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Prevalence And Antimicrobial Susceptibility Patterns of ESBL and AMPC B-Lactamase Producing Escherichia Coli and Klebsiella Pneumoniae Isolated from Clinical Samples in A Tertiary Care Centre, Raichur: A Cross-Sectional Study

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ABSTRACT

Background: The growing resistance of *Escherichia coli* and *Klebsiella pneumoniae* to commonly used antibiotics—particularly through the production of ESBLs and AmpC β-lactamases—has become a serious clinical challenge worldwide. These enzymes weaken the action of multiple β-lactam agents and drive multidrug resistance. This study investigated how frequently these mechanisms occur in a tertiary-care facility and assessed how these isolates responded to routinely used antimicrobial agents.

Materials and Methods: Two hundred unique isolates (140 *E. coli* and 60 *K. pneumoniae*) recovered from diverse clinical specimens were examined for further evaluation. ESBL activity was confirmed through phenotypic disc diffusion–based methods, while AmpC production was screened using cefoxitin discs and verified by the AmpC disc test. susceptibility testing was performed through a standardised disc-diffusion protocol following CLSI guidelines. statistical comparisons were carried out using χ^2 analysis, with significance defined at $p < 0.05$.

Results: ESBL production occurred in 41% of isolates, with *E. coli* presenting slightly higher positivity (42.8%) compared to *K. pneumoniae* (36.6%). AmpC β-lactamase activity was identified in 19.5%, more frequently in *K. pneumoniae* (21.6%) than *E. coli*. Co-production of both enzymes was noted in 9.5%. Urine specimens yielded the most ESBL producers, whereas pus samples contributed the highest number of AmpC-positive isolates. Marked resistance to cephalosporins (80–90%) and considerable fluoroquinolone resistance (55–65%) were observed. Carbapenems continued to show high efficacy (>95%), and nitrofurantoin remained highly active against urinary *E. coli*.

Conclusion: The findings reveal a notable burden of ESBL and AmpC producers among *E. coli* and *K. pneumoniae*, emphasising the need for routine enzyme detection, rational antimicrobial use, and robust infection-control measures to restrict further spread.

Keywords: *Escherichia coli; Klebsiella pneumoniae; ESBL; AmpC β-lactamase; Antimicrobial resistance.*

INTRODUCTION

The threat posed by antimicrobial resistance (AMR) has escalated globally, influencing clinical outcomes through prolonged disease, extended hospitalisation, increased financial burden, and higher mortality. Gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*, play a major role in this rise because of their capacity to acquire, retain, and transmit diverse resistance determinants. Among the numerous mechanisms reported in these organisms, β-lactamase families such as ESBLs and AmpC enzymes are especially impactful due to their ability to compromise widely used β-lactam antibiotics.[1,2]

ESBL-producing Enterobacterales can hydrolyse third-generation cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime; however, this activity can be neutralised by clavulanic acid.[3] The genes encoding ESBLs are frequently plasmid-borne and often accompany resistance markers against aminoglycosides, cotrimoxazole, and fluoroquinolones, contributing to multidrug resistance (MDR).[4,5] These plasmids are easily transferable, promoting the spread of resistant strains within healthcare centres as well as community settings.[6]

AmpC β-lactamases represent another important mechanism conferring resistance to β-lactams. Unlike ESBLs, AmpC enzymes degrade cephamycins and remain unaffected by β-lactamase inhibitors.[7] Although present on chromosomes in certain species, plasmid-mediated AmpC variants—which can move between *E. coli* and *K. pneumoniae*—pose a higher epidemiological risk.[8,9] Their presence can obscure ESBL detection and is associated with poor therapeutic response to cephalosporins.[10]

Global surveillance studies indicate a steady rise in these resistance mechanisms, with the highest prevalence documented in many regions of Africa, Asia, and South America.[11,12] India is among the most severely affected countries because of widespread antimicrobial exposure, dense patient populations, and inconsistent antimicrobial stewardship practices.[13] Multiple regional studies report that ESBL prevalence ranges from 40–70%, while AmpC production is seen in roughly 10–25% of isolates.[14,15]

These resistant pathogens are implicated in a variety of clinical conditions, affecting diverse sites such as the urinary tract, bloodstream, soft tissues, and respiratory system.[16] The scarcity of effective antibiotics often pushes clinicians toward carbapenems, contributing to the increasing appearance of carbapenem-resistant Enterobacterales (CRE).[17,18]

Accurate identification of ESBL and AmpC β-lactamases is essential for optimising treatment decisions and implementing targeted infection-control strategies. Despite advances in molecular diagnostics, phenotypic assays such as double-disc synergy tests, combined disc tests, cefoxitin screening, and AmpC disc methods remain widely adopted due to their reliability and cost-effectiveness.[19,20] Routine surveillance assists in guiding empirical therapy and restricting the spread of MDR organisms.[21]

This study was designed to determine the prevalence and antimicrobial susceptibility patterns of ESBL- and AmpC-producing *E. coli* and *K. pneumoniae* isolated from clinical samples in a tertiary-care hospital.

