

# Prevalence and Phenotypic Profile of Antimicrobial Resistance Pattern of Multidrug Resistant Uropathogens in a Rural Tertiary Care Hospital, Gujarat, India

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## ABSTRACT

**Introduction:** Multidrug Resistant (MDR) organisms have become a major problem for the treatment of various infections and are imposing the greatest challenge to public health worldwide. Uncomplicated Urinary Tract Infections (UTIs) are treated empirically sometimes with broad spectrum antibiotics without performing drug susceptibility tests that adds to drug resistance in bacteria.

**Aim:** To identify the current prevalence and evaluate phenotypic profile of antimicrobial resistance pattern of MDR uropathogens.

**Materials and Methods:** This was a cross-sectional descriptive study conducted from January 2019 to July 2021 at Parul Sevashram Hospital Vadodara, Gujarat, India. Total 960 uropathogens were analysed for prevalence, their phenotypic antimicrobial resistance mechanism and antibiotic sensitivity pattern. Isolated organisms, their phenotypic resistance pattern and antibiotic sensitivity data was entered in Microsoft excel and data were analysed using Statistical Package for the Social Sciences (SPSS) 25.0 version.

**Results:** During the study period, total 960 urinary isolates were analysed of which 891 (92.8%) were gram negative bacilli, 69 (7.2%) were gram positive cocci. Probable antimicrobial resistance pattern in gram negative isolates causing UTI were 317 (35.6%) of Extended Spectrum Beta Lactamase (ESBL) producer, While carbapenemase (+ or - ESBL) were 328 (36.8%) and impermeability carba (+ESBL or +High-Level Ampicillinase C (HL AmpC)) were 311 (34.9%). Amikacin was highly sensitive antibiotic in 378 (75.3%) of *Escherichia coli* (*E. coli*) and 111 (52.9%) of *Klebsiella pneumoniae* (*K. pneumoniae*) isolates causing UTI.

**Conclusion:** The study concluded that carbapenem resistance was more in *K. pneumoniae* isolates causing UTI than *E. coli*. Aminoglycosides like amikacin was highly effective for the treatment of UTI caused by *E.coli* and *K. pneumoniae*.

**Keywords:** *Escherichia coli*, *Klebsiella pneumoniae*, Multi drug resistant organism, Urinary tract infection

## INTRODUCTION

The MDR bugs have become a major problem in the treatment of various infections and are imposing the greatest challenge to public health worldwide. MDR bacteria cause around 700,000 deaths worldwide every year and it is estimated that they will cause 10 million deaths by 2050, with a greater loss of economic resources [1]. Antimicrobial resistance is also an increasing concern worldwide, especially in gram negative bugs where there is limited availability of new and effective antimicrobial agents. To prevent the problem of drug resistance, the World Health Organisation (WHO) has put various interventions in place which contain the formation of a national task force, the development of indicators to assess the effect of antimicrobial resistance; and designing microbiological baselines that effectively coordinates the surveillance of antibiotic resistance among common bacterial pathogens [2,3]. Although these interventions are found to be well employed in the developed countries because absence of resources has limited their execution in many developing countries where treatment opportunities are also limited.

UTI is the most frequent and common bacterial infections encountered in clinical settings [4]. Primary uncomplicated, community acquired UTIs are treated most of the times empirically with broad spectrum antibiotics. In such cases drug susceptibility tests are not performed and that may add to drug resistance in bacteria. *E. coli* is the predominant uropathogen responsible for (80%) UTIs followed by *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Enterococcus* [4]. The pathogens traditionally are associated with UTI pattern of antimicrobial resistance can

change and underlying other host factors [4]. Selective pressure of antibiotics has led to the emergence of various drug resistance mechanisms like production of different betalactamases viz. ESBL, Amp C  $\beta$ -lactamases and Carbapenemases and other mechanisms like efflux pumps [5]. Antimicrobial susceptibility testing doesn't only help to choose the appropriate antibiotic but it also helps to facilitate the empirical therapy by preparation of periodic antibiograms [6]. Studies done in a particular geographical area can help tackle detection of emerging antibiotic resistance, changes in the antibiotic resistance pattern and fosters antibiotic stewardship [7]. Present study was an attempt to do the same in a rural tertiary care hospital in Western India to identify the current prevalence and evaluate phenotypic profile of antimicrobial resistance pattern of MDR uropathogens amongst patients admitted in the hospital.

