UTIs. We found that only Tobramycin and Kanamycin antibiotics are effective against all uropathogenic bacteria. The unusual resistance to antibiotics more globally to other antibiotics observed in some strains is alarming and suggests the need for more judicious use of these antibiotics.

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