MATERIALS AND METHODS

Study Setting

This cross-sectional study was performed in the Department of Microbiology, Navodaya Medical College & Research Centre, Raichur, from July 2024 to June 2025.

Specimen Handling

Two hundred non-duplicate isolates were included in the analysis (140 *E. coli*, 60 *K. pneumoniae*). Samples such as urine, pus, blood, respiratory secretions, and sterile body fluids were collected aseptically and promptly transported to the microbiology laboratory. When brief delays occurred, samples were kept at room temperature.

Each specimen was cultured on MacConkey agar, Blood agar, and Chocolate agar and grown aerobically at 37°C for an overnight period. Organism identification was performed through standard laboratory procedures.

Antimicrobial Susceptibility Testing

Kirby–Bauer disc diffusion testing on Mueller–Hinton agar was used to determine susceptibility, and interpretation followed CLSI recommendations.

Screening and Confirmation Procedures

ESBL Screening

Strains showing reduced inhibition zones for key third-generation cephalosporins were flagged as potential ESBL candidates.

AmpC Screening

A cefoxitin inhibition zone <18 mm signified potential AmpC enzyme production.

Confirmatory Tests

Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

Cefotaxime and ceftazidime discs—with and without clavulanic acid—were positioned 30 mm apart on a lawn inoculated at 0.5 McFarland turbidity. An enhancement of ≥5 mm in the clavulanate-containing disc confirmed ESBL activity.

Double Disc Synergy Test (DDST):

Cephalosporin discs placed 15 mm from an amoxicillin–clavulanate disc were examined for synergy, indicated by a characteristic "keyhole" pattern.

AmpC Disc Test:

A lawn of *E. coli* ATCC 25922 was prepared on Mueller–Hinton agar, and a cefoxitin disc was placed alongside a blank disc inoculated with the test isolate. Distortion of the cefoxitin inhibition zone suggested AmpC β-lactamase production.

Quality Control

E. coli ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls, respectively. All media batches underwent sterility checks.

Statistical Analysis

Data were entered in Microsoft Excel and analyzed using **SPSS version 20.0**. Findings were expressed as frequencies, percentages, and proportions.Comparisons between ESBL and non-ESBL isolates, as well as AmpC and non-AmpC isolates, were performed using the **Chi-square (χ^2) test**. A **p-value <0.05** was considered statistically significant, and **95% confidence intervals** were calculated where applicable.

RESULTS

A total of **200 non-repetitive clinical isolates** were included in the present study, consisting of **140 (70%) *Escherichia coli*** and **60 (30%) *Klebsiella pneumoniae***. *E. coli* constituted the majority of isolates (70%), indicating its predominance as a clinical pathogen across different samples as shown in Table 1

Table 1: Distribution of Clinical Isolates (n = 200)

Organism	Number	Percentage
Escherichia coli	140	70%
Klebsiella pneumoniae	60	30%

ESBL production was more common in *E. coli* (42.8%) compared to *K. pneumoniae* (36.6%). The difference was statistically **not significant** ($p > 0.05$), although *E. coli* showed a slightly higher ESBL rate as shown in Table 2

Table 2: Prevalence of ESBL Producers

Organism	Total Isolates	ESBL Positive	Percentage
E. coli	140	60	42.8%
K. pneumoniae	60	22	36.6%
Total	200	82	41%

Statistical Test: $\chi^2 = 0.72$, $p > 0.05$ → **Not significant**

AmpC production was significant in both organisms, with *K. pneumoniae* showing slightly higher AmpC positivity (21.6%) than *E. coli* (18.5%). The difference was **not statistically significant** as shown in Table 3

Table 3: Prevalence of AmpC Producers

Organism	Total Isolates	AmpC Positive	Percentage
E. coli	140	26	18.5%
K. pneumoniae	60	13	21.6%
Total	200	39	19.5%

Statistical Test: $\chi^2 = 0.28$, $p > 0.05$ → **Not significant**

Combined ESBL and AmpC production was detected in both organisms. Co-production was slightly higher in *K. pneumoniae* (11.6%) compared to *E. coli* (8.5%) as shown in Table 4

Table 4: ESBL + AmpC Co-production

Organism	Total Isolates	Co-producers	Percentage
E. coli	140	12	8.5%
K. pneumoniae	60	7	11.6%
Total	200	19	9.5%

Urine yielded the highest number of ESBL producers.Pus/wound samples had the highest AmpC positivity as shown in Table 5

Table 5: Distribution of ESBL and AmpC Producers Based on Sample Type

Sample Type	Total Isolates	ESBL Positive	AmpC Positive
Urine	110	50	15
Pus/Wound	45	18	14
Blood	20	6	5
Respiratory (Sputum/ETA)	15	5	3
Body Fluids	10	3	2

Highest resistance to cephalosporins (80–90%). Moderate resistance to fluoroquinolones (60%). High sensitivity to carbapenems (98%). Nitrofurantoin was highly effective against urinary *E. coli* isolates as shown in Table 6

Table 6: Antimicrobial Susceptibility Pattern of *E. coli* (n = 140)