## MATERIALS AND METHODS

This was a cross-sectional descriptive study conducted from January 2019 to July 2021 at Parul Sevashram Hospital Vadodara, Gujarat, India. Study was duly approved by Institutional Ethics Committee (IEC) (IEC approval no: PUIECHR/PIMSR/00/011802/1402).

**Inclusion criteria:** All the adult patients admitted to Parul Sevashram Hospital who had symptoms of UTI during the study period (fever, burning micturition, frequency, urgency) were included.

**Exclusion criteria:** Outpatients and paediatric patients were excluded from in this study.

## METHODOLOGY

**Collection of specimens:** A 5 to 10 mL of clean-catch technique of midstream urine specimens were collected in a sterile container before starting new antibiotic therapy. Urine specimens of catheterised patients were obtained prior to catheter change or removal from each patient. A 5 to 10 mL of urine was obtained from the collection port of the catheter tube (after cleaning with an antiseptic) using a sterile needle and syringe into sterile universal container and transported to the microbiology laboratory for testing within one hour of collection.

During the study period urinary samples were tested by standard microbiological procedure [8]. The samples were plated on Nutrient Agar and MacConkey Agar media by the semi-quantitative plating method using the calibrated loop technique. Plates were incubated aerobically overnight at 37°C. Plates showing growth suggestive of significant bacteriuria (more than 10<sup>5</sup> colony forming unit (cfu)/mL) were subjected to identification and antibiotic sensitivity testing by Vitek 2.0 with Advanced Expert System™ which also allows determines probable phenotypic antimicrobial resistance mechanism expressed on the basis of sensitivity Minimum Inhibitor Concentration (MICs) of various antibiotics.

## STATISTICAL ANALYSIS

Data on vitek system like isolated organisms, their phenotypic resistance pattern and antibiotic sensitivity data was extracted and converted to Excel sheet and data were analysed in terms of frequency using SPSS 25.0 version.

## RESULTS

During the study period, total 960 urinary isolates were analysed. Among UTIs patients, 583 (60.7%) were females and 377 (39.3%) were males. In case of females, the UTIs were reported highest (41.7%) in the age group of 31-40 years, However, in case of males, infections were more prevalent (38.5%) in the age group of 61-70 years [Table/Fig-1]. Out of the 960 isolates, 891 (92.8%) were gram negative bacilli, 69 (7.2%) were gram positive cocci. Amongst the gram negative isolates, *Escherichia coli* 502 (56.3%) was predominant followed by *Klebsiella pneumoniae* 210 (23.6%) [Table/Fig-2]. Amongst gram positive isolates, *Enterococcus faecalis* 21 (30.4%) was predominant followed by *Enterococcus faecium* 18 (26.1%) [Table/Fig-3]. As far as antibiogram of gram-negative bacteria is concerned, amikacin was the most effective drug with sensitivity of (61.5%), followed by ertapenem (46.2%) and Nitrofurantoin (45%) [Table/Fig-4]. Linezolid (66.7%) was most sensitive antibiotic amongst gram positive isolates followed by Teicoplanin (60.9%) and Vancomycin (39.1%) [Table/Fig-5]. As far as mechanism of resistance is concerned, 35.6% of gram-negative isolates were Extended spectrum beta lactamase producer. While carbapenamase (+ or - ESBL) were 36.8% and impermeability carba (+ESBL or +HL Ampc) were 34.9 % [Table/Fig-6]. Mechanism of resistance in gram

Age (years)	Male n (%)	Female n (%)	Total n (%)
0-10	0	0	0
11-20	0	0	0
21-30	2 (0.5)	110 (18.9)	112 (11.7)
31-40	5 (1.3)	243 (41.7)	248 (25.8)
41-50	80 (21.2)	67 (11.5)	147 (15.3)
51-60	110 (29.2)	40 (6.8)	150 (15.6)
61-70	145 (38.5)	53 (9.1)	198 (20.6)
71-80	35 (9.3)	70 (12)	105 (11.0)
Total	377 (39.3)	583 (60.7)	960 (100)

[Table/Fig-1]: Age/Sex wise distribution of UTI cases.

