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ORIGINAL ARTICLE

Prevalence of Extended-Spectrum Beta-Lactamase Genes among Escherichia Coli and Klebsiella Pneumniae Clinical Isolates

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ABSTRACT

Background: Extended-spectrum beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae have become increasingly common in healthcare settings, posing a major threat to effective antibiotic therapy. These organisms can resist multiple classes of antibiotics, leading to limited treatment options and increased healthcare burdens. This study aimed to determine the prevalence of ESBL genes 'CTX-M, TEM, and SHV among clinical isolates of E. coli and K. pneumoniae' collected from a tertiary care hospital in Peshawar, Pakistan.

Methods: A cross-sectional study was conducted from January to June 2023 in the Department of Microbiology, Hayatabad Medical Complex, Peshawar. A total of 110 non-duplicate clinical isolates of E. coli and K. pneumoniae were processed. 'Phenotypic screening for ESBL production was done using the combination disk method'. Polymerase chain reaction (PCR) was performed to detect CTX-M, TEM, and SHV genes in phenotypically positive isolates. 'Statistical analysis was conducted using SPSS version 25, and associations were assessed using the chi-square test'.

Results: From a total of 110 isolates, 60% were found to be ESBL positive. The most prevalent was CTX-M (69.1%), followed by TEM (47.3%) and SHV (38.2%). Many of the isolates did contain several genes. The presence of prior antibiotic therapies, indwelling catheters, and being in an ICU was significantly correlated with having an ESBL gene ($p < 0.05$).

Conclusion: The high prevalence of ESBL genes, particularly CTX-M, highlights the urgent need for molecular surveillance and stricter antibiotic stewardship in clinical practice.

Keywords: ESBL, E. coli, K. pneumoniae, CTX-M, TEM, SHV, antibiotic resistance, PCR, Pakistan

INTRODUCTION

The emergence of Extended-Spectrum Beta-Lactamases (ESBL) has profoundly deepened the problems associated with modern medicine, especially in hospitals. 'ESBL-synthesising gram-negative bacteria like E. coli and Klebsiella pneumoniae are able to outsmart commonly circulated beta-lactam antibiotics such as penicillins and cephalosporins, rendering them useless'[1]. Subsequently, the number of treatment choices available for infections linked with these organisms is becoming more and more restricted. This will result in increased time spent recovering from these infections, increases health costs, and spike mortality rates[2,3].

'E. coli and K. pneumoniae are among the most frequently isolated gram-negative bacteria that serve as pathogens for urinary tract infections, bloodstream infections, pneumonia, and wound infections'. The emergence of community-associated strains of ESBL producing bacteria has been facilitated by the indiscriminate use of broad-spectrum antibiotics in both hospital and community settings (4, 5). These pathogens typically possess numerous resistance genes located on mobile genetic vectors, thereby enabling the rapid spread of resistance traits throughout healthcare systems.

Molecular investigations in the last few years have discovered many families of ESBL genes, including 'CTX-M, TEM, and SHV' which are noteworthy in the context of resistance in these pathogens. Although it is now accepted that CTX-M is the most prevalent ESBL type found in the world, these genes tend to differ considerably by region, bacterial species, and healthcare traditions. Determining the genetic makeup and local frequency of ESBL-producing isolates plays a major role in formulating precise strategies to manage infections and determining guidelines for empirical antibiotic treatment[6].

Even with the rising awareness of antimicrobial resistance, there is scarce information from this area of Pakistan in relation to the molecular 'characterisation of ESBL genes in clinical isolates of E. coli and K. pneumonia'. Literature has primarily focused on its phenotypic detection, thereby broadening the gap within the specific genetic underpinnings of the resistance.

This study addressed this gap by identifying and comparing the prevalence of ESBL genes specifically CTX-M, TEM, and SHV in E. coli and K. pneumoniae isolates obtained from various clinical specimens at a tertiary care center. By combining phenotypic detection with molecular analysis, this research aims to provide a clearer picture of resistance patterns and contribute to more effective infection management strategies in local healthcare settings.

METHODOLOGY

This cross-sectional study was conducted at the Department of Microbiology, Hayatabad Medical Complex, Peshawar, from January 2023 to June 2023. It was designed to estimate the frequency of 'extended-spectrum beta-lactamase (ESBL) genes in clinical isolates of Escherichia coli and Klebsiella pneumoniae'.

