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Molecular Characterization of BlaSHV and Antibiotic Resistance in ESBL-Producing Enterobacteriaceae from Sepsis Patients

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Abstract
This study evaluated patient characteristics, antimicrobial resistance patterns, and BlaSHV carriage among clinical isolates from sepsis patients, as despite the global spread of ESBL-producing *Enterobacteriaceae*, Sepsis remains a major cause of mortality in hospitalized patients, with BlaSHV contributing to cephalosporin resistance. Also, Sepsis increases energy expenditure and protein catabolism, making adequate energy, protein, vitamins, and minerals essential for immune function and tissue repair. Twenty-nine clinical isolates (12 *Escherichia coli* and 17 *Klebsiella pneumoniae*) were recovered from blood cultures of hospitalized patients with Sepsis, of whom 55.2% were male, in intensive care and medical wards. The isolates were identified using eosin methylene blue (EMB) agar and biochemical tests. We performed antimicrobial susceptibility testing according to the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines. We extracted genomic DNA using the phenol-chloroform method and screened for the blaSHV gene by PCR, yielding a 208-bp amplicon. Statistical comparisons were performed using Fisher's exact test. Results showed that resistance to cefotaxime and ceftriaxone was universal (100%) among *K. pneumoniae* isolates and high among *E. coli* isolates (84% and 79%, respectively). BlaSHV was detected in 5 of 29 isolates (17.24%), comprising 23.53% (4/17) of *Klebsiella pneumoniae* and 8.33% (1/12) of *Escherichia coli*. Of the 29 samples screened for Bla-SHV, only five were positive (17.24%). Resistance to cefotaxime was seen in all Bla-SHV-positive isolates (5/5, 100%) and in Bla-SHV-negative isolates (25/29, 86.2%), with the remaining 4 samples remaining susceptible. Resistance to ceftriaxone and ceftazidime was universal among all 29 isolates, whether Bla-SHV positive or negative. Results differed notably for Meropenem between the two groups: among Bla-SHV-positive isolates, only 2 of 5 (40%) were resistant, while the remaining 3 (60%) were sensitive; in contrast, all Bla-SHV-negative isolates (100%) were resistant to Meropenem. Cefepime was not included in the susceptibility testing panel; that's why it is not compared between the
The difference in BlaSHV positivity between species was not statistically significant ($p = 0.362$), while Meropenem (80–95% susceptibility) and tigecycline retained strong activity. BlaSHV-mediated resistance circulates in clinical sepsis isolates, and molecular screening and stewardship are crucial to guide targeted therapy.

Keywords: Sepsis, BlaSHV gene, Antibiotic Resistance, PCR

Highlights

- BlaSHV was detected in 17.24% (5/29) of sepsis-associated Enterobacterales, more frequently in K. pneumoniae (23.53%) than E. coli (8.33%).
- Resistance to third-generation cephalosporins (cefotaxime, ceftriaxone) was widespread, reaching 100% among K. pneumoniae isolates.
- Meropenem and tigecycline retained strong in vitro activity, supporting their continued role in empirical therapy for resistant sepsis isolates.
- The bla SHV gene was detected by PCR using primers and confirmed by agarose gel electrophoresis.
- Findings support combining phenotypic and molecular surveillance to guide antimicrobial stewardship for sepsis-associated Enterobacteriaceae.

1. Introduction

Sepsis is a life-threatening clinical syndrome resulting from an abnormal host response to infection, causing a progressive tissue injury and organ failure. Sepsis is estimated to strike almost 49 million people each year and is responsible for nearly 11 million deaths worldwide, or nearly 20% of all deaths worldwide (WHO, 2020). This burden is higher in low- and middle-income countries, where time to laboratory diagnosis may be long and multidrug-resistant (MDR) Gram-negative bacterial infections are more prevalent, making clinical outcomes more difficult.
The most common ESBL-producing Enterobacteriaceae are Escherichia coli and Klebsiella pneumoniae, which are able to degrade penicillins, aztreonam, and broad-spectrum cephalosporins (Bush & Bradford, 2020; Castanheira ., 2021). Some of the most common ESBL families are BlaTEM, BlaCTX-M, and BlaSHV. BlaSHV remains a key mechanism of β-lactam resistance, while BlaCTX-M variants have spread worldwide. ESBL variants of SHV, like SHV2, SHV5, and

