Original Article

DOI: 10.7860/JCDR/2026/77173.22303

Microbiology Section

Clinical Profile, Antimicrobial Resistance and Molecular Detection of ESBL, MBL and Other Carbapenemase Genes in *Klebsiella* Species-UTI Isolates from Chronic Kidney Disease Patients: A Cross-sectional Study

NITHYA KANGOSSERI[1], VIDYA PAI[2], BH SANTHOSH PAI[3], ADITYA KUMAR MISHRA[4], HAISAM BIN ABDUL KADHAR[5], PREEMA DSOUZA[6]

ABSTRACT

Introduction: Urinary Tract Infections (UTIs) are a common yet serious medical condition that can impact individuals of all ages and genders. UTIs in Chronic Kidney Disease (CKD) patients lead to a low quality of life, and the situation becomes worse when the pathogens exhibit antibiotic resistance due to Extended Spectrum Beta-Lactamases (ESBLs), Metallo-Beta-Lactamases (MBLs), and Carbapenemase genes.

Aim: To to evaluate the clinical profile, antibiotic resistance, and frequency of resistance genes in *Klebsiella pneumoniae* isolates from urine samples of suspected UTIs in CKD patients.

Materials and Methods: This cross-sectional study was conducted at Yenepoya Medical College Hospital in Mangalore, Karnataka, India, from January 2023 to January 2024. A total of 138 *Klebsiella* spp. isolates were collected and included in the study. Antimicrobial susceptibility was assessed using the Vitek2 method. The production of MBL, carbapenemase, and ESBL was confirmed by tests described in the Clinical and Laboratory Standards Institute (CLSI) 2023 document, and genes were detected using multiplex Polymerase Chain Reaction (PCR). Statistical analyses were expressed as percentages for all quantitative data, and categorical variables were compared using Fisher's exact test, with a p-value of <0.001 considered significant.

Results: Among the study participants, 79 (57.25%) were male, and approximately 84 (60.87%) were over 50 years of age. About 46 (33.33%) patients had a history of recurrent UTIs and stage 1 renal impairment. A total of 85 (61.59%) of *Klebsiella* spp. isolates exhibited Multidrug Resistance (MDR). The maximum resistance was observed against ceftazidime and cefepime, while lower resistance was noted for amikacin, gentamicin, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole. Eighteen of the MDR isolates (72%) carried β-lactamase genes, such as *bla*$_{-SHV}$ and *bla*$_{-CTX-M}$. Additionally, 33 (82.5%) had *bla*$_{-KPC}$, 21 (52.5%) had *bla*$_{-IMP}$, and OXA genes (58, 23, 51, and 48) were found in 2 (2.8%) isolates each.

Conclusion: The present study emphasises the significance of the co-occurrence of ESBL and carbapenemase-encoding genes in *K. pneumoniae* isolates implicated in UTIs among CKD patients, which could pose challenges for effective treatment options.

Keywords: Extended-spectrum beta-lactamases, Metallo beta lactamases, Urinary tract infections

INTRODUCTION

The UTIs are often serious medical conditions that affect both men and women and can manifest at various stages of life [1]. Numerous bacterial pathogens, such as *E. coli, Klebsiella* spp., Staphylococci, and yeast like *Candida* spp., are known to cause UTIs [2]. Several investigations have documented the presence of *Klebsiella pneumoniae* in high-grade infections of the upper or lower urinary tract. Various traits of these pathogenic bacteria enable them to infiltrate the host system and intensify their virulence [3]. They possess a remarkable capacity to adapt to various environments, thus promoting their role as virulent pathogens [4]. The intricate process of bacterial pathogenicity depends on both the host immune system and the bacterium's virulence [5]. Individuals with impaired immune systems may be more susceptible to infections due to diminished immune activity. Numerous research studies have linked UTIs in kidney patients to *Klebsiella pneumoniae*, which is an opportunistic bacterium that can cause nosocomial infections [6-9]. Most kidney patients undergo prolonged haemodialysis and are consequently at risk for reduced immunity, anaemia, malnutrition, inflammation, and vitamin deficiencies. Research on the prevalence of UTIs in kidney patients has documented a low quality of life [10]. Multiple virulence factors present in bacteria help them adapt to the host's immunological status [11]. Pathogens utilise virulence factors such as adhesins, invasins, secretion systems, outer membrane proteins, toxins, capsules, iron acquisition mechanisms, and biofilm formation to invade and persist within the human host [12]. This scenario is exacerbated when pathogens also carry genes for antimicrobial resistance, particularly ESBLs, MBLs and Carbapenemase genes [13].

Since the 1940s, beta-lactam antibiotics have been used in clinical settings. Over time, bacterial pathogens began to evolve beta-lactamases, with *Klebsiella pneumoniae*'s penicillin resistance being a significant example [14]. Potential genes of pathogenic bacteria have been identified as belonging to classes such as bla-SHV, bla-TEM, bla-CTXM, bla-NDM, bla-KPC, bla-VIM, and bla-IMP [15,16]. The presence of virulence genes is sufficient to evade host defense mechanisms and infect humans, while resistance genes provide survival strategies for the pathogen. Consequently, the pathogenic uniqueness of these bacteria increases, enabling them to cause severe infections in patients.

The current study aimed to determine the clinical profile, CKD staging, assess the pattern of antibiotic resistance, and molecularly

detect the frequency of ESBL, MBL, and carbapenem classes of genes in *Klebsiella pneumoniae* isolated from urine samples of suspected UTIs in CKD patients.