A non-probability consecutive sampling technique was employed. All 'clinical samples received in the microbiology laboratory during the study period, from which either E. coli or K. pneumoniae was isolated, were considered for inclusion'. Only one isolate per patient was included to avoid duplication. Samples with mixed growth or contamination were excluded.

Inclusion criteria Clinical samples (urine, blood, pus, sputum, etc.) yielding E. coli or K. pneumonia. Both inpatients and outpatients. Patients of all age groups and both sexes

Exclusion criteria Polymicrobial growth. Repeated isolates from the same patient. Incomplete patient data or lost isolates

One hundred and ten clinical isolates of E. coli and K. pneumoniae were confirmed and collected throughout the study period. They were categorized according to established procedures of microbiological identification.

All specimens were collected using standard aseptic techniques and processed according to routine microbiological protocols. Culture media included blood agar and MacConkey agar. Organism identification was performed using colony morphology, Gram staining, biochemical testing (e.g., indole, citrate, urease, TSI, and motility), and confirmed with API 20E identification strips when needed.

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All isolates underwent antimicrobial susceptibility testing using the modified Kirby-Bauer disk diffusion method as per CLSI guidelines (Clinical and Laboratory Standards Institute). Antibiotic disks tested included ceftazidime, cefotaxime, ceftriaxone, and cefpodoxime. ESBL screening was initially done phenotypically using combination disk methods (ceftazidime and ceftazidime-clavulanic acid).

Isolates that tested positive for ESBL production phenotypically were further subjected to molecular analysis. DNA was extracted using the boiling method, and PCR amplification was carried out 'to detect blaCTX-M, blaTEM, and blaSHV genes'. The thermal cycler was programmed for specific gene primers, and PCR products were visualized using agarose gel electrophoresis under UV light.

A structured data collection form was used to document demographic information (age, sex, hospital ward, residence), clinical history (comorbidities, antibiotic use, hospitalization history), specimen type, phenotypic resistance patterns, and the presence of ESBL genes. 'The presence or absence of individual genes (CTX-M, TEM, SHV) and their combinations were also recorded'.

To ensure reliability, trained personnel used standard operating protocols to carry out all culture and sensitivity testing procedures. PCR assays were run with both positive and negative controls to ensure accuracy. All results were reviewed independently by two microbiologists. Duplicate testing was done randomly for 10% of the isolates to ensure reproducibility.

The data were entered and analyzed using SPSS version 25. Descriptive statistics such as frequency and percentages were used for categorical variables. The chi-square test assessed associations between variables like ESBL gene presence and clinical factors. A p-value of less than 0.05 was considered statistically significant.

RESULT

Most patients in this study belonged to the 20–40-year age group, comprising nearly one-third of the total sample. A statistically significant relationship was observed between age groups and ESBL prevalence, with higher rates among middle-aged and elderly individuals (p=0.041), suggesting age-related risk. Gender distribution was fairly balanced, with a slightly higher proportion of male patients, 'although the difference was not statistically significant (p=0.316)'. Urban residents accounted for a larger portion of the sample, but no significant association with ESBL was noted (p=0.109). Interestingly, patients admitted to the inpatient department (IPD) showed a significantly higher likelihood of ESBL-positive isolates (p=0.032), indicating that prolonged or repeated healthcare exposure may increase the risk of resistant infections.

Table 1: Demographic Characteristics of Patients (n=110)

Variable	Category	Frequency (%)	p-value
Age Group	<20 years	18 (16.4%)	
	20–40 years	36 (32.7%)	
	41–60 years	30 (27.3%)	
	>60 years	26 (23.6%)	0.041*
Sex	Male	60 (54.5%)	
	Female	50 (45.5%)	0.316
Residence	Urban	68 (61.8%)	
	Rural	42 (38.2%)	0.109
Ward Type	ICU	22 (20.0%)	
	OPD	34 (30.9%)	
	IPD	54 (49.1%)	0.032*

Among clinical factors, prior use of antibiotics was highly prevalent (70.9%) and showed a strong association with ESBL positivity (p=0.001), emphasizing the role of antimicrobial misuse in resistance development. Nearly half of the patients had no comorbidities, but diabetes and immunosuppression were frequent among those with ESBL-producing infections, and the association was statistically significant (p=0.018). Indwelling devices such as catheters were significantly associated with ESBL infections (p=0.027), suggesting that invasive interventions may provide a route for colonization by resistant organisms. Though prior hospitalization within the last three months approached significance, it did not meet the statistical threshold (p=0.089).

