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A Cross- Sectional Study of Antimicrobial Resistance Patterns of *Klebsiella pneumoniae* and *Escherichia coli* isolates at a Tertiary Care Hospital in Gujarat, India

Dr. Gaurishanker Shrimali[1], Dr. Komal Patel[2], Dr. Rakesh Rajat[3]

[1]Professor, Department of Microbiology, GMERS Medical College and Hospital, Gandhinagar, Gujarat, India
[2]Associate Professor, Department of Microbiology, GMERS Medical Collegeand Hospital, Vadnagar, Gujarat, India
[3]Professor, Department of Microbiology, GMERS Medical Collegeand Hospital, Godhra, Gujarat, India

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*Corresponding Author:

Dr. Gaurishanker Shrimali
Professor, Department of Microbiology, GMERS Medical College and Hospital, Gandhinagar, Gujarat, India

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A B S T R A C T

BACKGROUND:: Antimicrobial resistance (AMR) is a growing global threat, with *Escherichia coli* and *Klebsiella pneumoniae* recognized as major pathogens causing urinary, respiratory, bloodstream, and wound infections. The increasing prevalence of extended-spectrum beta-lactamase (ESBL) producers and carbapenem-resistant Enterobacteriaceae has severely limited therapeutic options, especially in tertiary care hospitals. Continuous local surveillance is essential to guide empirical therapy and strengthen antibiotic stewardship.

OBJECTIVES: To determine the antimicrobial resistance patterns of *E. coli* and *K. pneumoniae* isolates from clinical specimens in a tertiary care hospital, and to estimate the prevalence of ESBL and carbapenemase production.

METHODOLOGY : This hospital-based cross-sectional study was conducted in the Department of Microbiology, GMERS Medical College, Gandhinagar, from January 2023 to January 2024. A total of 300 consecutive non-duplicate isolates, including 180 *E. coli* and 120 *K. pneumoniae*, were obtained from urine, blood, pus, respiratory samples, and body fluids. Identification was carried out by standard biochemical tests and confirmed by automated systems where required. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar as per CLSI 2023 guidelines. ESBL production was confirmed by the combined disk method, and carbapenemase production was detected using the Modified Hodge Test and Carbapenem Inactivation Method. Data were analyzed using descriptive statistics and chi-square test.

RESULTS: *E. coli* was the predominant isolate from urine specimens (61.1%), while *K. pneumoniae* was more common in respiratory (18.3%) and blood samples (26.7%). High resistance was observed to third-generation cephalosporins in both organisms (>70%), and fluoroquinolone resistance exceeded 65%. Carbapenem resistance was noted in 22% of *E. coli* and 31% of *K. pneumoniae* isolates. ESBL production was detected in 57.8% of *E. coli* and 64.2% of *K. pneumoniae*, while carbapenemase production was found in 16.1% and 24.2% respectively. Multidrug resistance was identified in 48.3% of *E. coli* and 55.8% of *K. pneumoniae* isolates.

CONCLUSION: The study revealed a high prevalence of ESBL-producing and carbapenem-resistant *E. coli* and *K. pneumoniae*, with a significant burden of multidrug resistance. These findings highlight the urgent need for robust antibiotic stewardship, strict infection control measures, and continuous AMR surveillance to prevent further escalation of resistance in tertiary care hospitals.

Keywords: Antimicrobial resistance, *Escherichia coli*, *Klebsiella pneumoniae*, ESBL, Carbapenemase, Multidrug resistance.

INTRODUCTION

Antimicrobial resistance (AMR) is recognized as one of the greatest challenges to global public health in the 21st century. According to the World Health Organization (WHO), AMR threatens the effective prevention and treatment of infections caused by bacteria, viruses, fungi, and parasites, and is responsible for significant morbidity, mortality, and

economic burden worldwide [1]. Among bacterial pathogens, *Escherichia coli* and *Klebsiella pneumoniae*, two important members of the Enterobacteriaceae family, have emerged as critical contributors to both community-acquired and hospital-acquired infections. These organisms are frequently implicated in urinary tract infections, bloodstream infections, pneumonia, and wound infections, making them a major focus of microbiological surveillance [2].