Isolated organism	Frequency (N)	Percentage (%)
<i>Escherichia coli</i>	502	56.3
<i>Klebsiella pneumoniae</i>	210	23.6
<i>Pseudomonas aeruginosa</i>	104	11.7
<i>Acinetobacter baumannii</i> cplx	18	2.0
<i>Enterobacter cloacae</i> complex	10	1.1
<i>Proteus mirabilis</i>	7	0.9
<i>Providentia rettgeri</i>	7	0.9
<i>Morganella morganii</i>	5	0.7
<i>Burkholderia cepacia</i>	3	0.3
<i>Citrobacter freundii</i>	3	0.3
<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i>	3	0.3
<i>Pseudomonas putida</i>	3	0.3
<i>Acinetobacter lwoffii</i>	2	0.2
<i>Aeromonas hydrophila/ Aeromonas caviae</i>	2	0.2
<i>Citrobacter koseri</i>	2	0.2
<i>Enterobacter aerogenes</i>	2	0.2
<i>Acinetobacter junii</i>	1	0.1
<i>Aeromonas sobria</i>	1	0.1
<i>Klebsiella oxytoca</i>	1	0.1
<i>Klebsiella ozaenae</i>	1	0.1
<i>Pantoea dispersa</i>	1	0.1
<i>Providencia stuartii</i>	1	0.1
<i>Raoultella ornithinolytica</i>	1	0.1
<i>Serratia fonticola</i>	1	0.1

[Table/Fig-2]: Prevalence of gram negative isolates causing UTI (N=891 isolates)

Gram positive isolates	Frequency (N)	Percentage (%)
<i>Enterococcus faecalis</i>	21	30.4
<i>Enterococcus faecium</i>	18	26.1
<i>Enterococcus</i> spp.	11	15.9
<i>Staphylococcus aureus</i>	8	11.6
Coagulase neg. <i>Staphylococcus</i>	3	4.3
<i>Enterococcus gallinarum</i>	2	2.9
<i>Staphylococcus lentus</i>	2	2.9
Coagulase pos. <i>Staphylococcus</i>	1	1.4
<i>Cronobacter sakazakii</i> group	1	1.5
<i>Staphylococcus epidermidis</i>	1	1.5
<i>Staphylococcus sciuri</i>	1	1.5

[Table/Fig-3]: Prevalence of gram positive isolates causing UTI (N=69 isolates)

Antibiotic	Sensitivity (N)	Sensitivity (%)
Ampicillin	118	13.2
Amoxicillin Clavulanic acid	274	30.8
Amikacin	548	61.5
Ceftazidime	219	24.6
Ciprofloxacin	156	17.5
Ceftriaxone	196	22
Ertapenem	412	46.2
Nitrofurantoin	401	45
Gentamycin	340	38.2
Nalidixic acid	110	12.3
Norfloxacin	184	20.7
Ofloxacin	160	18
Cotrimoxazole	258	29
Piperacillin tazobactam	246	27.6

[Table/Fig-4]: Antibiotic sensitivity of gram-negative isolates causing UTI (N=891 isolates).

positive isolates were following, high level resistant gentamicin was 65.2%, resistant (MLSB) was 42%, (modification of pbp) was 37.7% [Table/Fig-7]. Majority of *E. coli* (51.4%) causing UTI was ESBL producer [Table/Fig-8] while majority of *K. pneumoniae* (61.4%) causing UTI were carbapenemase (+ or - ESBL) producer [Table/Fig-9]. Amikacin was highly sensitive antibiotic in 75.3% of *E. coli* and 52.9% of *K. pneumoniae* [Table/Fig-10].

Antibiotic	Sensitivity (N)	Sensitivity (%)
Levofloxacin	7	10.1
Ciprofloxacin	9	13
Erythromycin	5	7.2
High level Gentamycin	7	10.1
Linezolid	46	66.7
Penicillin	14	20.3
Teicoplanin	42	60.9
Tetracyclin	22	31.9
Vancomycin	27	39.1

**[Table/Fig-5]:** Antibiotic sensitivity of gram-positive isolates causing UTI (N=69 isolates)

Mechanism of resistance	Frequency (N)	Percentage (%)
Carbapenemase (+ OR - *ESBL)	328	36.8
Extended spectrum beta-lactamase	317	35.6
Impermeability carba (+ESBL OR +HL AmpC)	311	34.9
Carbapenemase	61	6.8
ESBL (CTX-M LIKE)	53	5.9
High level cephalosporinase	50	5.6
Acquired penicillinase	41	4.6
HL Cephalosporinase (**AmpC)	50	4
ESBL + Impermeability (cephamycins)	27	3
Wild (penicillinase)	14	1.6
Acquired cephalosporinase (except ACC-1)	14	1.6
High level case (AmpC)	6	0.7
Wild (cephalosporinase)	5	0.6
ESBL (clavulanate inhibited)	5	0.6
Cephalosporinase (AmpC)	3	0.3

