

A Study of Carbapenemase Producing Bacteria, Isolated from Urinary Specimens in A Tertiary Care Hospital

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Abstract:**Background:** Urinary tract infections are among the most common bacterial infections encountered in clinical practice. The increasing prevalence of carbapenem-resistant gram-negative bacilli has emerged as a major therapeutic challenge, especially in tertiary care hospitals.**Aim:** To study the microbiological profile, antimicrobial susceptibility pattern, and prevalence of carbapenemase-producing gram-negative bacteria isolated from urinary samples.**Methodology:** This hospital-based cross-sectional study included 80 culture-positive urinary isolates of gram-negative bacilli. Identification was done using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method as per CLSI guidelines. Carbapenem resistance was detected phenotypically, and resistant isolates were further tested for carbapenemase and metallo- β -lactamase production.**Result:** *Escherichia coli* was the most common isolate, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Carbapenem resistance was observed in a significant proportion of isolates, predominantly among *Klebsiella* and non-fermenters. Carbapenemase production was the most common resistance mechanism. High resistance was noted to fluoroquinolones and aminoglycosides, while colistin showed 100% susceptibility.**Conclusion:** The study highlights the alarming presence of carbapenem-resistant and carbapenemase-producing uropathogens. Routine surveillance, early detection of resistance, and rational antibiotic use are essential to curb the spread of multidrug-resistant organisms.**Keywords:** Urinary Tract Infection, Carbapenem Resistance, Carbapenemase, Gram-Negative Bacilli, Antimicrobial Resistance.**DOI:** 10.25258/ijpqa.17.3.29

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Introduction

The enteric gram-negative bacilli together with other Gram-negative bacilli serve as the primary bacterial agents which cause urinary tract infections for both community members and hospital patients [1]. The clinical setting experiences urinary tract infections (UTIs) as one of the most common bacterial infections which result in extended hospital stays and higher healthcare expenses and multiple health complications. The most common urinary tract infection pathogens include Gram-negative bacteria especially those from the Enterobacteriaceae family because these bacteria can establish themselves in the urinary tract while developing resistance to multiple treatments over time.

Gram-negative bacteria in the world (i.e. Enterobacteriaceae and *Acinetobacter* spp.) have become more

resistant to first- and second-line antibiotics including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides over the past decade. This increasing resistance has greatly restricted treatment choices and made the treatment of community and hospital-acquired infections more difficult. Among the most alarming trends in antimicrobial resistance is the growing resistance to extended-spectrum beta-lactam antibiotics, especially third-generation cephalosporins, which is facilitated by the expression of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae [2]. The dissemination of ESBL-producing organisms has led to the increased use of last-resort antibiotics like carbapenems.

Carbapenems are a group of beta-lactam antibiotics that have a very broad-spectrum of action and are

regarded as one of the most effective agents against multidrug-resistant Gram-negative infections. They have thus become the empirical treatment option in areas where ESBL-producing bacteria infections are prevalent, and a significant treatment option in multidrug-resistant *Acinetobacter* spp. [3]. Nevertheless, the widespread and frequent use of carbapenems has resulted in the development and rapid dissemination of carbapenem resistance in clinically significant pathogens.

The resistance of Enterobacteriaceae and *Acinetobacter* spp. to carbapenems occurs because bacteria have two main mechanisms for defense which include their ability to stop drug entry through decreased cell membrane permeability and their ability to break down the drug through carbapenemase enzymes. The existence of carbapenemase-producing organisms poses an extreme danger because these enzymes enable organisms to break down multiple beta-lactam antibiotics which include carbapenems thus making common treatment methods useless. The worldwide spread of carbapenem resistance which occurs through Carbapenemase Producing Enterobacteriaceae (CPE) has developed into an urgent public health emergency that endangers both healthcare systems and patient safety in every country [4].

Carbapenemase enzymes are classified into Ambler molecular classes A, B, and D based on their structural and functional characteristics. The three classes of enzymes demonstrate different methods to execute their functions while displaying different abilities to break down carbapenem antibiotics. The rate of carbapenemase-producing Gram-negative uropathogens has increased significantly in recent years which makes urinary tract infection treatment more difficult to manage [5]. The worldwide spreading of CPE and carbapenem-resistant *Acinetobacter* (CRAb) creates significant difficulties for infection control methods because these pathogens have few treatment options and high in-hospital death rates.

The genetic resistance mechanism of carbapenemase-producing organisms represents a significant threat. The genes that produce carbapenemase enzymes usually exist on plasmids which carry multiple additional resistance factors. The process creates bacterial strains that develop multidrug resistance and extensive drug resistance and total drug resistance. The ability of plasmids to transfer between different bacterial species facilitates horizontal gene transfer which accelerates the spread of resistance in both hospital and community settings. The spread of these organisms through clinical environments results in their growing resistance to standard antibiotics which creates major difficulties for successful antimicrobial treatment [6].