Over the past two decades, both *E. coli* and *K. pneumoniae* have developed alarming levels of resistance to commonly used antibiotics, particularly third-generation cephalosporins, largely due to the widespread production of extended-spectrum β-lactamases (ESBLs). ESBL-producing strains are resistant to penicillins, cephalosporins, and aztreonam, leaving only limited therapeutic options [2]. In recognition of this growing problem, the WHO has listed carbapenem-resistant Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, in the **critical priority group** of pathogens for which new antibiotics are urgently needed [3].

Resistance in these organisms is mediated by multiple mechanisms. The production of ESBLs and AmpC β-lactamases confers resistance to extended-spectrum cephalosporins, while the acquisition of carbapenemase genes such as KPC, NDM, OXA-48, and VIM results in resistance even to carbapenems, which are considered last-resort drugs. Additional mechanisms, including porin mutations and efflux pump overexpression, further contribute to treatment failures [4]. The rapid spread of these resistance mechanisms has been facilitated by horizontal gene transfer via plasmids and transposons, resulting in outbreaks across hospitals worldwide [5].

Global surveillance studies have reported increasing resistance rates in Enterobacteriaceae. In many regions of Asia and Latin America, ESBL prevalence among *E. coli* exceeds 50%, and carbapenem resistance in *K. pneumoniae* has risen steadily over the last decade [6]. In India, the scenario is even more concerning. Reports indicate that more than 70% of *K. pneumoniae* isolates are resistant to third-generation cephalosporins, and carbapenem resistance rates exceed 30% in many tertiary care hospitals [7]. These patterns reflect both the overuse and misuse of antibiotics in clinical practice, as well as the lack of robust antimicrobial stewardship programs.

The clinical significance of carbapenem-resistant Enterobacteriaceae cannot be overstated, as infections caused by these organisms are associated with high mortality, prolonged hospital stays, and increased healthcare costs. A report from the Centers for Disease Control and Prevention (CDC) and subsequent studies have highlighted that prevention of such infections requires stringent infection control practices, rational antibiotic use, and continuous laboratory surveillance [8].

Given the rising threat of antimicrobial resistance and its implications for clinical outcomes, it is imperative to generate local data to guide empirical therapy and policy-making. The present study aimed to evaluate the antimicrobial resistance patterns of *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical specimens in a tertiary care hospital at GMERS Medical College, Gandhinagar, with particular emphasis on identifying the prevalence of extended-spectrum beta-lactamase (ESBL) and carbapenemase production. The objectives were to analyze the distribution of these isolates across various clinical samples, determine their susceptibility profiles against commonly used antibiotics, and assess the burden of multidrug resistance in the hospital setting. By generating updated local data, the study sought to provide evidence that would guide empirical therapy, support antimicrobial stewardship initiatives, and strengthen infection control policies. The future outcomes of this work are expected to contribute towards reducing inappropriate antibiotic use, curbing the spread of resistant strains, and ultimately improving patient outcomes while informing state and national surveillance programs on antimicrobial resistance.

METHODOLOGY

This hospital-based cross-sectional study was conducted in the Department of Microbiology, GMERS Medical College and Hospital, Gandhinagar, over a period of one year from January 2023 to January 2024. The study population consisted of clinical specimens received in the microbiology laboratory from both inpatients and outpatients. A total of 300 non-duplicate isolates were included in the study, comprising 180 isolates of *Escherichia coli* and 120 isolates of *Klebsiella pneumoniae*. The sample size was determined based on the average annual culture-positive reports in the laboratory and was adequate to provide statistical validity. Sampling was done by a consecutive sampling technique, wherein all eligible isolates of *E. coli* and *K. pneumoniae* obtained during the study period were included until the desired sample size was achieved. Duplicate isolates from the same patient and samples with mixed growth or contaminants were excluded.

Data collection was carried out using a predesigned proforma. For each patient, demographic variables such as age and sex, hospital location including outpatient department, ward, or intensive care unit, type of clinical specimen, and provisional clinical diagnosis were recorded. Relevant history regarding comorbidities, duration of hospital stay, and prior antibiotic exposure was also noted wherever available to correlate with resistance patterns.