The primary objectives include detailed observations on the clinical profile and CKD staging based on estimated Glomerular Filtration Rate (eGFR) as per the National Kidney Foundation reference guide on kidney disease screening criteria [17]. The study also aimed to assess the pattern of antibiotic resistance in *Klebsiella pneumoniae* isolates from urine samples of these patients.

The secondary objective was the molecular detection of the frequency of the ESBL, MBL, and carbapenem classes of genes in the study isolates.

MATERIALS AND METHODS

This cross-sectional study was performed at Yenepoya Medical College Hospital in Mangalore, Karnataka, India, from January 2023 to January 2024. Ethical clearance was approved by the Institutional Ethics Committee with protocol number YEC-1/2022/246, and patients were recruited for the study after obtaining informed consent.

Sample size: A total sample size of 138 *Klebsiella* species from CKD patients with UTIs was included in the study, based on an estimated prevalence of *Klebsiella* as a cause of UTI in CKD patients at 10%, with a standard normal coefficient of 1.96 [18]. Simple random sampling of urine culture-positive *Klebsiella* isolates from CKD patients was performed in this study.

Inclusion criteria: Urine cultures from CKD patients demonstrating significant growth (>10^5 cfu/mL) of *Klebsiella* spp. were included in the study.

Exclusion criteria: Urine samples with lower colony counts, significant counts of other genera of Enterobacteriaceae, or those harbouring more than one type of bacteria were excluded from the study.

Study Procedure

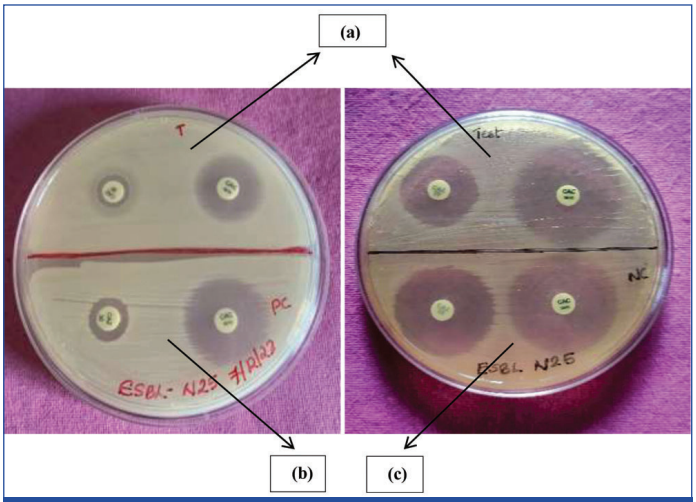
A total of 138 isolates of *Klebsiella* spp. from urine specimens of CKD patients were included in the study. The isolates were identified using standard microbiological techniques and the automated identification system, Vitek 2 [19]. For further experiments, the isolates were stored on semisolid Nutrient Agar (NA; HiMedia, India) [20]. The demographic profiles of patients were collected and analysed for risk factors. CKD stage classification was done based on the standard principles set forth by the National Kidney Foundation. The CKD stages were classified based on eGFR ranges reviewed in each patient and categorised as follows:

- ≥90 mL/min/1.73m² eGFR - CKD stage 1
- 60-90 mL/min/1.73m² eGFR - CKD stage 2
- 30-59 mL/min/1.73m² eGFR - CKD stage 3
- 15-29 mL/min/1.73m² eGFR - CKD stage 4
- <15 mL/min/1.73m² eGFR - CKD stage 5 [21].

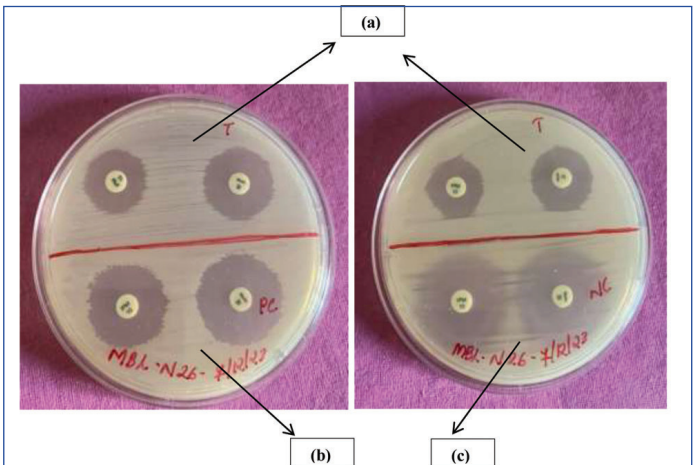
Antimicrobial susceptibility assessment: The susceptibility patterns of antimicrobials were determined using the Automated Bacterial AST system (Vitek 2). ID-GNB cards were employed to identify the isolates in accordance with the manufacturer's instructions. The cards were inoculated with a 0.5 McFarland suspension of the organisms prepared from an 18-20 hour-old 5% sheep blood agar plate and manually placed inside the Vitek 2. Fluorescence measurements were taken every 15 minutes, and identification findings were determined after 3-7 hours. For antimicrobial susceptibility testing, U235 panel cards were used, which included the following antibiotics:

- Ampicillin-sulbactam
- Ceftazidime
- Cefepime
- Ertapenem
- Imipenem
- Meropenem
- Piperacillin-tazobactam
- Amoxicillin-clavulanate
- Amikacin
- Gentamicin
- Ciprofloxacin
- Levofloxacin
- Nitrofurantoin
- Trimethoprim-sulfamethoxazole.