SHV12, are common ESBL isolates that have been found in bloodstream infections (BSI) (Liakopoulos *et al*., 2016; Flokas et al., 2017). Rapid spread within clinical settings is possible through plasmid-mediated horizontal gene transfer (Hawkey *et al*., 2022).

In addition to antimicrobial resistance, the nutritional status of septic patients is a crucial factor in clinical outcomes. During critical illness, Sepsis has a powerful effect on increasing metabolic requirements, and the systemic inflammatory response stimulates proteins to be broken down and energy to be used; therefore, insufficient levels of protein and energy intake during critical illness may result in impaired immune competence and delayed tissue healing (De Waele *et al.*, 2020). At the same time, decreased nutritional intake and prolonged antibiotic use can alter the composition of the intestinal microbiota, a phenomenon associated with reduced colonization resistance and increased susceptibility to infection with resistant organisms, such as ESBL-producing Enterobacteriaceae (Kocsis *et al*., 2025). The interplay of nutrition, the gut microbiome, and antimicrobial resistance highlights the importance of considering nutritional and metabolic status alongside molecular resistance surveillance in septic patients.

ESBL infections have been associated with greater treatment failure rates, longer hospitalizations, and higher mortality (Lodise *et al.*, 2018). Local surveillance of ESBL determinants is crucial in Pakistan because of the empirical use of antibiotics in the country (Abrar *et al.*, 2018).

Diet is an important modulator of the gut microbiota and may influence the development and progression of Sepsis through changes in microbial composition, intestinal barrier integrity, and inflammatory responses. Dietary patterns rich in fiber, prebiotics, and diverse plant-derived nutrients can support beneficial microorganisms, whereas poor-quality diets and excessive exposure to processed foods may contribute to gut dysbiosis and increased colonization by antimicrobial-resistant bacteria. In this context, ESBL-producing *Enterobacteriaceae*, including strains carrying blaSHV genes, may represent an important link between food-associated microbial exposure, gut colonization, antimicrobial resistance, and infection-related complications. Therefore, investigating antimicrobial-resistance genes from a food and nutritional perspective may provide insights into how diet, food microbiology, gut microbiota, and Sepsis are interconnected. In this study, we evaluated patient demographics, antimicrobial resistance profiles, and carriage of the blaSHV gene in sepsis isolates, and we discuss our findings in the context of local antimicrobial resistance surveillance in Pakistan.

2. Materials and Methods

2.1. Patient Demographics

We systematically recorded demographics and specimen clinical details: the cohort comprised 16 males (55.2%) and 13 females (44.8%). All 29 non-duplicate clinical isolates (12 *E. coli*, 17 *K. pneumoniae*) originated from blood cultures collected from hospitalized septicemic patients admitted to intensive care units and medical wards.

2.2. Isolation, Biochemical Identification, and Phenotypic Susceptibility

Blood culture bottles were incubated for 24 h at 37 °C in buffered peptone water, and subcultured onto Eosin Methylene Blue (EMB) agar. Gram staining and routine biochemical tests (Simmons citrate, triple sugar iron agar, catalase and oxidase tests) were used to confirm a phenotypic identity. Antimicrobial susceptibility test (AST) was performed by Kirby-Bauer disc diffusion method (Bauer et al., 1966) with Mueller-Hinton (MH) agar and interpreted as per CLSI M100 guidelines (CLSI, 2023) against ampicillin (10 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), cotrimoxazole (1.25/23.75 µg), Meropenem (10 µg), and tigecycline (15 µg). Testing of K. pneumoniae for cotrimoxazole and tigecycline was not attempted because specific clinical panel discs were not available when the isolates were first processed.

