Prevalence and Antibiotic Resistance Patterns of Extended-Spectrum Beta-Lactamase (ESBL)-Producing Pathogens: Insights from Clinical Isolates

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KEYWORDS

ESBL, antibiotic resistance, prevalence, carbapenem, clinical isolates, public health.

ABSTRACT

This study investigates the prevalence and antibiotic resistance patterns of Extended-Spectrum Beta-Lactamase (ESBL)-producing pathogens in a regional tertiary care hospital. A retrospective analysis of 444 clinical isolates from various specimen types was conducted, focusing on resistance to commonly used antibiotics and the presence of blaTEM and blaSHV genes. The study found a 30.63% ESBL prevalence, with higher rates among the elderly and in specimens such as endotracheal secretions and cerebrospinal fluid. Imipenem (IPM) showed the highest resistance rate (45.27%), while ceftriaxone (CTR), cefotaxime (CTX), and ceftazidime (CAZ) exhibited moderate resistance (~21-25%). Gentamicin (GEN) and streptomycin (S) showed no resistance. Molecular analysis revealed a high prevalence of blaTEM and blaSHV genes, often co-occurring within the same isolates. The findings highlight the need for enhanced antimicrobial stewardship programs, continuous surveillance, and alternative therapeutic strategies to combat rising antibiotic resistance.

1. Introduction

Extended-spectrum beta-lactamase (ESBL)-producing pathogens have emerged as a major global health concern, posing significant challenges to healthcare systems due to their ability to hydrolyze third-generation cephalosporins and other beta-lactam antibiotics. These enzymes, first described in the 1980s, have been increasingly associated with multidrug-resistant infections in both hospital and community settings [1]. The prevalence of ESBL-producing organisms, particularly *Escherichia coli* and *Klebsiella pneumoniae,* has been linked to prolonged hospital stays, increased healthcare costs, and higher mortality rates [2,3]. These infections disproportionately affect vulnerable populations, including the elderly, immunocompromised patients, and individuals in intensive care units (ICUs) [4]. One of the primary concerns associated with ESBL-producing pathogens is their propensity for horizontal gene transfer, enabling the rapid dissemination of resistance genes among bacterial populations [5]. This characteristic has fueled outbreaks in healthcare facilities, particularly in regions with inadequate infection control measures or excessive antibiotic use. The clinical implications of ESBL production are profound. Patients infected with these pathogens often require treatment with carbapenems, considered the last line of defense against multidrug-resistant Gram-negative bacteria [1]. However, the increasing reliance on carbapenems raises the risk of resistance development, highlighting the urgent need for alternative therapeutic strategies and robust antimicrobial stewardship programs [2]. ESBL-producing pathogens represent a critical threat to public health, necessitating coordinated efforts to improve infection control, enhance diagnostic capabilities, and develop novel interventions to mitigate their impact.

1.1 Global Trends in Antibiotic Resistance: Antibiotic resistance is a growing global health crisis, recognized as one of the top 10 threats to public health by the World Health Organization [6]. The emergence and dissemination of resistant pathogens, particularly those producing extended-spectrum beta-lactamases (ESBLs), have rendered many commonly used antibiotics ineffective, leading to higher morbidity, mortality, and healthcare costs [7]. Globally, resistance rates to third-generation cephalosporins among *E. coli* and *K. pneumoniae* have reached alarming levels, particularly in regions with limited resources and weak antibiotic stewardship programs[8]. In low- and middle-income countries, over 60% of *E. coli* isolates exhibit resistance to third-generation cephalosporins [9]. Similarly, carbapenem resistance in *K. pneumoniae* has surged in regions like South Asia and the Mediterranean, with rates exceeding 50% in some countries [10]. A significant driver of global antibiotic resistance is the inappropriate use of antibiotics in human medicine, agriculture, and veterinary practices. For instance, countries with higher antibiotic consumption report a stronger prevalence of resistance, underscoring the critical role of stewardship and policy reforms [11]. Additionally, the global spread of resistance genes, such as those encoding ESBLs, has been facilitated by international travel, trade, and inadequate infection control measures [12]. These examples highlight the importance of coordinated global action to mitigate the antibiotic resistance crisis. Antibiotic resistance, particularly among ESBL-producing pathogens, is a complex and multifaceted issue requiring sustained global efforts. Addressing this crisis involves improved surveillance, policy reforms, public awareness, and the development of innovative therapeutics.

1.2 Study Objectives: The growing prevalence of extended-spectrum beta-lactamase (ESBL)-producing pathogens and their associated antibiotic resistance necessitate targeted studies to understand their regional dynamics. Regional datasets provide critical insights into the epidemiology of resistance, which often varies due to differences in healthcare practices, antibiotic usage patterns, and socio-economic factors [13]. Following study objectives were set for the research:

1.2.1 Evaluate ESBL Prevalence: Assess the prevalence of ESBL-producing pathogens in a healthcare setting, stratified by age group, gender, and specimen type.

1.2.2 Analyze Antibiotic Resistance Patterns: Investigate the resistance rates of ESBL-producing isolates to commonly used antibiotics, including cephalosporins and carbapenems.

1.2.3 Identify Specimen-Specific Risk: Determine which clinical specimen types show the highest prevalence of ESBL-producing pathogens to guide infection control priorities.

1.2.4 Support Local Antibiotic Stewardship: Generate evidence to inform regional antibiotic stewardship programs and improve prescribing practices.