Results were characterised as resistant, intermediate, or sensitive according to CLSI criteria. In this survey, *Klebsiella* spp. isolates that displayed resistance to more than three classes of antibiotics were classified as MDR [22]. The isolates exhibiting the MDR profile were chosen for phenotypic identification of ESBL, MBL, and carbapenemase production using the double disc synergy test with Ceftazidime and Ceftazidime plus clavulanic acid, Imipenem and Imipenem-EDTA, and the modified Hodge test [Table/Fig-1-3], as described in CLSI guidelines [22]. The MDR strains of *K. pneumoniae* were further evaluated for the presence of antibiotic resistance genes using a Multiplex PCR approach.

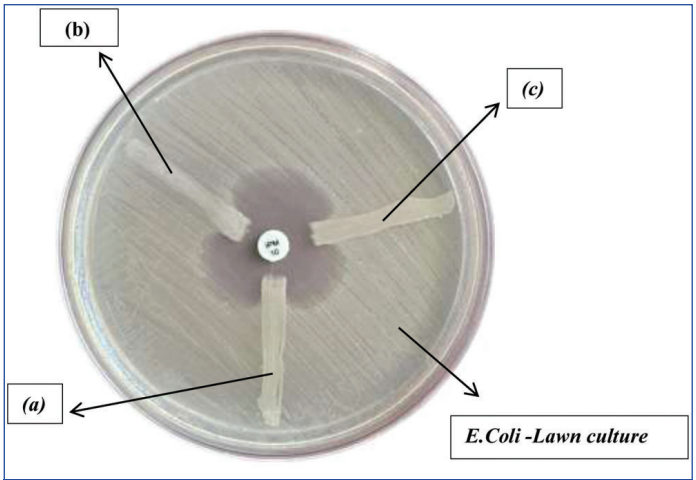


[Table/Fig-1]: Double disc synergy test for Extended Spectrum Beta-Lactamase (ESBLs).
Extended spectrum beta-lactamase producing K.spp: (a) Test organism; (b) Positive control: *Klebsiella pneumoniae* ATCC 700603; (c) Negative control: *Escherichia coli* ATCC 25922. Discs used: Ceftazidine (CAZ) and Ceftazidine clavulinic acid (CAC).



[Table/Fig-2]: Double disc synergy test for Metallo Beta-Lactamse (MBL).
Metallo beta lactamase producing K.spp: (a) Test organism; (b) Positive control: *Pseudomonas aeruginosa* ATCC 27853; (c) Negative control: *Escherichia coli* ATCC 25922. Discs used: Imipenem (IMP) and Imipenem-EDTA (IE).

DNA extraction method [23]: Each isolate of *Klebsiella* spp. was cultivated in Luria-Bertani broth (HiMedia, India) and incubated in



[Table/Fig-3]: Carbapenemase production in *Klebsiella* species identified by Modified Hodge test.
Detection of Carbapenemase production in *Klebsiella* spp using Modified Hodge test-*Klebsiella* spp producing carbapenemase enzyme shown as clover leaf shape appearance on *Escherichia coli* lawn culture plate after 24 hrs incubation at 37°C: (a) Test organism 1; (b) Test organism 2; (c) Test organism 3. Disc used: Imipenem (Imp).

an aerobic environment at 37°C for 24 hours. Pelletisation of the overnight bacterial broth culture was performed by centrifugation at 13,000 rpm for two minutes at room temperature to extract the cells. DNA was extracted and purified using the HiPuraA® Bacterial genomic DNA purification kit-MB505 (HiMedia, India) [24]. The purity of the DNA was estimated to be approximately 1.63 by Nanodrop. The extracted DNA was then used for the detection of genes coding for ESBL, MBL, and carbapenemase in *Klebsiella* spp.

Multiplex PCR method [25]: The current investigation utilised the Hi-PCR® ESBL gene quantification probe kit-MBPCR226 (HiMedia, India) [26]. The purpose of this kit was to identify the specific gene regions encoding ESBLs involved in the detection of *bla*$_{-SHV}$, *bla*$_{-CTX-M}$, and *bla*$_{-OXA}$ 10/11. All target primer sequences, as well as the internal and positive controls for the multiplex PCR mixture, were preinstalled in the kit. The recommended PCR program involved an initial denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds. Annealing and extension were performed at 60°C for 30 seconds, repeated for a total of 45 cycles.

The Hi-PCR Carbapenemase gene (Multiplex) probe PCR kit-MBPCR 132 from HiMedia, India [27] provides two reaction mixtures for both sets of genes employed in the multiplex PCR kit targeting MBL and carbapenemase genes. This kinetic PCR, conducted with master mix-1, under the MBL genes section, includes the following genes: *bla*$_{-NDM,}$ *bla*$_{-KPC,}$ *bla*$_{-VIM,}$ and *bla*$_{-IMP}$. Additionally, carbapenemase genes OXA-51, OXA-23, OXA-58, and OXA-48 were included in master mix-2. The PCR cycle was programmed with an initial denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for five seconds. Annealing and extension occurred at 60°C for one minute for a total of 45 cycles. Results were interpreted according to the protocol and guidelines provided by the HiMedia Multiplex-PCR kit [27].

STATISTICAL ANALYSIS

The data obtained were analysed using the IBM Statistical Package for the Social Sciences (SPSS) application, version 23.0 All quantitative data were expressed as percentages. Categorical variables were compared using Fisher's exact tests. A p-value of <0.001 considered significant.

RESULTS

A total of 138 kidney patients with UTI, in whom urine culture yielded significant growth of *Klebsiella* spp., were recruited for the study. Male participants accounted for 79 (57.25%) of the total, while 59 (42.75%) were female patients. The age distribution of patients enrolled in the study was as follows: 13 (9.42%) patients were <30 years, 84 (60.87%) were >50 years, and 41 (29.71%) were between 31 and 50 years.

