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Original Research Article

RISING RESISTANCE IN THE HILLS OF KUMAUN: A STUDY OF ESBL-PRODUCING PATHOGENS FROM A NEW TEACHING HOSPITAL

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ABSTRACT

Background: Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales represent a major public health concern due to their role in multidrug resistance and limited therapeutic options. This study aimed to determine the prevalence and distribution of ESBL-producing pathogens isolated from clinical samples in hospitals of the Kumaun region. **Materials and Methods:** A total of 1,708 non-duplicate clinical samples (urine, blood, sputum, pus, and others) were collected aseptically from patients with suspected bacterial infections and processed using standard microbiological techniques. Identification of isolates was performed using conventional biochemical methods and the VITEK 2 Compact system. Screening for ESBL production was based on reduced susceptibility to third-generation cephalosporins, followed by confirmation using the phenotypic combination disk method as per CLSI guidelines. **Results:** Of the total samples, 570 (33.4%) were culture-positive, while 1,138 (66.6%) showed no growth or contamination. Among culture-positive samples, Enterobacterales accounted for 213 (12.5%) isolates. ESBL production was detected in 108 (50.7%) of these isolates. The majority of ESBL-producing isolates were obtained from inpatient departments (56.5%) compared to outpatient settings (43.5%), though the difference was not statistically significant (p = 0.178). *Escherichia coli* was the predominant ESBL producer (54.6%), followed by *Klebsiella* spp. (29.6%) and *Citrobacter* spp. (9.3%). Urine samples constituted the most common source (37.0%), followed by sputum (23.1%) and blood (20.4%). **Conclusion:** A high prevalence of ESBL-producing Enterobacterales was observed, with *E. coli* as the leading pathogen. The substantial burden in both inpatient and outpatient settings highlight the need for continuous surveillance, antimicrobial stewardship, and strict infection control measures to mitigate the spread of resistant organisms.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health threats, significantly compromising the effective management of infectious diseases. Among the various resistance mechanisms, the production of extended-spectrum beta-lactamases (ESBLs) by Gram-negative bacteria represents a critical concern due to their ability to inactivate a wide range of beta-lactam antibiotics, including third-generation cephalosporins and monobactams.[1,2] ESBL-producing organisms, particularly *Escherichia coli* and *Klebsiella pneumoniae*, are increasingly implicated in both community-acquired and hospital-associated infections, thereby limiting therapeutic options and necessitating the use of more expensive or toxic alternatives.[2,3]

The burden of ESBL-producing pathogens is especially high in hospital settings, where factors such as invasive procedures, prolonged hospital stays, and extensive antibiotic exposure facilitate their emergence and transmission.[3,4] Infections caused by these resistant organisms are associated with adverse clinical outcomes, including increased morbidity, mortality, and healthcare costs.[4] Moreover, the rapid dissemination of ESBL genes through mobile genetic elements such as plasmids accelerates the spread of resistance across different bacterial species, posing a serious challenge to infection control practices.[5]

In recent years, developing countries like India have reported a substantial rise in the prevalence of ESBL-producing isolates, driven by inappropriate antibiotic

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usage, over-the-counter availability of drugs, and limited implementation of antimicrobial stewardship programs.[6,7] Despite growing national data, there remains considerable regional variability in resistance patterns, underscoring the need for localized epidemiological studies. Such data are essential for guiding empirical therapy, optimizing antibiotic policies, and strengthening infection prevention strategies.

The Kumaun region of northern India presents unique healthcare challenges due to its difficult terrain, variable access to medical facilities, and limited laboratory infrastructure in certain areas. These factors may influence both the detection and management of resistant infections. However, there is a scarcity of updated and region-specific data on the prevalence of ESBL-producing pathogens in hospitals within this area. Understanding the local resistance profile is therefore crucial for improving clinical outcomes and supporting evidence-based decision-making.

In this context, the present study aims to determine the prevalence of ESBL-producing pathogens isolated from clinical samples in hospitals of the Kumaun region. The study also seeks to contribute to the existing body of knowledge by providing recent and region-specific insights into antimicrobial resistance trends, which may aid in the development of targeted infection control measures and rational antibiotic use policies.

MATERIALS AND METHODS

Study Design and Setting

This hospital-based cross-sectional study was conducted over a period of one year in newly developed tertiary care teaching hospital of the Kumaun region, Uttarakhand, India. The primary objective was to determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacterial isolates obtained from various clinical specimens.

Sample Collection and Processing

A total of 1,708 non-duplicate clinical samples, including urine, blood, sputum, pus, and other relevant specimens, were collected aseptically from patients with suspected bacterial infections. All samples were transported without delay to the microbiology laboratory and and processed according to standard microbiological procedures. Bacterial isolates were identified based on colony morphology, Gram staining, and conventional biochemical tests or automated VITEK 2 Compact system (BioMérieux, France).[8]

Inclusion and Exclusion Criteria:

All clinically significant, non-duplicate Gram-negative bacterial isolates obtained during the study period were included. Duplicate isolates from the same patient and samples deemed contaminated or clinically insignificant were excluded.