**[Table/Fig-6]:** Mechanism of resistance in gram negative isolates causing UTI (N=891 isolates)

\*ESBL= Extended Spectrum Beta-Lactamase, \*\*HL-AmpC = High-Level Ampicillinase C

Mechanism of resistance	Frequency (N)	Percentage (%)
Family -oxazolidinone – 2	50	72.5
High Level Resistant Gentamicin	45	65.2
Resistant *(MLSB)	29	42
Resistant (Modification of **PBP)	26	37.7
Resistant (van A Like)	18	26.1
Resistant (van B Like)	11	15.9
Modification of PBP (mecA)	9	13

**[Table/Fig-7]:** Mechanism of resistance in gram positive isolates causing UTI (N=69 isolates).

\*MLSB = Macrolide-lincosamide-streptogramin B, \*\*PBP = Penicillin binding protein

Mechanism of resistance	Frequency (N)	Percentage (%)
Extended Spectrum Beta-Lactamase	258	51.4
Carbapenemase (+ Or - *ESBL)	183	36.5
HL Cephalosporinase (**AmpC)	36	7.2
ESBL (Ctx-M Like)	36	7.2
Acquired Penicillinase	14	2.8
Impermeability carba (+ESBL OR +HL Ampc)	182	36.3
Cephalosporinase (AmpC)	3	0.6

**[Table/Fig-8]:** Mechanism of resistance in *E. coli* causing UTI (N= 502 isolates)

\*ESBL= Extended Spectrum Beta-Lactamase, \*\*AmpC = Ampicillinase C

Mechanism of resistance	Frequency (N)	Percentage (%)
Extended Spectrum Beta-Lactamase	49	23.3
Carbapenemase (+ Or - *ESBL)	129	61.4
ESBL + Impermeability (Cephamycins)	27	12.9
ESBL (Ctx-M Like)	14	6.7
Acquired Penicillinase	8	3.8
Impermeability carba (+ESBL OR+HL **AmpC)	126	60

**[Table/Fig-9]:** Mechanism of resistance in *Klebsiella pneumoniae* causing UTI (N=210)

\*ESBL= Extended Spectrum Beta-Lactamase, \*\*AmpC = Ampicillinase C

Antibiotic	Sensitivity			
	<i>E. coli</i> sensitivity (N)	<i>E. coli</i> sensitivity (%)	<i>K. pneumoniae</i> sensitivity (N)	<i>K. pneumoniae</i> sensitivity (%)
Ampicillin	54	10.8	1	0.5
Amoxicillin Clavulanic acid	175	34.9	30	14.3
Amikacin	378	75.3	111	52.9
Ceftazidime	115	22.9	31	14.8
Ciprofloxacin	69	13.7	28	13.3
Ceftriaxone	103	20.5	40	19
Ertapenem	309	61.6	74	35.2
Nitrofurantoin	329	65.5	52	24.8
Gentamycin	248	49.4	74	35.2
Nalidixic acid	40	8	49	23.3
Norfloxacin	113	22.5	50	23.8
Ofloxacin	95	18.9	45	21.4
Cotrimoxazole	174	34.7	65	31
Piperacillin tazobactam	201	40	39	18.6

**[Table/Fig-10]:** Antibiotic sensitivity of *E.coli* and *K. pneumoniae* causing UTI.

## DISCUSSION

Effective management for the treatment of UTI is commonly depending on the identification of disease causing type of organism and the choice of suitable antibiotic for the treatment. Development of resistance to commonly used antibiotics is a major concern worldwide causing failure of treatment in different types infections including UTI [9]. In present study, *E. coli* was the most frequent and predominant isolate followed by *K. pneumoniae*, similar findings were observed by Flores-Mireles A et al., Shiralizadeh S et al., Al-Zahrani J et al., Akter ML et al., and Ravishankar U et al., in many previous studies [10-14]. The series of causative agents for complicated UTIs following uropathogenic *E. coli* as highly common, they are *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and Group B *Streptococcus* [10, 11]. In further previous studies, *E.coli* (70.4%), and *Klebsiella* spp (21.2%) found to be highly isolated microbes [12]. In one more study done by Akter ML et al., *E. coli* was found to be the leading uropathogen isolated from 118 (59.30%) samples [13]. Amongst gram positive bacteria *Enterococcus* species (75.3%) was most common bacteria causing UTI in present study. Most UTI were due to either *E. faecalis* (30.4%) or *E. faecium* (26.1%). Similar findings were reported by Goel V et al., in which most common enterococcal isolate causing UTI was *E. faecalis* (61/115 [53%]), followed by *E. faecium* (42/115 [36.5%]) [15]. Silverman J et al., also illustrated out of total 100 isolates from stool culture, 73 (68%) were *E. faecalis*, followed by *E. faecium* 26 (24%), *Enterococcus gallinarum* 5 (4%) and others [16]. Most of the earlier studies done on enterococci support the similar findings which could be because of the predominance of *E. faecalis* in the endogenous flora of the body