In this study, the most common co-morbidities were Diabetes Mellitus (DM) in 25 participants (31.25%) and Hypertension (HTN) in 32 (40%). Additionally, 16 (20%) had both DM and HTN. Minor co-morbidities were noted in only 7 (8.75%) patients. Notably, 42.03% of the patients (n=58) had no co-morbidities.

CKD staging was assessed for all participants, with the majority (n=46) diagnosed at stage 1, accounting for 33.33% of the total. Patients with CKD stages 2, 3, and 4 were represented by n=20 (14.49%), n=25 (18.12%), and n=19 (13.77%) respectively, while 28 patients (20.29%) were at stage 5 CKD. The demographic clinical profile is summarised in [Table/Fig-4].

Factor		n (%)
Sex	Male	79 (57.25%)
	Female	59 (42.75%)
Age (years)	0-30	13 (9.42%)
	31-50	41 (29.71%)
	above 50	84 (60.87%)
Co- morbidities	Yes	80 (57.97%)
	No	58 (42.03%)
Type of co-morbidities	Hypertension (HTN)	32 (40.0%)
	Diabetics Melittus (DM)	25 (31.25%)
	HTN/DM	16 (20.0%)
	Others	7 (8.75%)
CKD- Stages	CKD stage 1	46 (33.33%)
	CKD stage 2	20 (14.49%)
	CKD stage 3	25 (18.12%)
	CKD stage 4	19 (13.77%)
	CKD stage 5	28 (20.29%)
History of Incidence of UTI	First time	15 (10.87%)
	2-3 times	23 (16.67%)
	more than 3	27 (19.56%)
	Recurrently	73 (52.89%)

[Table/Fig-4]: Demographic clinical profile of CKD- UTI patients selected for the study was summarised and tabulated as percentage; CKD: Chronic kidney disease; UTI: Urinary tract infection.

In this investigation, 15 individuals experienced a UTI for the first time, while 23 and 27 individuals had UTIs 2-3 times and >3 times, respectively. Recurrent UTIs were identified in 73 CKD patients (52.89%).

Frequency of UTI in different CKD stages: The study found that recurrent UTIs were more common in CKD stage 1, followed by stage 5 [Table/Fig-5].

CKD stage	Incidence UTI				Total
	First time	2-3 times	More than 3 times	Recurrently	
CKD Stage 1	2	2	16	27	47
CKD Stage 2	5	3	4	7	19
CKD Stage 3	3	2	0	20	25
CKD Stage 4	0	7	2	10	19
CKD Stage 5	5	9	5	9	28
Total	15	23	27	73	138

[Table/Fig-5]: Frequency of UTI in different stages of CKD analysed using Fisher's-Exact test.
UTI: Urinary tract infection; CKD: Chronic kidney disease**.** Fisher's-Exact test p-value is <0.001

Bacterial species identification and AST pattern analysis: In the present study, *Klebsiella pneumoniae* was identified in 137 urine culture isolates, while only one was *Klebsiella oxytoca*. AST,

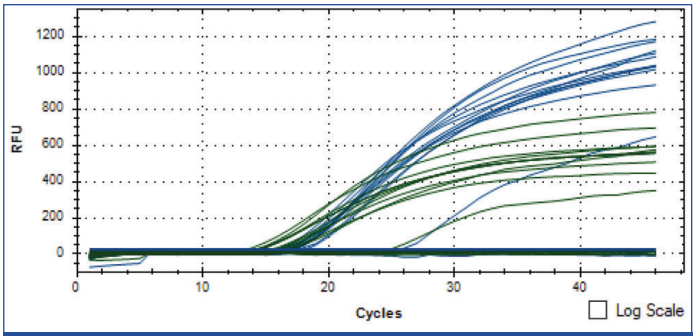
performed using the Automated Bacterial AST system (Vitek 2) according to CLSI 2023 guidelines [22], showed that 85 (61.59%) isolates exhibited MDR phenotype, while the remaining 53 (38.41%) were susceptible to many antibiotics tested. Among the MDR isolates, 15 were resistant to the Quinolone, Aminoglycoside, and Cotrimoxazole drug classes, yet were sensitive to Beta-lactams. Seventy isolates were considered MDR due to their resistance to carbapenems, cephalosporins, and penicillins. These 70 MDR isolates were further tested for confirmation of resistance mechanisms by phenotypic methods and for drug resistance genes by PCR. Using the double disc synergy test, it was determined that n=25 (35.71%), n=34 (28%), and n=37 (52.85%) of the MDR *Klebsiella* spp. produced the ESBL, MBL, and carbapenemase phenotypes, respectively [28-30].

Resistance to antibiotics: Resistance to ceftazidime, ciprofloxacin, and cefepime was high among the tested isolates. The AST pattern revealed that most isolates were susceptible to trimethoprim-sulfamethoxazole (SXT), amikacin, gentamicin, and piperacillin-tazobactam. The antibiotic resistance profile is shown in [Table/Fig-6].