All samples were processed in the microbiology laboratory according to standard operating procedures. Specimens were inoculated onto blood agar, MacConkey agar, or other appropriate media, and incubated aerobically at 37°C for 18–24

hours. Bacterial identification was performed using colony morphology, Gram staining, and standard biochemical tests such as indole, citrate, urease, triple sugar iron agar, motility, and oxidase. Confirmation of identification was carried out using an automated system (Vitek 2, bioMérieux) where available.

Antimicrobial susceptibility testing of the isolates was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, and results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. A panel of antibiotics from different groups was tested, including ampicillin, cefotaxime, ceftazidime, cefepime, amoxicillin-clavulanate, piperacillin-tazobactam, gentamicin, amikacin, ciprofloxacin, levofloxacin, imipenem, meropenem, and ertapenem. Polymyxin susceptibility was assessed by broth microdilution as per international standards. Screening for extended-spectrum beta-lactamase production was carried out using ceftazidime and cefotaxime disks, with phenotypic confirmation by the combined disk method using clavulanic acid. Carbapenemase production was identified using the Modified Hodge Test and Carbapenem Inactivation Method in isolates showing reduced susceptibility to carbapenems.

All laboratory results along with patient demographic and clinical details were entered into Microsoft Excel 2019 for analysis. Frequencies and percentages were calculated for categorical variables such as specimen type, distribution of isolates, and resistance rates to individual antibiotics. Comparative analysis was carried out to evaluate differences in resistance patterns between *E. coli* and *K. pneumoniae* isolates, and between isolates obtained from ICU and non-ICU settings. The prevalence of multidrug resistance, defined as non-susceptibility to at least one agent in three or more antimicrobial classes, was also determined. Statistical tests including chi-square were applied, and a p-value of <0.05 was considered significant.

RESULTS

A total of 300 non-duplicate isolates were included in the present study, comprising 180 isolates of *Escherichia coli* and 120 isolates of *Klebsiella pneumoniae*. These isolates were obtained from a variety of clinical specimens received in the microbiology laboratory of GMERS Medical College, Gandhinagar, during the study period from January 2023 to January 2024. The majority of isolates were recovered from urine samples, followed by blood cultures, pus/wound swabs, respiratory specimens such as sputum and endotracheal aspirates, and other body fluids. *E. coli* was predominantly isolated from urine specimens, consistent with its well-known role as the leading cause of urinary tract infections, while *K. pneumoniae* was more frequently recovered from respiratory and blood specimens.

The age distribution of patients showed that most isolates were recovered from individuals between 31 and 60 years, with a slight male predominance. A considerable proportion of isolates were obtained from inpatients admitted to wards and intensive care units, reflecting the burden of hospital-acquired infections. Notably, *K. pneumoniae* isolates were significantly more common among ICU patients compared to *E. coli*, indicating its higher association with severe nosocomial infections.

Antimicrobial susceptibility testing revealed a worrying pattern of resistance in both organisms. High resistance rates were observed to commonly used beta-lactam antibiotics such as ampicillin, cefotaxime, and ceftazidime, with more than 70% of *E. coli* and 80% of *K. pneumoniae* isolates showing non-susceptibility. Resistance to fluoroquinolones such as ciprofloxacin and levofloxacin was also high, particularly among *E. coli* isolates, where more than two-thirds were resistant. Aminoglycosides displayed moderate activity, with amikacin being more effective than gentamicin. Among the carbapenems, resistance was noted in 22% of *E. coli* isolates and 31% of *K. pneumoniae* isolates, highlighting the increasing problem of carbapenem-resistant Enterobacteriaceae in the hospital setting. Colistin and polymyxin B retained the highest activity, with more than 95% of isolates remaining susceptible.