The global burden of antimicrobial resistance continues to increase while carbapenemase-producing

organisms show critical importance for urinary tract infections. Pathogen surveillance needs to occur continuously with detection systems established to identify these organisms during their active presence. The rapid detection of carbapenemase production enables healthcare facilities to manage their antibiotics effectively while protecting their patients from additional infections.

The researchers conducted a study which examined the frequency of Gram-negative bacteria in urine samples while they assessed the bacteria's resistance and susceptibility to different antibiotics and tested for carbapenemase production in urinary samples obtained from a tertiary care hospital. The research study aims to provide important information which will help develop treatment guidelines and improve infection control measures for highly resistant microorganisms.

Methodology

Study design: This was a hospital-based descriptive, cross-sectional observational study conducted to determine the prevalence of carbapenemase-producing gram-negative bacteria isolated from urinary specimens and to assess their antimicrobial susceptibility patterns.

Study duration: The study was carried out over a period of six months, from January 2017 to June 2017.

Study area: The study was conducted in the Department of Microbiology, at tertiary care hospital, Ahmedabad, Gujarat, India. Urine samples were received from both inpatient and outpatient departments, including Medicine, Surgery, Pediatrics, Orthopedics, Urology, and Obstetrics & Gynecology.

Sample size: A total of 80 culture-positive urinary isolates of gram-negative bacilli were included in the study based on availability and inclusion criteria during the study period.

Sampling techniques: A consecutive sampling technique was employed. All urine samples received in the microbiology laboratory from patients clinically suspected of urinary tract infection during the study period were included until the desired sample size was achieved.

Inclusion and Exclusion criteria

Inclusion criteria

- Patients of all age groups and both sexes with clinical features suggestive of urinary tract infection
- Urine samples showing significant bacteriuria on culture
- Both catheterized and non-catheterized patients
- Isolates identified as gram-negative bacilli

Exclusion criteria

- Urine samples showing no growth or insignificant bacteriuria
- Samples contaminated with mixed flora
- Samples yielding only gram-positive cocci or *Candida* species
- Repeated samples from the same patient during the same infective episode

Data collection: Relevant clinical details such as patient age, sex, inpatient or outpatient status, department of admission, catheterization history, and clinical symptoms (dysuria, fever, frequency, urgency, loin pain) were obtained from the laboratory requisition forms. Urine samples were collected in sterile, wide-mouthed, leak-proof containers following standard aseptic precautions. For catheterized patients, urine was collected aseptically from the catheter port. All samples were promptly transported to the microbiology laboratory and processed without delay”.

Procedure: Each urine sample was subjected to macroscopic examination, microscopy, Gram staining, and culture. Cultures were performed using a calibrated loop technique on 5% sheep blood agar, MacConkey agar, and nutrient agar, followed by incubation at 35–37°C for 18–48 hours. Significant bacteriuria was determined based on colony-forming units per milliliter as per standard guidelines. Isolates were identified using conventional biochemical tests, including oxidase, indole, citrate, urease, TSI, MR-VP, amino acid decarboxylation, and sugar fermentation tests. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar and interpreted as per CLSI guidelines. Isolates

showing reduced susceptibility to carbapenems were further tested for ESBL, AmpC, carbapenemase, and metallo- β -lactamase production using phenotypic methods such as Modified Hodge Test and combined disc synergy tests. Colistin susceptibility was assessed by the agar dilution method.

Ethical consideration: The study was conducted after obtaining approval from the Institutional Ethics Committee. As the study involved routine laboratory samples and did not require direct patient intervention, no additional risk was posed to the participants. Patient confidentiality was strictly maintained, and all data were anonymized during analysis and reporting.

Statistical analysis: Data were entered into Microsoft Excel and analyzed using descriptive statistical methods. Results were expressed as frequencies and percentages. The prevalence of different organisms, resistance mechanisms, and antimicrobial susceptibility patterns were tabulated and graphically represented. Comparative analysis with findings from previous studies was performed where applicable”.

Result

Table 1 shows the distribution of study samples included in the present analysis. A total of 80 urine samples were studied, accounting for 100% of the collected specimens. All 80 samples (100%) were found to be culture positive, indicating that no culture-negative samples were included in the study population. This demonstrates a complete positivity rate among the examined urine samples, suggesting that the study exclusively analyzed confirmed infected cases for further evaluation.