Antibiotics	Names	Sensitive (%)	Intermediate (%)	Resistant (%)
Beta-Lactam antibiotics	Ampicillin	15 (10.86%)	8 (5.79%)	115 (83.33%)
Cephalosporins antibiotics	Ceftazidime	42 (30.43%)	4 (2.89%)	92 (66.66%)
	Cefepime	52 (37.68%)	21 (15.21%)	65 (47.10%)
Carbapenem antibiotics	Ertapenem	47 (34.05%)	6 (4.34%)	85 (61.59%)
	Imipenem	57 (41.30%)	10 (7.24%)	71 (51.44%)
	Meropenem	59 (42.75%)	8 (5.79%)	71 (51.44%)
BL-BLI Antibiotics	Piperacillin - tazobactam	56 (40.57)	7 (5.07%)	75 (54.34%)
	Amoxicilin - clavunate	49 (35.50%)	8 (5.79%)	81 (58.69%)
Aminoglycoside Antibiotics	Amikacin	57 (41.30%)	10 (7.24%)	71 (51.44%)
	Gentamicin	52 (37.68%)	11 (7.97%)	75 (54.34%)
Quinilones antibiotics	Ciprofloxacin	37 (26.81%)	6 (4.34%)	95 (68.84%)
	Levofloxacin	32 (23.18%)	23 (16.66%)	83 (60.14%)
Others	Nitrofurantoin	48 (34.78%)	13 (9.42%)	77 (55.79%)
	Trimethoprim/ Sulfamethoxazole SXT	61 (44.20%)	6 (4.34%)	71 (51.44%)

[Table/Fig-6]: Antimicrobial susceptibility pattern of *Klebsiella* spp. expressed as percentage (%) on the sensitive, intermediate and resistant group.

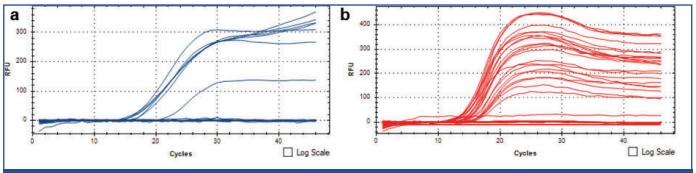
Phenotypic and genotypic detection of drug resistance in MDR isolates: Out of 70 isolates that were resistant to BL, BLI and carbapenemase classes of antibiotics as determined by Vitek 2, 25 were phenotypically positive for ESBL, while 45 were negative. Eighteen out of the 25 ESBL producers tested positive for the bla SHV and bla CTX-M genes, whereas 15 out of the 45 non ESBL producers were also positive for these genes. PCR results for 15 MDR isolates that were sensitive to beta-lactams via Vitek 2 revealed that two isolates had the *bla* $_{-SHV}$ gene, and three had the *bla* $_{CTX-M}$ gene detected [Table/Fig-7a,b]. In this study, 40 out of 70 and 3 out of 70 isolates were tested phenotypically positive for carbapenemase and MBL production, respectively. The results of gene detection for these isolates are depicted in [Table/Fig-8a-c].



[Table/Fig-7a]: PCR amplification result for bla_{SHV} (blue curve) and bla_{CTX-M} (green curve) detected after 45 cycles of PCR program. Ct value ≤33 is considered positive for bla_{SHV} and bla_{CTX-M} genes.
bla_{SHV} genes detected by FAM Detector (blue curve)
bla_{CTX-M} genes detected by HEX Detector (green curve)

	Number of isolates	Number of isolates	*bla* $_{SHV}$	*bla* $_{CTX-M}$	OXA 10/11
MDR (BL, BLI, Carba class of antibiotics)	70	25 (Phenotypic test+ve)	18	18	Nil
		45 (Phenotypic test-ve)	15	15	Nil
MDR (other class of antibiotics)	15	Nil (Phenotypic test)	2	3	Nil
Total MDR	85				

[Table/Fig-7b]: Extended Spectrum Beta Lactamase (ESBLs) gene identification by PCR detection method.



[Table/Fig-8a,b]: PCR amplification result for MBL and Carbapenemase genes.
a MBL genes detected by FAM Detector
b Carbapenemase genes detected by Texas Red Detector

DISCUSSION

The UTIs are among the most common bacterial infections encountered by humans. Certain populations, such as infants, pregnant women, the elderly, individuals with multiple sclerosis, diabetics, patients with renal injury, and those with underlying urological abnormalities, exhibit an increased risk of developing UTIs [31]. The necessity for vascular access for haemodialysis and renal replacement therapy, combined with low immunity and severe clinical conditions, places patients with renal impairment at a heightened risk for urinary infections.

To date, only a few studies have been published on CKD patients with urinary infections. According to two reports, *Klebsiella pneumoniae* is the second most common uropathogen after *E. coli*, predominantly affecting individuals with compromised immune systems [32,33]. Dimitrijevic Z et al.'s study on risk factors for CKD patients with urinary infections reported that most patients were older women; however, the present study primarily included older men [34]. The majority of the patients were in Stage 1 renal impairment (33.33%), and those patients also had a history of recurrent urinary infections (n=27). There has been no targeted study that closely examines the incidence of UTIs in relation to the stages of CKD.

	Number of isolates	Number of isolates	*bla* $_{NDM}$	*Bla* $_{KPC}$	*Bla* $_{IMP}$	*Bla* $_{VIM}$	OXA 58	OXA 23	OXA 51	OXA 48
MDR (BL,BLI,Carba class of antibiotics)	70	40 (Phenotypic test+ve)	3	33	21	Nil	2	2	2	2
		30 (Phenotypic test-ve)	Nil	5	4	Nil	Nil	Nil	Nil	Nil
MDR (other class of antibiotics)	15	Nil (Phenotypic test)	Nil	2	1	Nil	Nil	Nil	Nil	Nil
Total MDR	85									

[Table/Fig-8c]: Multiplex PCR method displayed detected MBL and Carbapenemase genes in *Klebsiella* spp. MDR: Muti-drug resistant.

According to the AST pattern analysis, 61.59% of *Klebsiella* species were MDR, with the highest resistance noted to ceftazidime, ciprofloxacin, and cefepime. Amikacin, gentamicin, piperacillin-tazobactam, and trimethoprim/sulfamethoxazole demonstrated good activity against most of the isolates. A similar AST pattern for *Klebsiella* species has been reported by Saha AK, [35].