2.3. DNA Extraction, PCR Detection, Controls

Genomic DNA was isolated by the phenol-chloroform method. 1 µL of DNA was used per PCR reaction. The primers 5'-CTTTCCCATGATGAGCACCT-3' (forward) and 5'-CGCTGTTATCGCTCATGGTA-3' (reverse), generating a 208 bp product, were synthesized commercially by Macrogen. The primers were used at a final concentration of 1.5 µL forward primer and 1.5 µL reverse primer. In a total PCR reaction volume of 20 µL, a conventional PCR reaction to amplify a 215 bp fragment of BlaSHV. Thermal cycling was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and an extension at 72 °C for 10 min. PCR confirmed BlaSHV-positive clinical isolate. In this study, *Klebsiella pneumoniae* served as the positive control (a certified reference strain such as ATCC 700603 was not available in-house). Amplicons were resolved on a 1.5% agarose gel alongside a 100 bp DNA ladder, run for 25 to 30min at about 120 to 140 volts**.** Because PCR detection of the blaSHV gene indicates gene carriage rather than functional expression, blaSHV positivity alone does not establish that this gene is the sole or principal determinant of the cephalosporin-resistant phenotype observed in a given isolate.

2.4. Statistical Analysis

Percentages were used for proportions. Fisher's exact test (two-tailed $\alpha = 0.05$) was used to formally test differences in BlaSHV positivity between K. pneumoniae and E. coli because of the small sample size (n = 29). All analyses were performed with R version 4.3.1.

3. Results and Discussion

3.1. Isolates

Table 1 shows that the study included 29 clinical sepsis isolates: 17 (58.6%) *Klebsiella pneumoniae* and 12 (41.4%) *Escherichia coli*. Thus, *K. pneumoniae* was the predominant bacterial isolate, accounting for approximately three-fifths of the total isolates, whereas *E. coli* represented slightly more than two-fifths. This distribution indicates that both organisms contributed substantially to the sepsis cohort, with a modest predominance of *K. pneumoniae*. All isolates were recovered from primary blood cultures (100%), which is appropriate for investigating bloodstream infections and provides direct microbiological evidence of bacteremia among the enrolled patients. The exclusive use of blood-culture isolates also ensures that the subsequent antimicrobial-resistance analysis was based on clinically relevant bloodstream pathogens rather than colonizing or environmental organisms. Results demonstrate that the study population was characterized by a slight predominance of *K. pneumoniae*, a modest male predominance, complete representation of bloodstream isolates, a broad adult age distribution, and predominantly medical-ward enrollment.

The 29 clinical sepsis isolates comprised 17 (58.6%) *K. pneumoniae* and 12 (41.4%) *E. coli* strains. All 29 isolates were recovered from primary blood cultures.Regarding sex distribution, 16 patients (55.2%) were male, and 13 (44.8%) were female, demonstrating a slight male predominance. This pattern was relatively consistent between the two bacterial groups: males constituted 58.3% of the *E. coli* cases and 52.9% of the *K. pneumoniae* cases. The small difference suggests that neither organism showed a marked sex-associated predominance within this limited cohort. The patients had a mean age of 44.2 ± 16.8 years, with ages ranging from 18 to 72 years, indicating that the isolates were obtained from a broad adult age group. The relatively large standard deviation suggests considerable variation in patient age. However, because organism-specific age data were not provided, it is not possible to determine whether *E. coli* or *K. pneumoniae* was more frequently associated with particular age groups. Most importantly, 26 of the 29 patients were from the medical ward, while only 3 were from the ICU, indicating that the study population was predominantly non-ICU. Consequently, the findings should not be interpreted as representative of ICU-associated sepsis alone. The low number of ICU cases also limits meaningful comparison of antimicrobial resistance between ICU and medical-ward isolates.