Antimicrobial Susceptibility Testing (AST):

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar and microbroth dilution during the initial phase of the study, once facility became available shifted to the VITEK 2 automated system, and results were interpreted according to the current Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] The antibiotics tested included third-generation cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone, along with other relevant antimicrobial agents as per the laboratory standard operating procedures.

Screening and Confirmation of ESBL Production:

Special emphasis was placed on the detection and confirmation of ESBL production. Initial screening for ESBL was based on reduced susceptibility patterns to third-generation cephalosporins (zone diameter below CLSI recommended cut-off values or as identified by the VITEK 2 system). Isolates flagged as potential ESBL producers were further evaluated using confirmatory methods.

Definitive confirmation of ESBL production was performed using the phenotypic combination disk method in accordance with CLSI recommendations. This involved testing ceftazidime and cefotaxime alone and in combination with clavulanic acid. An increase of ≥5 mm in the zone diameter for either antimicrobial agent tested in combination with clavulanate compared to the zone when tested alone was interpreted as confirmatory evidence of ESBL production.[9,10]

Additionally, the VITEK 2 system's Advanced Expert System (AES) was utilized to support ESBL detection by analyzing susceptibility patterns and providing interpretive comments, thereby enhancing the accuracy and reliability of ESBL identification.[8] This dual approach—automated detection along with phenotypic confirmation—ensured robust and precise identification of ESBL-producing isolates.

Quality Control

Quality control procedures were strictly followed using standard reference strains, including Escherichia coli ATCC 25922 (ESBL-negative control) and Klebsiella pneumoniae ATCC 700603 (ESBL-positive control), as recommended by CLSI guidelines.[9]

Data Analysis

Data were compiled and analyzed using statistical software such as SPSS version 22. The prevalence of ESBL-producing isolates was calculated as a percentage of the total number of Gram-negative isolates. Descriptive statistical methods were applied, and results were presented in tables and graphical formats where appropriate.

Ethical Considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee. Patient confidentiality was strictly maintained, and all data were anonymized.

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RESULTS

A total of 1,708 clinical samples were processed during the study period. Of these, 1,138 (66.6%) samples showed no growth or were reported as contaminated and were excluded from further microbiological characterization.

Among the remaining samples, microbial growth was observed as follows: fungal isolates were recovered in 53 (3.1%) samples, Gram-positive bacteria in 227 (13.3%) samples, non-fermenting Gram-negative bacilli (NFGNB) in 77 (4.5%) samples, and members of the Enterobacterales group in 213 (12.5%) samples.

Table 1: Distribution of Clinical Samples and Microbial Growth Pattern (N = 1708)

Parameter	Number (n)	Percentage (%)
Total samples processed	1708	100
No growth / Contaminated samples	1138	66.6
Culture positive samples	**570**	**33.4**
Type of isolates (n = 570)		
Fungal isolates	53	3.1
Gram-positive bacteria	227	13.3
Non-fermenting Gram-negative bacilli (NFGNB)	77	4.5
Enterobacterales	213	12.5

This table presents the overall distribution of clinical samples processed during the study period and the proportion yielding microbial growth, along with classification of isolates.

Extended-spectrum beta-lactamase (ESBL) production was detected in 108 isolates, representing 50.7% of the Enterobacterales isolates.

Table 2: Prevalence and Distribution of ESBL-Producing Enterobacterales (n = 213)

Parameter	Number (n)	Percentage (%)
ESBL-producing isolates	108	50.7
Non-ESBL isolates	105	49.3
Patient location (n = 108)		
Inpatient Department (IPD)	61	56.5
Outpatient Department (OPD)	47	43.5

This table shows the prevalence of ESBL production among Enterobacterales isolates and their distribution according to patient care settings. Chi-square test was applied to compare IPD vs OPD distribution ($\chi^2 = 1.81$, df = 1, p = 0.178), indicating no statistically significant difference.

Among the 108 ESBL-producing isolates, 61 (56.5%) were obtained from inpatient department (IPD) samples, while 47 (43.5%) were from outpatient department (OPD) samples.

The species distribution of ESBL-producing isolates revealed that *Escherichia coli* was the most predominant organism (n = 59, 54.6%), followed by *Klebsiella species* (n = 32, 29.6%), *Citrobacter species* (n = 10, 9.3%), *Enterobacter species* (n = 3, 2.8%), *Salmonella* Typhi (n = 2, 1.9%), and *Morganella morganii* (n = 1, 0.9%).

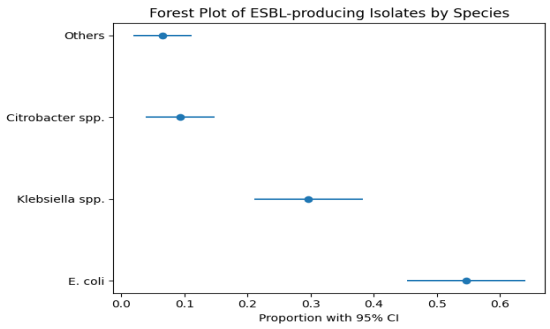

Figure 1: Forest plot showing the proportion of ESBL-producing isolates by species with 95% confidence intervals

Escherichia coli demonstrated the highest proportion, followed by *Klebsiella* spp., while other organisms contributed relatively smaller proportions. The confidence intervals indicate variability in the distribution across species.