[16]. In current study, probable antimicrobial resistance pattern in gram negative isolates causing UTI were 35.6% of ESBL producer, while carbapenemase (+ or - ESBL) were 36.8% and impermeability carba (+ESBL or +HL Ampc) were 34.9%. Majority of *E. coli* (51.4%) causing UTI was ESBL producer compared to *Klebsiella pneumoniae* which accounts for only 23.3%. ESBL production was observed in 45.51% of *E. coli* isolates causing UTI in a study by Jain R et al., [17]. Such finding was also seen in a study done by Abayneh M et al., where *E. coli* accounts for greater number of urinary isolates as well as higher numbers of ESBL production 76.5% was observed than *K. pneumoniae* which was 23.5% [18]. In an another study by Malik S et al., observed 83% of the collected Uropathogenic *Escherichia coli* (UPEC) isolates revealed MDR pattern and observed highest resistance to cephalosporins and fluoroquinolones; and showed maximum susceptibility toward tigecycline (100%), followed by to amikacin, colistin, fosfomycin, and nitrofurantoin (90.5%, 96.2%, 86.7%, 84.9%) [19]. The likelihood of simple UPEC isolates transforming into MDR strains over a long period of time depends on several factors namely biofilm formation in the bladder, rise of ESBL producing strains, inappropriate use of antibiotics by physicians or unqualified practitioners, and easy accessibility of antibiotics [20]. Present study found that majority of *Klebsiella pneumoniae* (61.4%) causing UTI were carbapenemase (+ or - ESBL) producer. In a Spanish study published in 2014, 50 cases of Carbapenem resistant *Klebsiella* infections were treated with aminoglycosides group of antibiotics (Gentamicin) observed a statistically significant reduction in mortality [21]. Present study revealed that amikacin was the most effective drug with sensitivity of (61.5%). followed by Ertapenem (46.2%) and Nitrofurantoin (45%) in gram negative isolates. In another study carried out by Kande S et al., the majority of the pathogens found susceptible to nitrofurantoin, gentamicin and amikacin (80.8%, 76.8%, 72.1%) [22]. As far as gram positive isolates are concerned, linezolid (66.7%) was most sensitive antibiotic followed by teicoplanin (60.9%) and vancomycin (39.1%).

Amikacin was highly sensitive antibiotic in 75.3% of *E. coli* and 52.9 % of *K. pneumoniae* isolates causing UTI. Amikacin susceptibility was also much higher which was noticed in previous study [23]. Possible reason for this higher susceptibility to amikacin could be because of the absence of routine use of amikacin as empirical therapy because of the nephrotoxicity and its absence of considerable cross-resistance with other groups of antimicrobial agents.

### Limitation(s)

The study limits to phenotypic expression of antibiotic resistance pattern. Further studies are needed to detect antibiotic resistance encoding genes (ARGs) of various uropathogens that will reveal the genetic resistance pattern as it is more specific since some of the genetic resistance like Amp C is expressed under selective pressure and might lead to treatment failure if it is based only on phenotypic studies. There is also a need to study the increase in MIC values over a period of time developed by different bacterial isolate in particular geographic location.

### CONCLUSION(S)

The study showed high rate of resistance in uropathogens with empiric antibiotic treatment, including fluoroquinolones, ampicillin and cotrimoxazole. This illustrates that the use of these antibiotics is not good as empiric treatment for UTIs. Carbapenem resistance was more in *K. pneumoniae* isolates causing UTI than *E. coli*.

Aminoglycosides like amikacin was highly effective for the treatment of UTI caused by *E. coli* and *K. pneumoniae*.

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**PLAGIARISM CHECKING METHODS:** [\[Jain H et al.\]](#)

- Plagiarism X-checker: Feb 25, 2022
- Manual Googling: Apr 18, 2022
- iThenticate Software: Apr 22, 2022 (24%)

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- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

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