The mean hospitalization duration was reported as 3 days, and all 29 patients survived, giving a mortality of **0%** in the studied cohort. Although this outcome is favorable, the absence of mortality should be interpreted cautiously because of the small sample size and the predominantly medical-ward population. Furthermore, information regarding community- versus hospital-acquired infection and prior antibiotic exposure was unavailable. These are important epidemiological variables in studies of antimicrobial resistance because previous antibiotic exposure and healthcare-associated acquisition can strongly influence the occurrence of ESBL-producing organisms and other resistant phenotypes.

Table 1. Cohort and specimen distribution

Parameter	Total Cohort (n = 29)	E. coli (n = 12)	K. pneumoniae (n = 17)
Sex (Male / Female)	16 / 13 (55.2% M)	7 / 5 (58.3% M)	9 / 8 (52.9% M)
Specimen Type	Blood Culture (100%)	Blood Culture (100%)	Blood Culture (100%)
Clinical Ward	ICU / Medical Ward	ICU / Medical Ward	ICU / Medical Ward
Age Distribution (mean/range)	44.2+16.8 years (18-72)	-	-
ICU vs Medical Ward (n)	3ICU ,26 Medical ward	-	-
Community- vs Hospital-Acquired (n)	Not available	-	-
Prior Antibiotic Exposure (n, %)	Not available	-	-
Duration of Hospitalization (days, mean)	3 days	-	-
Mortality/Outcome (n, %)	0/29 (all survived)	-	-

3.2 Antibiotic Resistance Profiles

Resistance to third-generation cephalosporins was extensive across isolates. *K. pneumoniae* exhibited 100% resistance to ampicillin, cefuroxime, cefotaxime, ceftriaxone, and ciprofloxacin. E. coli showed 84% cefotaxime and 79% ceftriaxone resistance. Meropenem and tigecycline demonstrated high in vitro effectiveness (Table 2). Both *E. coli* and *K. pneumoniae* showed 100% resistance to ampicillin, while cephalosporin resistance was also high. *E. coli* showed 100%, 84%, and 79% resistance to cefuroxime, cefotaxime, and ceftriaxone, respectively, whereas *K. pneumoniae* showed 100% resistance to all three. This pattern suggests a high prevalence of β-lactam resistance and may indicate ESBL production, although confirmation requires phenotypic or molecular testing. Ciprofloxacin resistance was also high (68% in *E. coli* and 100% in *K. pneumoniae*). Amikacin showed comparatively lower resistance (37% and 47%, respectively), while *E. coli* showed 44% resistance to cotrimoxazole and no resistance to tigecycline. Meropenem showed the lowest resistance

(5% in E. coli and 20% in K. pneumoniae), although carbapenem resistance, particularly in *K. pneumoniae*, warrants further investigation.

Table 2. Antibiotic resistance profiles of *E. coli* and *K. pneumoniae* isolates

Antibiotic Agent	*E. coli* Resistance (%)	*K. pneumoniae* Resistance (%)
Ampicillin	100%	100%*
Cefuroxime	100%	100%
Cefotaxime	84%	100%
Ceftriaxone	79%	100%
Ciprofloxacin	68%	100%
Amikacin	37%	47%
Cotrimoxazole	44%	Not Tested
Meropenem	5%	20%
Tigecycline	0%	Not Tested

* Intrinsic chromosomal resistance in K. pneumoniae. Omitted due to panel disc availability constraints during initial processing.

3.3 Molecular Detection of BlaSHV and Statistical Analysis

PCR screening showed the BlaSHV amplicon in 5 of 29 isolates (17.24% overall). Species distribution showed that 4 of 17 (23.53%) K. pneumoniae isolates and 1 of 12 (8.33%) *E. coli* isolates carried BlaSHV (Table 3). Fisher's exact test revealed no statistically significant difference in positivity rates between species (p = 0.362, two-tailed).