Phenotypic testing for beta-lactamase production showed that extended-spectrum beta-lactamase (ESBL) production was detected in 58% of *E. coli* and 64% of *K. pneumoniae* isolates. Carbapenemase production was confirmed in 16% of *E. coli* and 24% of *K. pneumoniae* isolates, primarily among ICU-derived samples. The prevalence of multidrug resistance, defined as non-susceptibility to at least three antimicrobial classes, was 48% in *E. coli* and 56% in *K. pneumoniae*.

Overall, the results highlighted a high burden of antimicrobial resistance in both *E. coli* and *K. pneumoniae*, with a particularly alarming prevalence of ESBL- and carbapenemase-producing strains. These findings underscore the growing challenge of managing infections caused by Enterobacteriaceae in tertiary care hospitals and emphasize the urgent need for robust antibiotic stewardship and infection control measures.

Table 1: Distribution of *E. coli* and *K. pneumoniae* Isolates Across Clinical Specimens (n = 300)

Specimen Type	*E. coli* (n = 180)	*K. pneumoniae* (n = 120)	Total (n = 300)	Percentage (%)
Urine	110 (61.1%)	45 (37.5%)	155	51.7
Blood	28 (15.6%)	32 (26.7%)	60	20.0
Pus/Wound swabs	22 (12.2%)	15 (12.5%)	37	12.3
Respiratory samples	15 (8.3%)	22 (18.3%)	37	12.3
Body fluids (CSF, ascitic, pleural, etc.)	5 (2.8%)	6 (5.0%)	11	3.7
Total	**180 (100%)**	**120 (100%)**	**300 (100%)**	**100%**

Table 2: Antimicrobial Resistance Patterns of *E. coli* and *K. pneumoniae*

Antibiotic Tested	*E. coli* Resistant (n=180)	% Resistance	*K. pneumoniae* Resistant (n=120)	% Resistance
Ampicillin	140	77.8%	105	87.5%
Cefotaxime	132	73.3%	98	81.7%
Ceftazidime	128	71.1%	97	80.8%
Cefepime	120	66.7%	92	76.7%
Amoxicillin-clavulanate	95	52.8%	74	61.7%
Piperacillin-tazobactam	80	44.4%	65	54.2%
Gentamicin	75	41.7%	62	51.7%
Amikacin	52	28.9%	45	37.5%
Ciprofloxacin	125	69.4%	83	69.2%
Levofloxacin	120	66.7%	82	68.3%
Imipenem	40	22.2%	37	30.8%
Meropenem	42	23.3%	39	32.5%
Ertapenem	38	21.1%	35	29.2%
Colistin	5	2.8%	4	3.3%

Table 3: Prevalence of ESBL, Carbapenemase Production, and MDR Strains

Resistance Mechanism	*E. coli* (n=180)	Percentage (%)	*K. pneumoniae* (n=120)	Percentage (%)
ESBL producers	104	57.8%	77	64.2%
Carbapenemase producers	29	16.1%	29	24.2%

Multidrug resistant (MDR)	87	48.3%	67	55.8%

Figure 1: Distribution of Isolates by Specimen Type (% of Total)