Table 2: Clinical Characteristics of Patients

Variable	Category	Frequency (%)	p-value
Prior Antibiotic Use	Yes	78 (70.9%)	
	No	32 (29.1%)	0.001*
Comorbidities	Diabetes Mellitus	28 (25.5%)	
	CKD	12 (10.9%)	
	Immunocompromised	16 (14.5%)	
	None	54 (49.1%)	0.018*
Indwelling Devices	Present	44 (40.0%)	
	Absent	66 (60.0%)	0.027*
Previous Hospitalization	Yes	36 (32.7%)	
	No	74 (67.3%)	0.089

E. coli was the more frequently isolated organism, accounting for over half of the isolates, followed by K. pneumoniae. A statistically significant difference was found between species distribution and ESBL positivity (p=0.003), with K. pneumoniae being more strongly linked with ESBL gene presence. 'Urine was the most common specimen type, followed by sputum and blood. ESBL production was notably higher in samples from blood and sputum, reflecting their association with more severe infections (p=0.014)'. Overall, 60% of all isolates tested positive for phenotypic ESBL activity, and this proportion was significantly higher than expected by chance (p<0.001).

Table 3: Microbiological Profile of Isolates

Variable	Category	Frequency (%)	p-value
Bacterial Species	E. coli	64 (58.2%)	
	K. pneumoniae	46 (41.8%)	0.003*
Sample Type	Urine	40 (36.4%)	
	Blood	24 (21.8%)	
	Pus	20 (18.2%)	
	Sputum	26 (23.6%)	0.014*
ESBL Phenotype Positive	Yes	66 (60.0%)	
	No	44 (40.0%)	<0.001*

Among 'ESBL-positive isolates, CTX-M was the most frequently detected gene, present in 69.1% of cases, with no significant difference between the two organisms (p=0.374)'. SHV was found 'significantly more often in K. pneumoniae isolates (p<0.001), aligning with prior literature highlighting SHV's predominance in this species'. TEM gene presence was similar across both bacteria (p=0.918). Notably, 'almost half the isolates harbored more than one ESBL gene, and this pattern was significantly more common in K. pneumoniae than in E. coli (p=0.031)' which might explain its higher resistance profile.

Table 4: Distribution of ESBL Genes among Isolates

ESBL Gene Type	Detected in E. coli (n=64)	Detected in K. pneumoniae (n=46)	Total (n=110)	p-value
CTX-M	42 (65.6%)	34 (73.9%)	76 (69.1%)	0.374
TEM	30 (46.9%)	22 (47.8%)	52 (47.3%)	0.918
SHV	14 (21.9%)	28 (60.9%)	42 (38.2%)	<0.001*
Multiple Genes	28 (43.8%)	26 (56.5%)	54 (49.1%)	0.031*

Several clinical factors showed strong associations with the presence of ESBL genes. Patients with prior antibiotic exposure were significantly more likely to harbor ESBL-producing organisms (p<0.001), reaffirming the risks of empirical and repeated antibiotic use. ICU admissions were also associated with higher rates of ESBL positivity (p=0.012), likely due to longer hospital stays and invasive interventions. Similarly, comorbid conditions (p=0.006) and the use of indwelling devices (p=0.022) were significant predictors of ESBL gene carriage, highlighting the importance of antimicrobial stewardship and infection control measures.

Table 5: Association between ESBL Gene Presence and Risk Factors

Risk Factor	ESBL Gene Present (n=66)	ESBL Gene Absent (n=44)	p-value
Prior Antibiotic Use	58 (87.9%)	20 (45.5%)	<0.001*
ICU Admission	18 (27.3%)	4 (9.1%)	0.012*
Comorbid Conditions	36 (54.5%)	12 (27.3%)	0.006*
Indwelling Devices	32 (48.5%)	12 (27.3%)	0.022*