Antibiotic	Sensitive (%)	Resistant (%)
Cefotaxime	20	80
Ceftazidime	18	82
Ceftriaxone	22	78
Cefoxitin	65	35
Gentamicin	55	45
Amikacin	70	30
Ciprofloxacin	40	60
Levofloxacin	45	55
Piperacillin-Tazobactam	82	18
Imipenem	98	2
Meropenem	97	3
Nitrofurantoin* (urine)	92	8

Klebsiella isolates showed higher resistance than *E. coli* for most antibiotics, reflecting their stronger resistance mechanisms (AmpC, ESBL, efflux pumps) as shown in Table 7

Table 7: Antimicrobial Susceptibility Pattern of *K. pneumoniae* (n = 60)

Antibiotic	Sensitive (%)	Resistant (%)
Cefotaxime	18	82
Ceftazidime	15	85
Ceftriaxone	20	80
Cefoxitin	60	40
Gentamicin	50	50
Amikacin	65	35
Ciprofloxacin	35	65
Levofloxacin	38	62
Piperacillin–Tazobactam	80	20
Imipenem	95	5

Antibiotic	Sensitive (%)	Resistant (%)
Meropenem	93	7

ESBL producers showed significantly higher resistance to fluoroquinolones and aminoglycosides (**p < 0.05**) as shown in Table 8

Table 8: Antibiotic Resistance in ESBL vs. Non-ESBL Isolates

Antibiotic	ESBL Producers (%)	Non-ESBL (%)	p-value
Cephalosporins	100	48	<0.001*
Ciprofloxacin	78	45	0.02*
Gentamicin	62	38	0.04*
Amikacin	45	25	0.05*
Carbapenems	10	4	0.20 (NS)

*Significant at $p < 0.05$

AmpC producers showed significantly higher resistance to cefoxitin and aminoglycosides as shown in Table 9

Table 9: Resistance in AmpC vs Non-AmpC Producers

Antibiotic	AmpC Resistant (%)	Non-AmpC Resistant (%)	χ^2	p-value
Cefoxitin	100	20	110.8	<0.001*
Gentamicin	72	40	11.5	0.002*
Ciprofloxacin	80	55	7.1	0.03*
Carbapenems	12	5	2.1	0.14 (NS)

DISCUSISON

In this study, *E. coli constituted the larger share of isolates in this study*, a pattern consistent with earlier studies describing it as a frequent cause of urinary and wound infections (22,23). Its dominance may be linked to its intestinal carriage and its exceptional ability to accumulate resistance genes (24).

ESBL Prevalence

The 41% ESBL rate observed closely matches reports from several regions where prevalence typically ranges from 35–60% (23,24). A slightly greater proportion of *E. coli* isolates manifested ESBL activity than in *K. pneumoniae*, a trend reported previously by Paterson and Bonomo (25). Shaikh et al. also identified *E. coli* as a major ESBL contributor worldwide (26).

AmpC Prevalence

AmpC production (19.5%) aligned with previously reported national estimates reporting 15–25% positivity (27). Slightly higher AmpC positivity in *K. pneumoniae* may reflect its ability to harbour both plasmid-mediated and chromosomal AmpC determinants (28,29). Since AmpC expression can go unnoticed in routine testing, it may lead to inappropriate therapy (30).

Co-production of ESBL and AmpC

Co-expression of both enzymes (9.5%) aligns with earlier reports indicating 5–15% prevalence, particularly in hospital-acquired infections (30,31). Such isolates often exhibit expanded resistance patterns, complicating antimicrobial decision-making.

Distribution by Sample Type

Urine samples yielded the greatest number of ESBL-positive isolates, reflecting the prominence of uropathogenic *E. coli* (22). AmpC-producing strains, however, were most common in pus samples, which may be associated with prolonged antibiotic exposure (32).

Drug Resistance Trends

Resistance levels to cephalosporins and fluoroquinolones were notably high, reflecting widespread antimicrobial pressure and consistent with the growing use and misuse of these antibiotics (33,34). Amikacin showed comparatively better activity than gentamicin, in line with previous findings (34). Carbapenems remained the most effective class (>95% sensitivity), though the appearance of carbapenem-nonsusceptible strains warns of emerging resistance (35). Nitrofurantoin remained a highly effective option for urinary *E. coli* (36).

Resistance in ESBL and AmpC Producers

ESBL-producing isolates exhibited significantly higher resistance to aminoglycosides and fluoroquinolones ($p < 0.05$), likely owing to the presence of co-located resistance genes on plasmids (37). AmpC producers showed characteristic resistance to cefoxitin and increased resistance to gentamicin and ciprofloxacin, consistent with earlier findings (38).

CONCLUSION

This study reveals a considerable burden of isolates expressing ESBL and AmpC enzymes among *E. coli* and *K. pneumoniae*, together with marked resistance to cephalosporins and fluoroquinolones. Co-production of these β-lactamases further exacerbates multidrug resistance. Although carbapenems remain the most effective agents, early detection, ongoing surveillance, responsible antibiotic use, and strengthened infection-control practices are essential to curb further spread of these resistant organisms.

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