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Antibiotic susceptibility pattern of Extended-spectrum Beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from indoor patients of SRG Hospital, Jhalawar

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Abstract:

Background
Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae represent a major cause of antimicrobial resistance, particularly in hospital settings.

Methods
This laboratory-based observational study included 385 non-duplicate isolates of *Escherichia coli* and *Klebsiella pneumoniae* from hospitalized patients over one year. Identification was performed using standard biochemical tests. ESBL screening and phenotypic confirmation were carried out using disc diffusion and combined disc tests according to CLSI M100 (2023) guidelines. Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method.

Results
Of 385 isolates, *E. coli* accounted for 68% and *K. pneumoniae* for 32%. ESBL production was confirmed in 158 isolates (41.04%), with a higher prevalence in *E. coli* (50%) than *K. pneumoniae* (21.95%). ESBL producers exhibited markedly higher resistance to third-generation cephalosporins and fluoroquinolones, while susceptibility to piperacillin–tazobactam and carbapenems remained higher.

Conclusion
A considerable prevalence of ESBL-producing isolates among hospitalized patients highlights the need for routine ESBL detection and antimicrobial stewardship to limit resistance spread.

Keywords:
ESBL, Escherichia coli, Klebsiella pneumoniae, Antimicrobial resistance, Combined disk diffusion, CLSI M100 2023

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Introduction

Antimicrobial resistance driven by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae continues to undermine effective infection management globally. ESBL enzymes, particularly the CTX-M family, hydrolyze third-generation cephalosporins and aztreonam, severely restricting β-lactam treatment options. The rapid spread of CTX-M variants, especially CTX-M-15, is facilitated by plasmids and other mobile genetic elements, enabling efficient horizontal transfer within and between bacterial species.

Among Enterobacteriaceae, Escherichia coli and Klebsiella pneumoniae remain the most frequent ESBL producers encountered in clinical infections, including urinary tract infections, bloodstream infections, and hospital-acquired pneumonia. Their increasing resistance is often accompanied by co-resistance to fluoroquinolones, aminoglycosides, and trimethoprim–sulfamethoxazole, leading to multidrug-resistant (MDR) phenotypes. As a result, carbapenems and select β-lactam/β-lactamase inhibitor combinations have become key therapeutic options; however, rising carbapenem use has accelerated the emergence of carbapenem-resistant Enterobacteriaceae (CRE), particularly in India.

India consistently reports some of the highest ESBL prevalence rates worldwide, driven by empirical antibiotic use, limited antimicrobial stewardship enforcement, and environmental dissemination pathways. Despite this burden, regional resistance data remain variable, and localized surveillance is critical to inform rational antibiotic therapy.

This study assesses the prevalence of ESBL-producing E. coli and K. pneumoniae among clinical isolates from hospitalized patients in a tertiary care center in Jhalawar, Rajasthan, and evaluates their antimicrobial susceptibility patterns. These findings aim to support evidence-based antibiotic selection and strengthen institutional antimicrobial stewardship practices.

Material and Methods

Study design and setting

This laboratory-based descriptive observational study was conducted in the Department of Microbiology, Jhalawar Medical College and attached tertiary care hospitals, Jhalawar, Rajasthan. The study period spanned one year, from 16 August 2023 to 15 August 2024.

Study Population and Sample Size

A total of 385 non-duplicate clinical isolates of Escherichia coli and Klebsiella pneumoniae obtained from indoor patients were included. Isolates were recovered from urine, pus, sputum, endotracheal tube aspirates, blood, and other clinical specimens. Outpatients and improperly collected or labeled samples were excluded.

Isolation and Identification of Bacterial Isolates

Specimens were cultured on Blood Agar and MacConkey Agar (Hi-Media, Mumbai) and incubated at 37°C for 18–24 hours. Lactose fermenting Gram-negative bacilli were further identified based on colony morphology, Gram staining, motility, and standard biochemical reactions including indole, methyl red, Voges–Proskauer, citrate utilization, urease activity, and triple sugar iron tests.

ESBL Screening and Phenotypic Confirmation

Screening for ESBL production was performed using cephalosporin antibiotic discs (cefpodoxime 10 µg, ceftazidime 30 µg, cefotaxime 30 µg, ceftriaxone 30 µg, and aztreonam 30 µg) on Mueller–Hinton Agar by the Kirby–Bauer disc diffusion

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method. Isolates with reduced susceptibility to any screening agent were subjected to phenotypic confirmatory testing using the combined disc method with ceftazidime (30 µg) and ceftazidime–clavulanate (30/10 µg), and cefotaxime (30 µg) and cefotaxime–clavulanate (30/10 µg). An increase of ≥5 mm in zone diameter in the presence of clavulanate was interpreted as ESBL production. Interpretation followed CLSI M100 (2023) guidelines.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton Agar, and results were interpreted according to CLSI M100 (2023) criteria. The antibiotic panel included piperacillin–tazobactam, nitrofurantoin, ciprofloxacin, norfloxacin, gentamicin, amoxicillin–clavulanate, cefpodoxime, ceftriaxone, ceftazidime, tobramycin, and imipenem.