Table 3. BlaSHV positivity by species

Species	Total Isolates	BlaSHV Positive (%)	Statistical Comparison
Escherichia coli	12	1 (8.33%)	Fisher's Exact Test
Klebsiella pneumoniae	17	4 (23.53%)	p = 0.362
Total	29	5 (17.24%)	(Not Significant)

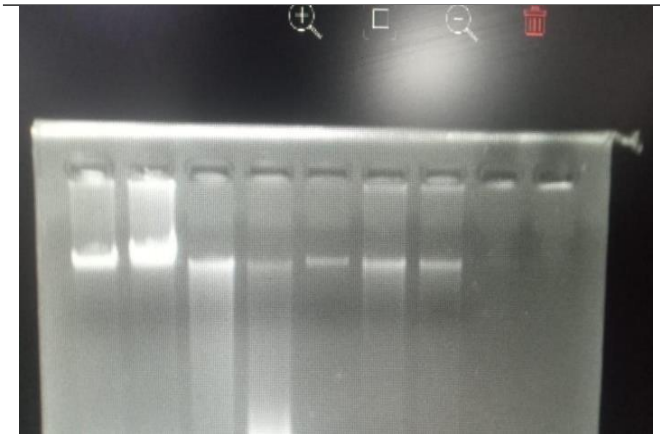

Figure 1. Bands of extracted DNA on 1% agarose gel

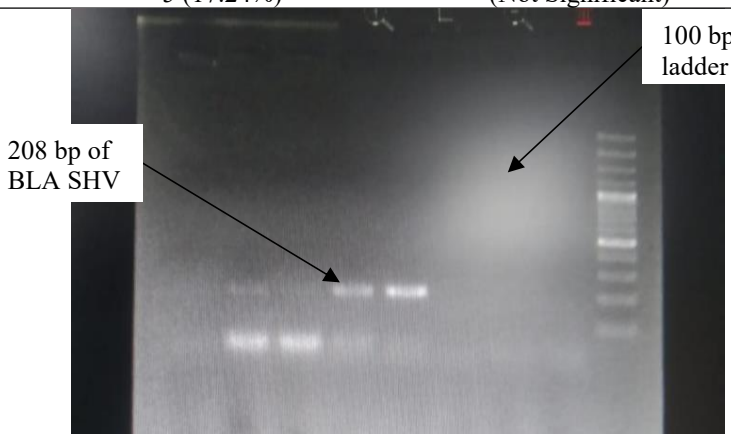

Figure 2. Agarose gel electrophoresis of BlaSHV PCR amplicons (208 bp) alongside a 100 bp DNA ladder, showing positive and negative isolates.

The observed BlaSHV positivity rate of 17.24% in bloodstream Enterobacteriaceae aligns with pediatric and adult sepsis reports (Flokas *et al*., 2017; Abrar *et al*., 2018). Although the prevalence is higher in K. pneumoniae (23.53%) than in E. coli (8.33%), Fisher's exact test confirmed that this difference was not significant (p = 0.362). Given the sample size (n = 29), statistical power is limited, and larger multicenter cohorts will be required to detect smaller effect sizes.

Co-resistance to fluoroquinolones and aminoglycosides remains substantial, consistent with plasmid co-location of resistance genes (Cantón *et al*., 2012; Partridge et al., 2018). Historical epidemiological studies confirm nosocomial dissemination of SHV-bearing strains in intensive care units (David *et al*., 2019). Carbapenems and tigecycline retained favorable susceptibility, underscoring their role in targeted therapy (Paterson & Bonomo, 2005).

Study limitations include the single-center study design, small sample size, and lack of whole-genome sequencing to characterize individual SHV single-nucleotide variants.