It has been noted that the prevalence of the *bla* $_{CTX-M}$ gene among humans has significantly increased over time in many parts of the world. CTX-M remains the most important enzyme in *K. pneumoniae* due to its extensive diffusion and association with human infections [36]. Consequently, its global spread is largely attributed to gene transfer between bacteria, mediated by conjugative plasmids. The *bla* $_{SHV}$ ESBL gene is often associated with other ESBL genes of the CTX-M type, both in human and animal settings.

Among the ESBL-screen-positive isolates, only 35% were confirmed positive by the confirmatory test. Out of 70 potential carbapenemase producers identified by screening, only 40 (57.2%) were confirmed positive by the confirmatory method. The ESBL genes selected for testing in this study using real-time PCR were *bla* $_{SHV,}$ *bla* $_{CTX-M,}$ OXA-10, and OXA-11. The results indicated that two isolates (8%) were positive for the *bla* $_{SHV}$ gene alone, 18 isolates (72%) had both *bla* $_{SHV}$ and *bla* $_{CTX-M,}$ genes, and none had OXA-10/11 genes. Additionally, among the non ESBL producers, eight isolates (17.1%) were positive for the *bla* $_{SHV}$ gene alone, three isolates (6.66%) had both *bla* $_{SHV}$ and *bla* $_{CTX-M}$ genes, and none had the OXA-10/11 genes.

Carbapenems are widely considered the most effective antibacterial agents and the first-choice treatment for infections caused by ESBL-producing Enterobacteriaceae. Among the studied isolates, only three MDR isolates were found to be MBL producers, with bla-NDM detected in these isolates.

The Modified Hodge test identified 37 (52.85%) carbapenemase-producing isolates. Among these, 33 (82.5%) had *bla* $_{KPC}$, 21 (52.5%) had *bla* $_{IMP}$, and OXA-58, -23, -51, and -48 were found in 2 (2.8%) isolates each. Some international studies have reported the presence of blaOXA-48 and OXA-181 genes among hospitalised patients [37].

Urmi UL et al., identified 58 *K. pneumoniae* from 142 acute UTI cases. Their results indicated that the prevalence rates of *bla* $_{KPC,}$ *bla* $_{IMP,}$ *bla* $_{NDM-1}$, and *bla* $_{VIM}$ were 15.5% (9), 10.3% (6), 22.4% (13), and 19% (11), respectively [38].

The PCR results also revealed that *bla* $_{KPC}$ and *bla* $_{IMP}$ genes were present in a small proportion of carbapenemase non producers (4.28%) and among isolates that were sensitive to beta-lactam drugs (5%). This finding likely indicates that these bacteria carry the resistance genes but do not express them phenotypically. This could facilitate the lateral spread of resistance genes.

Interestingly, 21 (30%) isolates in the present study possessed at least one ESBL gene and one carbapenemase gene. This may be attributed to the presence of multiple resistance genes on the same genetic cassette. The plasmids that harbour ESBL genes often carry resistance genes for other antibiotic classes such as aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and tetracycline. Thus, gram-negative bacilli bearing these plasmids are frequently multidrug-resistant.

The present findings emphasise the significance of the co-occurrence of ESBL and carbapenemase-encoding genes in *K. pneumoniae* isolates implicated in UTIs among ckd patients, posing challenges for effective treatment options.

Limitation(s)

The study did not collect data on antibiotic usage among the participants. Further experiments to determine the location of the genes (plasmid or chromosome) were not conducted, as this aspect would have provided insight into the spread of antimicrobial resistance.

CONCLUSION(S)

The study concludes that UTIs are more frequent in patients with CKD stages 1 and 5, although they can occur at any stage of the disease. It also highlights that a majority of UTI-causing *Klebsiella* spp. isolates are MDR, which may warrant further research into alternative treatments for such infections. Among the drug resistance genes carried by these bacteria, *bla* $_{SHV,}$ *bla* $_{KPC,}$ and *bla* $_{-NDM}$ are prevalent in *Klebsiella pneumoniae* isolated from urine samples of suspected UTIs in CKD patients. Improved surveillance and focused antibiotic stewardship are recommended to combat multidrug-resistant bacterial infections, particularly in immunocompromised CKD patients.

Acknowledgement

Author is deeply grateful to the Department of Microbiology, the Department of Nephrology, and the technical staff members for their constant support, helpful criticism, and encouragement, all of which significantly improved the outcome of this research. I also value the expert advice and knowledgeable counsel of author's coworkers and fellow researchers at the Yenepoya Research Centre. Author would like to acknowledge Yenepoya (Deemed to be) University for providing the institutional seed grant fund. Author also express author's gratitude to Yenepoya (Deemed to be) University for their assistance and insights throughout the course of this research.

REFERENCES

[1] Dillirani V, Suresh R. A study on prevalence and antimicrobial resistance pattern of urinary *Klebsiella pneumoniae* in a tertiary care centre in south India. Int J Curr Microbiol Appl Sci [Internet]. 2018;7(04):969-73. Available from: http://dx.doi.org/10.20546/ijcmas.2018.704.103.

[2] Haider JS. Frequency of urinary tract bacterial infection and their susceptibility patterns among hemodialysis patients in zliten hospital. J Microbiol Exp [Internet]. 2016;3(3). Available from: http://dx.doi.org/10.15406/jmen.2016.03.00093.

[3] Ding Y, Wang H, Pu S, Huang S, Niu S. Resistance trends of Klebsiella pneumoniae causing urinary tract infections in Chongqing, 2011-2019. Infect Drug Resist [Internet]. 2021;14:475-81. Available from: http://dx.doi.org/10.2147/IDR.S295870.