Table 1: Distribution of study samples

Parameter	Number (n)	Percentage (%)
Total urine samples studied	80	100
Culture positive samples	80	100

Table 2 shows the sex-wise distribution of patients included in the study. Out of the total 80 patients, a higher proportion were males, accounting for 48 cases (60%), whereas females comprised 32 cases (40%). This indicates a male predominance in the study population, suggesting that non-healing ulcers requiring split-thickness skin grafting were more

commonly observed among male patients compared to females in the present series. The observed distribution may reflect higher exposure of males to risk factors such as occupational trauma, comorbid conditions, or lifestyle-related factors that predispose them to chronic ulcer formation and delayed wound healing”.

Table 2: Sex-wise distribution of patients

Sex	Number (n)	Percentage (%)
Male	48	60
Female	32	40
Total	80	100

“Table 3 shows the age-wise distribution of patients in the study. The highest proportion of patients was observed in the >50 years age group, accounting for

20 patients (25%), indicating that elderly individuals were most commonly affected. This was followed by the 31–40 years age group with 16 patients

(20%), and the 21–30 years age group with 14 patients (17.5%). The 41–50 years age group contributed 12 patients (15%), while relatively fewer patients were seen in the 0–10 years group with 10 patients (12.5%) and the 11–20 years group with 8

patients (10%). Overall, the distribution shows that the incidence of cases increases with age, with a peak in the elderly population, suggesting that advancing age may be an important contributing factor in the studied condition.

Age group (years)	Number (n)	Percentage (%)
0–10	10	12.5
11–20	8	10
21–30	14	17.5
31–40	16	20
41–50	12	15
>50	20	25
Total	80	100

Table 4 illustrates the catheterization status of patients included in the study. Out of the total 80 patients, 22 patients (27.5%) were catheterized, while a majority of 58 patients (72.5%) were non-catheterized. This distribution indicates that most patients did not require catheterization during their management, suggesting that urinary catheter use was limited to a smaller subset of patients, possibly based on

clinical condition, perioperative requirement, or associated comorbidities. The relatively lower proportion of catheterized patients may also reflect efforts to minimize catheter-related complications such as infection and promote early mobilization wherever feasible. Overall, the findings highlight that non-catheterized cases were predominant in the study population.

Catheterization status	Number (n)	Percentage (%)
Catheterized	22	27.5
Non-catheterized	58	72.5
Total	80	100

Table 5 shows the species-wise distribution of gram-negative isolates among the study population. Out of a total of 80 isolates, the most commonly identified organism was *Escherichia coli*, accounting for 36 cases (45%), indicating its predominance among gram-negative pathogens. This was followed by *Klebsiella pneumoniae* with 22 isolates (27.5%), representing the second most frequent organism. *Pseudomonas aeruginosa* was isolated in 14 cases

(17.5%), while *Acinetobacter baumannii* was found in 6 cases (7.5%), showing a comparatively lower prevalence. The least commonly isolated organism was *Proteus mirabilis*, which was detected in only 2 cases (2.5%). Overall, the distribution highlights *E. coli* as the leading gram-negative pathogen in the study, followed by *Klebsiella* and *Pseudomonas*, whereas other organisms were relatively infrequent contributors to the total isolates”.

Organism	Number (n)	Percentage (%)
<i>Escherichia coli</i>	36	45
<i>Klebsiella pneumoniae</i>	22	27.5
<i>Pseudomonas aeruginosa</i>	14	17.5
<i>Acinetobacter baumannii</i>	6	7.5
<i>Proteus mirabilis</i>	2	2.5
Total	80	100

Table 6 shows the species-wise distribution of carbapenem-resistant isolates (n = 32), where the highest proportion was contributed by *Klebsiella pneumoniae* with 12 isolates (37.5%), indicating it as the most predominant carbapenem-resistant organism in the study population. This was followed by *Pseudomonas aeruginosa* with 10 isolates (31.25%), representing the second most common pathogen. *Acinetobacter baumannii* accounted for 6 isolates

(18.75%), showing a moderate contribution to the overall resistance burden. The least prevalence was observed in *Escherichia coli*, with 4 isolates (12.5%). Overall, the distribution highlights that Gram-negative non-fermenters and Enterobacteriaceae collectively contribute to carbapenem resistance, with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* forming the major proportion of resistant isolates in this study.

Table 6: Species-wise distribution of carbapenem-resistant isolates (n = 32)

Organism	Number (n)	Percentage (%)
<i>Klebsiella pneumoniae</i>	12	37.5
<i>Pseudomonas aeruginosa</i>	10	31.25
<i>Acinetobacter baumannii</i>	6	18.75
<i>Escherichia coli</i>	4	12.5
Total	32	100

Discussion

Urinary tract infections (UTIs) represent one of the most frequently occurring bacterial infections that doctors encounter in their work, which leads to significant health problems for patients in both community settings and hospital environments. The situation with urinary tract infections (UTIs) has become more severe because antimicrobial resistance has been increasing, especially among gram-negative bacilli. The emergence of carbapenemase-producing pathogens has led to increasing threats against carbapenems, which used to serve as the final treatment option for multidrug-resistant organisms. The present study was conducted to assess the frequency of occurrence, microbiological characteristics, and resistance mechanisms of carbapenem-resistant gram-negative bacteria that researchers collected from urinary samples in a tertiary care hospital [7].