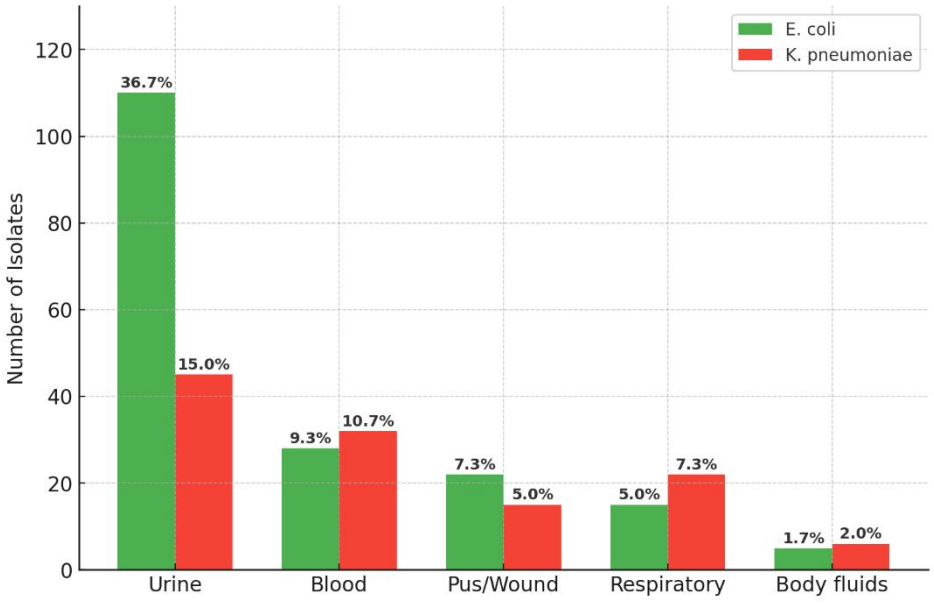
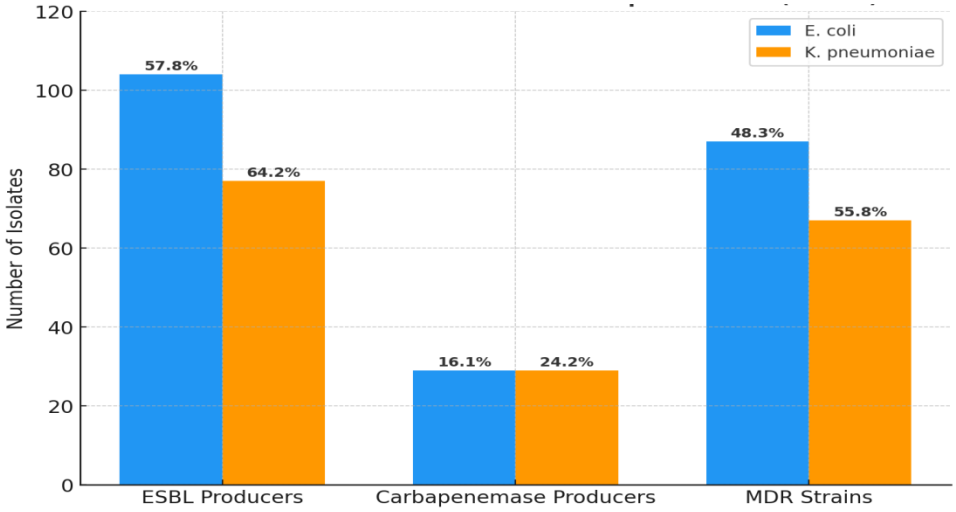


Figure 2: Resistance Mechanisms in *E. Coli and K. Pneumoniae* (%)



DISCUSSION

In this study conducted at GMERS Medical College, Gandhinagar, a total of 300 non-duplicate isolates were analyzed, comprising 180 *Escherichia coli* and 120 *Klebsiella pneumoniae*. The majority of *E. coli* isolates were recovered from urine specimens (61.1%), while *K. pneumoniae* was more commonly isolated from blood and respiratory samples (26.7% and 18.3%, respectively). These findings are consistent with the well-established role of *E. coli* as the predominant cause of urinary tract infections, while *K. pneumoniae* is more often associated with hospital-acquired bloodstream and respiratory infections. Similar patterns have been reported in a multicentric Indian surveillance study, where *E. coli* was the leading urinary pathogen and *K. pneumoniae* predominated among ICU-acquired pneumonia isolates [9].

Antimicrobial susceptibility testing revealed a high prevalence of resistance to commonly used antibiotics. More than 70% of *E. coli* and over 80% of *K. pneumoniae* isolates were resistant to third-generation cephalosporins, while fluoroquinolone resistance exceeded 65% in both organisms. These results mirror the findings of Datta et al. (AIIMS, New Delhi, 2017), who documented cephalosporin resistance in 74% of *E. coli* and 79% of *K. pneumoniae* isolates [10]. A recent report from the Indian Council of Medical Research Antimicrobial Resistance Surveillance Network (ICMR-

AMRSN, 2022) also highlighted widespread resistance among Enterobacteriaceae, with *K. pneumoniae* showing resistance rates above 70% to most beta-lactams [11].