[4] Sodhi K, Mittal V, Arya M, Kumar M, Phillips A, Kajla B. Pattern of colistin resistance in *Klebsiella* isolates in an Intensive Care Unit of a tertiary care hospital in India. J Infect Public Health [Internet]. 2020;13(7):1018-21. Available from: http://dx.doi.org/10.1016/j.jiph.2019.10.013.

[5] Cubero M, Marti S, Domínguez MÁ, González-Díaz A, Berbel D, Ardanuy C. Hypervirulent *Klebsiella pneumoniae* serotype K1 clinical isolates form robust biofilms at the air-liquid interface. PloS One [Internet]. 2019;14(9):e0222628. Available from: http://dx.doi.org/10.1371/journal.pone.0222628.

[6] Cristea OM, Avramescu CS, Bălăşoiu M, Popescu FD, Popescu F, Amzoiu MO. Urinary tract infection with *Klebsiella pneumoniae* in patients with chronic kidney disease. Curr Health Sci J [Internet]. 2017;43(2):137-48. Available from: http://dx.doi.org/10.12865/CHSJ.43.02.06.

[7] Krawczyk B, Wysocka M, Michalik M, Gołębiewska J. Urinary tract infections caused by *K. pneumoniae* in kidney transplant recipients-epidemiology, virulence and antibiotic resistance. Front Cell Infect Microbiol [Internet]. 2022;12:861374. Available from: http://dx.doi.org/10.3389/fcimb.2022.861374.

[8] Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev [Internet]. 1998;11(4):589-603. Available from: http://dx.doi.org/10.1128/CMR.11.4.589.

[9] Pruss A, Kwiatkowski P, Sienkiewicz M, Masiuk H, Łapińska A, Kot B, et al. Similarity analysis of *Klebsiella pneumoniae* producing carbapenemases isolated from UTI and other infections. Antibiotics (Basel) [Internet]. 2023;12(7). Available from: http://dx.doi.org/10.3390/antibiotics12071224.

[10] Yang DC, Chao JY, Hsiao CY, Tseng CT, Lin WH, Kuo TH, et al. Impact of urinary tract infection requiring hospital admission on short-term, mid-term and long-term renal outcomes in adult CKD patients - A potentially modifiable factor for CKD progression. J Infect Public Health. 2025;18(5):102712. Available from: http://dx.doi.org/10.1016/j.jiph.2025.102712.

[11] Wang G, Zhao G, Chao X, Xie L, Wang H. The characteristic of virulence, biofilm and antibiotic resistance of *Klebsiella pneumoniae*. Int J Environ Res Public Health [Internet]. 2020;17(17):6278. Available from: http://dx.doi.org/10.3390/ijerph17176278.

[12] Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, et al. Virulence factors and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from neonates with sepsis. Front Med (Lausanne) [Internet]. 2018;5:225. Available from: http://dx.doi.org/10.3389/fmed.2018.00225.