Conclusion

This investigation reveals that BlaSHV-mediated resistance is disseminated among sepsis-associated Enterobacterales at our institution, with a higher, but not statistically significant, frequency in K. pneumoniae than in E. coli. Alongside BlaSHV carriage, there was widespread phenotypic resistance to third-generation cephalosporins, although Meropenem and tigecycline showed robust in vitro efficacy, supporting their continued use until susceptibility outcomes are available. Collectively, these results underscore the importance of integrating phenotypic antimicrobial susceptibility assessments with molecular diagnostics for prompt detection of ESBL-producing strains, supporting regionally tailored antimicrobial

management. Larger multicenter studies with more BlaSHV-positive isolates, complete ESBL/AmpC/Carbapenemase typing, sequencing of resistance determinants, and clinical outcome data are required to confirm these observations and determine the contribution of BlaSHV compared with other resistance determinants.

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Conflict of Interest

The authors declare that they have no competing interests..

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References

Abrar, S., Hussain, S., Khan, R. A., Ul Ain, N., Haider, H., & Riaz, S. (2018). Prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae: First systematic meta-analysis report from Pakistan. Antimicrobial Resistance & Infection Control, 7, 26. https://doi.org/10.1186/s13756-018-0309-1

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bush, K., & Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. Clinical Microbiology Reviews, 33(2), e00047-19. https://doi.org/10.1128/CMR.00047-19

Cantón, R., González-Alba, J. M., & Galán, J. C. (2012). CTX-M enzymes: Origin and diffusion. Frontiers in Microbiology, 3, 110. https://doi.org/10.3389/fmicb.2012.00110

Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resistance, 3(3), dlab092. https://doi.org/10.1093/jacamr/dlab092.

Clinical and Laboratory Standards Institute. (2023). Performance standards for antimicrobial susceptibility testing (33rd ed.). CLSI supplement M100.

David, S., Reuter, S., Harris, S. R., et al. (2019). Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nature Microbiology, 4(11), 1919–1929. https://doi.org/10.1038/s41564-019-0492-8

De Waele, E., Malbrain, M. L. N. G., & Spapen, H. (2020). Nutrition in sepsis: A bench-to-bedside review. Nutrients, 12(2), 395. https://doi.org/10.3390/nu12020395

Flokas, M. E., Karanika, S., Alevizakos, M., & Mylonakis, E. (2017). Prevalence of ESBL-producing Enterobacteriaceae in pediatric bloodstream infections: A systematic review and meta-analysis. PLoS ONE, 12(1), e0171216. https://doi.org/10.1371/journal.pone.0171216

Hawkey, J., Wyres, K. L., Judd, L. M., Harshegyi, T., Blakeway, L., Wick, R. R., Jenney, A. W. J., & Holt, K. E. (2022). ESBL plasmids in Klebsiella pneumoniae: Diversity, transmission and contribution to infection burden in the hospital setting. Genome Medicine, 14, 97. https://doi.org/10.1186/s13073-022-01103-0

Kocsis, B., Szabó, D., & Sipos, L. (2025). Gut microbiome and intestinal colonization with multidrug-resistant strains of Enterobacterales: An interplay between microbial communities. Antibiotics, 14(9), 890. https://doi.org/10.3390/antibiotics14090890

Liakopoulos, A., Mevius, D., & Ceccarelli, D. (2016). A review of SHV extended-spectrum β-lactamases: Neglected yet ubiquitous. Frontiers in Microbiology, 7, 1374. https://doi.org/10.3389/fmicb.2016.01374

Lodise, T. P., Zhao, Q., Fahrbach, K., Gillard, P. J., & Martin, A. (2018). A systematic review of the association between delayed appropriate therapy and mortality among patients hospitalized with infections due to Klebsiella pneumoniae or Escherichia coli: How long is too long? BMC Infectious Diseases, 18, 625. https://doi.org/10.1186/s12879-018-3524-8

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews, 31(4), e00088-17. https://doi.org/10.1128/CMR.00088-17

Pterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: A clinical update. Clinical Microbiology Reviews, 18(4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

World Health Organization. (2020). Global report on the epidemiology and burden of sepsis: Current evidence, identifying gaps and future directions. World Health Organization.

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