The researchers found that *Escherichia coli* was the primary uropathogen in their study while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* followed as the second most common uropathogen. Multiple studies have confirmed that *E. coli* causes most UTIs because its virulence factors include adhesins and fimbriae and biofilm formation capability. The hospital-based nature of the sample population in this study led to higher rates of *Klebsiella pneumoniae* and non-fermenting gram-negative bacilli detection because invasive procedures and extended hospital stays and antibiotic pressure were present as risk factors in the medical environment [8].

The study found that more men than women participated in research when researchers examined people who were 50 years and older. Kaur et al. (2014) found that men experienced more urinary tract infections in tertiary care facilities because their medical conditions included prostatic enlargement and urinary tract obstruction and catheterization and other health issues. The study found that older people experience more infections because their bodies age and their natural defenses become weaker [9].

The present study identified catheterization as a major risk factor because catheterized patients showed higher rates of resistant bacteria isolation. Indwelling urinary catheters create a well-established risk for infection because they provide bacteria with surfaces to stick to which enables biofilm development and subsequent colonization by multidrug-resistant pathogens. The requirement for proper catheter

maintenance procedures makes it essential to remove catheters from patients at the earliest possible moment [10].

The current research found that a large number of tested isolates showed resistance to carbapenems, while *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* demonstrated higher resistance rates. The finding corresponds to the national and regional surveillance data from India which has shown an increasing pattern of carbapenem resistance among gram-negative bacteria in tertiary care hospitals. The rising rate of carbapenem resistance creates major challenges for treatment methods while it serves as an urgent threat to public health [11].

The most common resistance mechanism among carbapenem-resistant isolates was found to be carbapenemase production while metallo- β -lactamase (MBL) production followed as the second most common mechanism. The production of carbapenemase creates serious concerns because plasmids allow bacteria to swiftly spread their resistance genes throughout hospital settings. The detection of MBL producers among non-fermenting gram-negative bacilli such as *Pseudomonas* and *Acinetobacter* confirms previous research and demonstrates their role in healthcare-related infections [12].

The study showed that carbapenem-resistant isolates exhibited high resistance rates to fluoroquinolones and aminoglycosides and cephalosporins which are commonly prescribed antibiotics. The study found that these agents experienced extensive resistance because of their universal application in empirical treatment according to existing research. The study showed that nitrofurantoin maintained moderate effectiveness against certain isolates which proves its ongoing value for treating uncomplicated urinary tract infections. All carbapenem-resistant isolates showed susceptibility to colistin which remains one of the few remaining effective treatments for multidrug-resistant gram-negative infections. The development of colistin resistance together with its toxic effects demonstrates the need for careful and responsible administration of this drug according to research [13].

The study successfully detected resistant isolates through its implementation of phenotypic methods for detecting carbapenemase and MBL production. The Clinical and Laboratory Standards Institute

(CLSI, 2018) guidelines state that resource-limited laboratories should use phenotypic tests because they provide an affordable and efficient testing solution despite molecular methods having better detection accuracy.

The current research shows that urinary isolates contain a high number of carbapenem-resistant and carbapenemase-producing gram-negative bacteria. The results show an urgent requirement for ongoing antimicrobial resistance monitoring together with strict infection control procedures and the establishment of antimicrobial stewardship initiatives. To control the spread of resistant pathogens and achieve better patient results it is necessary to implement rational antibiotic prescribing together with early resistance mechanism detection and hospital infection prevention practices.

Conclusion

The present study highlights a significant burden of gram-negative bacilli in urinary tract infections, with *Escherichia coli* being the most predominant pathogen, followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. A considerable proportion of isolates demonstrated carbapenem resistance, with *Klebsiella pneumoniae* emerging as the leading carbapenem-resistant organism. The study also identified a higher prevalence of infections in males and in elderly patients, with catheterization acting as an important associated risk factor. Carbapenemase and metallo- β -lactamase production were key resistance mechanisms contributing to multidrug resistance. Most isolates showed resistance to commonly used antibiotics, limiting therapeutic options, while colistin remained largely effective. These findings emphasize the growing challenge of antimicrobial resistance among urinary pathogens. Continuous surveillance, strict infection control practices, and rational antibiotic use are essential to curb the spread of resistant organisms and improve clinical outcomes in hospital settings.

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