[13] Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant *Klebsiella pneumoniae* isolated from bloodstream infection: Indian experience. Pathog Glob Health [Internet]. 2017;111(5):240-46. Available from: http://dx.doi.org/10.1080/20477724.2017.1340128.
[14] Saga T, Yamaguchi K. History of antimicrobial agents and resistant bacteria. Jpn Med Assoc J. 2009;52:103-08.
[15] Ugbo EN, Anyamene CO, Moses IB, Iroha IR, Babalola OO, Ukpai EG, et al. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among extended spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* of clinical origin. Gene Rep [Internet]. 2020;21(100909):100909. Available from: http://dx.doi.org/10.1016/j.genrep.2020.100909.
[16] Spyropoulou A, Papadimitriou-Olivgeris M, Bartzavali C, Vamvakopoulou S, Marangos M, Spiliopoulou I, et al. A ten-year surveillance study of carbapenemase-producing *Klebsiella pneumoniae* in a tertiary care Greek university hospital: Predominance of KPC-over VIM-or NDM-producing isolates. J Med Microbiol [Internet]. 2016;65(3):240-46. Available from: http://dx.doi.org/10.1099/jmm.0.000217.
[17] Kidney Foundation. Quick reference guide: Kidney disease screening. Available from: https://www.kidney.org/quick-reference-guide-kidney-disease-screening.
[18] Shankar M, Narasimhappa S, Madhura NS. Urinary tract infection in chronic kidney disease population: A clinical observational study. Cureus [Internet]. 2021;13(1):e12486. Available from: http://dx.doi.org/10.7759/cureus.12486.
[19] Joyanes P, del Carmen Conejo M, Martínez-Martínez L, Perea EJ. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. J Clin Microbiol [Internet]. 2001;39(9):3247-53. Available from: http://dx.doi.org/10.1128/JCM.39.9.3247-3253.2001.
[20] Manjana A, Banjana A, Arijitmajumdar D, Nikhil Tudu J. Selection of storage methods for maintenance of different stock cultures. Int J Curr Microbiol Appl Sci [Internet]. 2016;5(10):1097-104. Available from: http://dx.doi.org/10.20546/ijcmas.2016.510.115 .
[21] Chen TK, Knicely DH, Grams ME. Chronic kidney disease diagnosis and management: A review. JAMA [Internet]. 2019;322(13):1294-304. Available from: http://dx.doi.org/10.1001/jama.2019.14745.
[22] Clinical and Laboratory Standards Institute. Susceptibility Testing Subcommittees, Volume 8, Issue 1. CLSI [Internet]. 2023 Jun. Available from: https://clsi.org/meetings/susceptibility-testing-subcommittees/
[23] Ugwu MC, Shariff M, Nnajide CM, Beri K, Okezie UM, Iroha IR, et al. Phenotypic and molecular characterization of β-lactamases among Enterobacterial uropathogens in southeastern Nigeria. Can J Infect Dis Med Microbiol [Internet]. 2020;2020:5843904. Available from: http://dx.doi.org/10.1155/2020/5843904.
[24] HiMedia Laboratories. HiPurA™ Bacterial Genomic DNA Purification Kit [Internet]. Available from: https://www.himedialabs.com/us/mb505-hipura-bacterial-genomic-dna-purification-kit.html.
[25] Pattolath A, Adhikari P, Pai V. Clinical and molecular profile of carbapenem resistant *Klebsiella pneumoniae* infections in a tertiary care hospital-Mangalore. Infect Drug Resist [Internet]. 2023;16:4335-48. Available from: http://dx.doi.org/10.2147/IDR.S411056.
[26] HiMedia Laboratories. Hi-PCR™ Extended Spectrum Beta-Lactamases (ESBLs) Gene Quantification Probe PCR Kit [Internet]. Available from: https://www.himedialabs.com/us/mbpcr226-hi-pcr-extended-spectrum-beta-lactamases-esbls-gene-quantification-probe-pcr-kit.html.
[27] HiMedia Laboratories. Hi-PCR™ Carbapenemase Gene Multiplex Probe PCR Kit [Internet]. Available from: https://www.himedialabs.com/in/mbpcr132-hi-pcr-carbapenemase-gene-multiplex-probe-pcr-kit.html.
[28] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
[29] Radhika A, Lakshmi JT, Ariyanachi K, Sakthivadivel V. Detection of metallo beta-lactamase (MBL) producing *Pseudomonas aeruginosa* in a tertiary care hospital, Ghanpur, Medchal, India. Maedica (Buchar) [Internet]. 2022;17(1):134-42. Available from: http://dx.doi.org/10.26574/maedica.2022.17.1.134.
[30] Clinical and Laboratory Standards Institute CLSI. Screening and Confirmatory Tests for Suspected Carbapenemases Production. 2010.
[31] Gilbert DN. Urinary tract infections in patients with chronic renal insufficiency. Clin J Am Soc Nephrol [Internet]. 2006;1(2):327-31. Available from: http://dx.doi.org/10.2215/CJN.01931105.
[32] Scherberich JE, Fünfstück R, Naber KG. Urinary tract infections in patients with renal insufficiency and dialysis-epidemiology, pathogenesis, clinical symptoms, diagnosis and treatment. GMS Infect Dis [Internet]. 2021;9:Doc07. Available from: http://dx.doi.org/10.3205/id000076.
[33] Soni P, Nerurkar A, Gandhi V. *Klebsiella pneumoniae*, an important uropathogen: Prevalence and antimicrobial susceptibility pattern. Trop J Pathol Microbiol [Internet]. 2019;5(3):171-76. Available from: http://dx.doi.org/10.17511/jopm.2019.i03.10.
[34] Dimitrijevic Z, Paunovic G, Tasic D, Mitic B, Basic D. Risk factors for urosepsis in chronic kidney disease patients with urinary tract infections. Sci Rep [Internet]. 2021;11(1):14414. Available from: http://dx.doi.org/10.1038/s41598-021-93912-3.
[35] Saha AK. Pattern of antimicrobial susceptibility of *Klebsiella pneumoniae* isolated from urinary samples in urinary tract infection in a tertiary care hospital, kishanganj, Bihar, 5 years' experience. Int J Contemp Med Res [IJCMR] [Internet]. 2019;6(12). Available from: http://dx.doi.org/10.21276/ijcmr.2019.6.12.42.
[36] Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Mmbaga BT, Lund O, et al. Prevalence and risk factors for CTX-M gram-negative bacteria in hospitalized patients at a tertiary care hospital in Kilimanjaro, Tanzania. Eur J Clin Microbiol Infect Dis [Internet]. 2018;37(5):897-906. Available from: http://dx.doi.org/10.1007/s10096-018-3196-8.
[37] Hassan MI, Alkharsah KR, Alzahrani AJ, Obeid OE, Khamis AH, Diab A. Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. J Infect Dev Ctries [Internet]. 2013;7(8):618-29. Available from: http://dx.doi.org/10.3855/jidc.2919.
[38] Urmi UL, Nahar S, Rana M, Sultana F, Jahan N, Hossain B, et al. Genotypic to phenotypic resistance discrepancies identified involving β-lactamase genes, blaKPC, blaIMP, blaNDM-1, and blaVIM in uropathogenic *Klebsiella pneumoniae*. Infect Drug Resist [Internet]. 2020;13:2863-75. Available from: http://dx.doi.org/10.2147/idr.s262493.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Microbiology, Yenepoya Medical College, Mangalore, Karnataka, India.
2. Professor, Department of Microbiology, Yenepoya Medical College, Mangalore, Karnataka, India.
3. Professor, Department of Nephrology, Yenepoya Medical College, Mangalore, Karnataka, India.
4. Assistant Professor, Department of Nephrology, Yenepoya Medical College, Mangalore, Karnataka, India.
5. Assistant Professor, Department of Nephrology, Yenepoya Medical College, Mangalore, Karnataka, India.
6. Senior Resident, Department of Nephrology, Yenepoya Medical College, Mangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Ms. Nithya Kangosseri,
Research Scholar, Department of Microbiology, Yenepoya Medical College,
Mangalore, Karnataka, India.
E-mail: nithyachandran01@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 07, 2024
- Manual Googling: Jun 28, 2025
- iThenticate Software: Jul 01, 2025 (10%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Dec 06, 2024**
Date of Peer Review: **Mar 28, 2025**
Date of Acceptance: **Jul 03, 2025**
Date of Publishing: **Jan 01, 2026**