Table 3: Distribution of ESBL-Producing Isolates by Sample Type (n = 108)

Sample Type	Number (n)	Percentage (%)
Urine	40	37.0
Sputum	25	23.1
Blood	22	20.4
Pus	13	12.0
Sterile body fluids	7	6.5
Endotracheal aspirate	1	0.9

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This table illustrates the distribution of ESBL-producing isolates across different clinical sample types.

Overall, ESBL-producing pathogens were predominantly associated with urinary and respiratory tract infections, with a notable proportion also isolated from bloodstream infections, underscoring their clinical significance in both community and hospital settings.

DISCUSSION

The present study demonstrated a culture positivity rate of 33.4%, which is consistent with recent reports from tertiary care settings in India and other low- and middle-income countries, where positivity rates range between 30% and 50%.[11-12] A high proportion of samples (66.6%) yielded no growth or were contaminated, which may be attributed to prior empirical antibiotic use, improper sampling techniques, or delays in specimen transport—factors commonly reported in resource-limited settings.[13-14]

Among the isolates, Enterobacterales constituted a significant proportion (12.5%), with 50.7% demonstrating ESBL production, highlighting a considerable burden of antimicrobial resistance. This prevalence aligns with recent surveillance data from India and other South Asian countries, where ESBL rates among Enterobacterales have been reported between 40% and 70%.[15-16] Globally, however, ESBL prevalence varies considerably, with lower rates reported in high-income countries such as the United States and parts of Europe (10%–25%), and higher rates in Asia, Africa, and Latin America (30%–70%).[17-18] This disparity reflects differences in antibiotic stewardship practices, infection control measures, regulatory policies, and over-the-counter availability of antibiotics. The relatively higher prevalence in India highlights the urgent need for strengthened antimicrobial stewardship and regulatory interventions.

Species-wise analysis revealed that *Escherichia coli* (54.6%) was the predominant ESBL-producing organism, followed by *Klebsiella* spp. (29.6%). This finding is consistent with multiple recent studies identifying *E. coli* as the leading ESBL producer in both community and hospital settings.[16,19-21] The predominance of *E. coli*, especially in urine samples, suggests a strong association with community-acquired infections such as urinary tract infections, while the predominance of *Klebsiella* spp. in hospital-associated infections further underscores its role in nosocomial transmission and its association with invasive procedures and prolonged hospitalization.[22]

The distribution of ESBL-producing isolates between IPD (56.5%) and OPD (43.5%) was not statistically significant ($p = 0.178$), indicating a comparable burden of ESBL organisms in both hospital and community settings. This observation is particularly concerning, as it reflects the spillover of resistant strains from healthcare facilities into the community, often linked to prior antibiotic exposure, self-medication, and environmental reservoirs.[23-24] The absence of a significant difference also suggests that empirical therapy protocols should consider local resistance patterns in both settings.

With respect to sample type, the majority of ESBL-producing isolates were recovered from urine (37.0%), followed by sputum (23.1%) and blood (20.4%). Similar trends have been reported in multiple studies, emphasizing the role of ESBL-producing organisms in urinary tract infections as well as severe invasive infections such as bacteremia and pneumonia.[20,25] The presence of ESBL producers in blood and respiratory samples is clinically significant, as these infections are associated with higher morbidity, mortality, and healthcare costs.[26]

The detection of ESBL production in less common organisms such as *Citrobacter*, *Enterobacter*, and *Morganella morganii* further highlights the horizontal dissemination of resistance genes, particularly plasmid-mediated β-lactamases, across different genera.[27] This genetic mobility contributes to the rapid spread of resistance and limits therapeutic options.

Furthermore, the co-selection of resistance due to antibiotic pressure plays a crucial role in maintaining and amplifying ESBL-producing strains in both hospital and community environments.[28] The widespread use of third-generation cephalosporins and other broad-spectrum antibiotics has been identified as a major driver of this phenomenon.[18]

Limitation

Molecular characterization of ESBL genes was not performed; therefore, the specific genetic mechanisms underlying ESBL production and their clonal dissemination could not be determined.

The detailed patient-level clinical data (such as prior antibiotic exposure, co-morbidity, duration of hospitalization, and clinical outcomes) were not analyzed, which limited the ability to identify risk factors associated with ESBL infections.

CONCLUSION

The high burden of ESBL producing Enterobacterales, indicating a substantial burden of beta-lactam resistance within the study setting and suggests that ESBL-producing organisms are not confined to hospital environments, but are also well established in the community. This pattern reflects a shifting epidemiology where resistance is increasingly encountered at the point of first clinical contact.

Overall, the findings reinforce the importance of context-specific microbiological surveillance and evidence-based antibiotic policies, particularly in settings with high empirical antibiotic use. Strengthening diagnostic stewardship and aligning empirical therapy with local resistance patterns will

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be critical to improving clinical management and further curb the spread of resistant pathogens.

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