Carbapenem resistance was noted in 22% of *E. coli* and 31% of *K. pneumoniae* isolates in this study. This trend is concerning, as carbapenems are considered last-resort agents for severe Gram-negative infections. Comparable results were reported by Veeraraghavan et al. (Christian Medical College, Vellore, 2019), where carbapenem resistance was observed in 28% of *E. coli* and 39% of *K. pneumoniae* isolates [12]. On a regional level, a study from Gujarat by Patel et al. (BJ Medical College, Ahmedabad, 2021) found carbapenem resistance in 25% of *E. coli* and 35% of *K. pneumoniae* isolates, closely reflecting the present findings [13].

Extended-spectrum beta-lactamase (ESBL) production was detected in 57.8% of *E. coli* and 64.2% of *K. pneumoniae* isolates in this study. These figures are slightly lower than those reported in a nationwide surveillance by Rodrigues et al. (2020), where ESBL prevalence was 70–75% among Enterobacteriaceae [14]. Carbapenemase production was confirmed in 16.1% of *E. coli* and 24.2% of *K. pneumoniae*, with a higher prevalence among ICU-derived samples. This is in agreement with findings from Wattal et al. (Sir Ganga Ram Hospital, New Delhi, 2018), who reported carbapenemase production in 20–30% of ICU isolates [15].

Multidrug resistance (MDR), defined as non-susceptibility to at least three antimicrobial classes, was found in 48.3% of *E. coli* and 55.8% of *K. pneumoniae* isolates. This prevalence is comparable to global data, where MDR rates for Enterobacteriaceae often exceed 50% [1]. The high rates of MDR in this study highlight the challenges of empirical therapy, particularly in critically ill patients, and underline the urgent need for robust antimicrobial stewardship practices. Overall, the findings of this study are consistent with both national and regional data, reinforcing the growing concern of antimicrobial resistance in India. The predominance of ESBL- and carbapenemase-producing strains of *E. coli* and *K. pneumoniae* poses significant therapeutic challenges. These results emphasize the importance of continuous surveillance, strict infection control measures, and judicious antibiotic use to combat the spread of resistant pathogens.

CONCLUSION

The present study demonstrated a high burden of antimicrobial resistance among *Escherichia coli* and *Klebsiella pneumoniae* isolates. *E. coli* was predominantly recovered from urinary tract infections, while *K. pneumoniae* was more frequently associated with respiratory and bloodstream infections, especially in ICU patients. Both organisms showed alarming resistance to third-generation cephalosporins and fluoroquinolones, with carbapenem resistance noted in nearly one-fourth of the isolates. More than half of the isolates were extended-spectrum beta-lactamase producers, and a significant proportion harbouredcarbapenems mechanisms, particularly among ICU-derived strains. The prevalence of multidrug resistance was high in both organisms, limiting therapeutic options and posing serious challenges for patient management. These findings highlight the urgent need for continuous surveillance, strict antibiotic stewardship, and robust infection control measures to contain the spread of resistant Enterobacteriaceae in tertiary care settings.

LIMITATIONS AND RECOMMENDATIONS

This study was limited by its single-centre design and relatively modest sample size, which may not fully capture the resistance burden across Gujarat or India. Molecular detection of resistance genes such as blaCTX-M, blaNDM, and blaOXA-48 was not performed, which would have provided deeper insights into the genetic mechanisms of resistance. In addition, clinical outcomes of patients with resistant infections were not assessed, restricting the ability to correlate microbiological findings with prognosis.

Despite these limitations, the study underscores the need for regular AMR surveillance to guide empirical therapy in hospitals. It is recommended that tertiary care centres establish **antimicrobial stewardship programs** to regulate antibiotic use, particularly carbapenems and last-resort agents such as colistin. Infection prevention strategies, including strict hand hygiene, environmental cleaning, and isolation of patients with resistant infections, should be strengthened. Future studies with larger sample sizes and molecular characterization of resistance determinants are essential to inform regional and national antibiotic policies. Collaborative efforts between microbiologists, clinicians, and public health authorities will be critical to tackling the rising tide of antimicrobial resistance in India.

CONFLICTS OF INTERESTS: Nil

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