Quality Control

Escherichia coli ATCC 25922 (ESBL-negative control) and Klebsiella pneumoniae ATCC 700603 (ESBL-positive control) were used for internal quality control during ESBL screening and susceptibility testing.

Results

In this study, a total of 385 clinical isolates of Escherichia coli and Klebsiella pneumoniae were included. E. coli constituted the majority of isolates (Table 1).

Table 1. Distribution of Clinical Isolates.

Organism	Number of Isolates	Percentage
Escherichia coli	**262**	**68%**
Klebsiella pneumoniae	**123**	**32%**

ESBL screening was positive in 285 isolates (74.03%), while phenotypic confirmatory testing identified ESBL production in 158 isolates (41.04%) (Table 2).

Table 2. ESBL Screening and Confirmation Results.

Category	Number of Isolates	Percentage
ESBL Screen Positive	**285**	**74.03%**
ESBL Confirmed (DDST)	**158**	**41.04%**
Organism	**Number of ESBL Confirmed Isolates**	**Percentage Among Species**
E. coli	**131**	**50%**
K. pneumoniae	**27**	**21.95%**

Overall, ESBL-producing isolates exhibited substantially reduced susceptibility to cephalosporins and fluoroquinolones, while susceptibility to carbapenems and piperacillin–tazobactam remained comparatively preserved (Table 3).

Table 3. Antibiotic Susceptibility Comparison for ESBL and Non-ESBL E. coli.

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Antibiotic	Sensitivity (%) Non-ESBL	Sensitivity (%) ESBL
Piperacillin–Tazobactam	100	85.57
Nitrofurantoin	77.58	75
Ciprofloxacin	68.96	45.19
Norfloxacin	63.79	42.30
Gentamicin	82.75	68.26
Amoxicillin–Clavulanate	46.55	31.73
Cefpodoxime	100	45.19
Ceftriaxone	100	19.23
Ceftazidime	100	45.19
Tobramycin	94.82	54.80
Imipenem	72.41	47.11
Klebsiella pneumoniae		
Antibiotic	Sensitivity (%) Non-ESBL	Sensitivity (%) ESBL
Piperacillin–Tazobactam	97.61	77.77
Nitrofurantoin	71.43	29.62
Ciprofloxacin	83.33	53.70
Norfloxacin	88.09	35.18
Gentamicin	92.85	48.14
Amoxicillin–Clavulanate	30.95	7.40
Cefpodoxime	100	42.59
Ceftriaxone	100	22.22
Ceftazidime	100	33.33
Tobramycin	97.61	38.88
Imipenem	73.80	42.59

Discussion

The present study demonstrated a high prevalence of ESBL-producing E. coli and K. pneumoniae among clinical isolates obtained from hospitalized patients in a tertiary care hospital in Jhalawar, Rajasthan. The overall ESBL confirmation rate of 41.04% aligns with the increasing burden of ESBL-mediated resistance reported across India. Comparable prevalence rates have been documented in studies from Delhi, Gujarat, and Madhya Pradesh, where ESBL production among Enterobacteriaceae ranged from 35% to 55%, reflecting the widespread dissemination of ESBL genes in hospital environments.

In the current study, ESBL production was more common in E. coli (50%) than in K. pneumoniae (21.95%), which is consistent with findings from other tertiary care centers in northern and western India. This pattern may be attributed to the extensive presence of CTX-M-type ESBL genes in community-acquired E. coli, coupled with plasmid-mediated transmission. Additionally, the high burden of urinary isolates in hospitalized patients likely contributed to the predominance of E. coli in this study.

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Resistance to fluoroquinolones and third-generation cephalosporins was significantly higher in ESBL-producing isolates compared to non-ESBL isolates. Similar high-level resistance patterns have been reported from studies conducted in Jaipur, Bhopal, and Ahmedabad. Frequent empirical use of cephalosporins and fluoroquinolones in both inpatient and outpatient settings may be driving this co-selection of resistance. In contrast, relatively better susceptibility to piperacillin–tazobactam and carbapenems observed in this study is consistent with previous Indian reports and supports their continued role in the treatment of ESBL-associated infections.

The findings emphasize the need for routine ESBL detection in clinical laboratories, rational antibiotic prescribing, and strict implementation of antimicrobial stewardship policies. Regional antibiogram data should be regularly updated to guide empirical therapy, especially in high-burden settings.

Conclusion

This study highlights a substantial prevalence of ESBL-producing E. coli and K. pneumoniae among hospitalized patients in a tertiary care setting in Rajasthan. The significantly reduced susceptibility to third-generation cephalosporins and fluoroquinolones among ESBL producers underscores the clinical challenge they pose. However, the comparatively preserved activity of piperacillin–tazobactam and carbapenems suggests that these agents remain valuable therapeutic options. Routine ESBL screening, judicious antibiotic use, and strengthened antimicrobial stewardship programs are essential to curb the further spread of resistance.

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