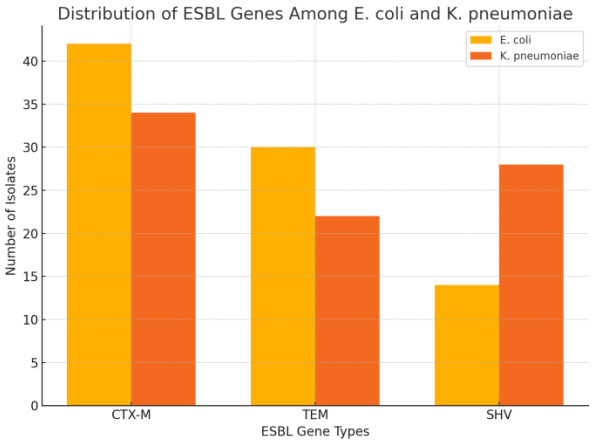

Figure 1: The graph shows how common three ESBL genes—CTX-M, TEM, and SHV are in E. coli and K. pneumoniae isolates. 'In E. coli, CTX-M was most common followed by TEM, whereas SHV was the least detected'. This suggests CTX-M is the dominant gene type in E. coli. In K. pneumoniae, CTX-M was also the most common, but SHV was detected more often than TEM. This pattern shows that SHV is more closely linked to K. pneumoniae than E. coli. The graph highlights that CTX-M is widely present in both species, while SHV is especially prominent in 'K. pneumoniae'. These differences highlight the importance of monitoring ESBL genes by bacterial type to guide effective treatment.

DISCUSSION

This study highlights the significant burden of ESBL-producing E. coli and K. pneumoniae in clinical settings, with a phenotypic ESBL prevalence of 60% among the isolates. 'These findings were consistent with regional and international trends, where the rise of multidrug-resistant gram-negative organisms has emerged as a major public health concern' [7-9]. The predominance of 'E. coli as this study's most frequently isolated species reflects its well-established role as a leading cause of urinary and systemic infections'. However, K. pneumoniae showed a higher association with certain ESBL genes, particularly SHV, indicating species-specific genetic profiles.

Among the genes analyzed, CTX-M was the most prevalent, detected in over two-thirds of all isolates. This pattern aligns with studies from various parts of Asia and Europe where CTX-M has become the dominant ESBL gene due to its rapid dissemination through mobile genetic elements[10-12]. Studies reported similar findings in Norwegian clinical isolates, highlighting the global reach of CTX-M–mediated resistance[13, 14]. TEM and SHV genes were also identified, with SHV being significantly more common in K. pneumoniae, consistent with earlier studies reported SHV's ancestral link with this species[15,16].

Our study's association between prior antibiotic use and ESBL production was statistically significant. This supports evidence from multiple studies that show irrational and repeated use of broad-spectrum antibiotics as a key driver of resistance. ICU admissions and the presence of indwelling devices such as urinary catheters and central lines were also associated with increased ESBL gene carriage, likely due to prolonged exposure to healthcare environments and biofilm formation[17-19].

Interestingly, nearly half of the isolates in our study harbored multiple ESBL genes, a finding that raises concern regarding treatment options. Co-carriage of genes like CTX-M and TEM or CTX-M and SHV suggests the potential for extensive resistance, limiting the efficacy of commonly used beta-lactam antibiotics and increasing reliance on carbapenems and colistin[20-22].

A few constraints must be taken into account. The research was performed in one tertiary care center, which might not be representative of community practices. The molecular analysis was restricted to the three most frequent ESBL genes, and other mechanisms of resistance such as AmpC or carbapenemases were not evaluated. Furthermore, the clinical outcomes of patients with infections were not monitored, which may have elucidated the role of resistance in morbidity and mortality.

Even with these shortcomings, the research contributes important understanding of the molecular profile of ESBL-producing microbes in northern Pakistan. It highlights the importance of continuous monitoring and strict policies regarding the use of antibiotics.

CONCLUSION

This research supports the 'dominant prevalence of ESBL producing E.coli and K. pneumoniae at a tertiary care facility with CTX-M emerging as the most prevalent gene'. Identifying multiple ESBL genes in almost fifty percent of the isolates is concerning and shows signs of an increasing resistance problem. The correlation of ESBL production with the previous uses of antibiotics, admission to ICU, and presence of invasive devices underlines the need for stronger infection control policies and more judicious use of antibiotics in hospitals. These results highlight the need for molecular screening, surveillance programs, and an integrated plan to control antimicrobial resistance at healthcare institutions and on a national scale.

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