1.2.5 Provide a Baseline for Future Studies: Establish baseline resistance data to track trends over time and evaluate the impact of interventions.

1.2.6 Importance of the Regional Dataset: Resistance patterns are influenced by localized factors such as healthcare infrastructure, regulatory policies, and antibiotic availability. By focusing on a regional healthcare setting, this study addresses gaps in global surveillance efforts, which often underestimate resistance in low- and middle-income countries [9]. Additionally, region-specific data empower healthcare providers to develop tailored infection control protocols, optimize empirical antibiotic therapy for common pathogens, monitor the effectiveness of implemented stewardship programs [14]. The findings from this study will complement global surveillance initiatives, such as the WHO Global Antimicrobial Resistance and Use Surveillance System "Global Antimicrobial Resistance and Use Surveillance System [15], which emphasize the importance of regional data in combating resistance [16].

2. Materials and Methods

2.1 Retrospective Analysis of Clinical Isolates: This study involved a retrospective analysis of clinical specimens obtained from a regional tertiary care hospital located at Chhatrapati Sambhajinagar district of state of Maharashtra, India. over a period of 12 months. The specimens were obtained from patients presenting with infections, including urinary tract infections, bloodstream infections, respiratory tract infections, and central nervous system infections. The inclusion criteria were based on the availability of complete patient demographics, clinical data, and specimen information, ensuring the reliability of the dataset (CLSI, 2021).

2.2 Sample Collection and Processing: Specimens included aspirates, urine, blood, pus, sputum, endotracheal secretions, pleural fluids, cerebrospinal fluid (CSF) and other fluids. The distribution of specimens was representative of a diverse patient population in terms of age, gender, and clinical conditions. Bacterial isolation was conducted using conventional culture techniques on MacConkey agar media. Isolates showing resistance to third-generation cephalosporins (cefotaxime, ceftazidime, or ceftriaxone) were selected for further analysis [1]. Demographic information, including age and gender, was collected anonymously in compliance with ethical guidelines [17]. Approval of the study was obtained from the institutional committee, ensuring adherence to the Declaration of Helsinki. No identifiable patient information was used, maintaining strict confidentiality. The antibiotic susceptibility of the clinical isolates was determined following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021).

2.3 Antimicrobial Susceptibility Testing: To conduct **antibiotic susceptibility testing**, a bacterial suspension is created and adjusted to a 0.5 McFarland standard, which is about $1x10^8$ CFU/mL [18]. The Kirby-Bauer disk diffusion method is then used on Mueller Hinton agar, with antibiotic disks like Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Gentamycin (50 µg) and Streptomycin (25 µg) applied**1**. After incubating the plates at 37°C for 16–18 hours, the zone diameters are measured in millimeters, and results are interpreted as susceptible, intermediate, or resistant based on CLSI standards (CLSI, 2021).

2.4 ESBL Confirmation: Isolates showing resistance to third-generation cephalosporins were further subjected to ESBL confirmation tests. Improved ESBL detection Ezy MIC™ Strip (HiMedia Laboratories) having Ceftazidime, Cefotaxime & Clavulanic acid (MIX+) with 0.032-4 µg/ml concentration range on one end of the strip and Ceftazidime & Cefotaxime (MIX) with 0.125-16 µg/ml concentration range on the other end was used to confirm ESBL status. A ≥5 mm increase in the inhibition zone with clavulanic acid confirmed ESBL production [1]. The following interpretive criteria was used for classifying

ESBL positive strain: To determine if a strain is **ESBL positive**, the ratio of the value obtained for MIX to the value of MIX with Clavulanic acid (MIX+) must be greater than 8, or there should be no zone for MIX but a zone for MIX+. A strain is considered **ESBL negative** if this ratio is less than or equal to 8, or if both zones are below the lowest concentration. If there is no zone of inhibition on either side, the ESBL result is **non-conclusive**, suggesting resistance may be due to other mechanisms. Control strains like E. coli ATCC 25922 and K. pneumoniae ATCC 700603 are used in each test batch to ensure testing accuracy [19].

2.5 Statistical Methods: Robust statistical analysis was employed to evaluate the relationships between demographic variables, specimen types, and antibiotic resistance patterns. The statistical methods included chi-square tests, confidence interval estimation, and Cramér's V[20] to measure effect sizes. These techniques are standard for categorical data analysis and ensure reliability and reproducibility of results [21].

Chi-Square test was performed to test the independence of categorical variables such as age group, gender, and specimen type with ESBL prevalence. For instance, the test evaluated whether ESBL prevalence was associated with specific age groups. $\chi^2 = \sum \frac{(O-E)^2}{E}$

O: Observed frequency, *E*: Expected frequency. A p-value < 0.05 was considered statistically significant, indicating an association between variables. The chi-square test was performed using contingency tables. Confidence Intervals were analyzed to estimate the precision of the prevalence and resistance rates.

For a proportion (p^):

$\mathrm{CI} = p\hat{} \pm \mathrm{Z}.\sqrt{\frac{p\hat{}(1-p\hat{})}{N}}$ where *Z = 1.96 for 95*% confidence.

Narrow confidence intervals indicate precise estimates, while wide intervals suggest variability in the data [22]. For finding the Effect Size Cramér's V analysis was performed to measure the strength of association between categorical variables identified as statistically significant by the chi-square test.

$V = \sqrt{\frac{\chi^2}{N\,.\,(k-1)}}$

where, *N* is the total sample size, and *k* is the smaller of the number of rows or columns in the contingency table. Interpretation was made with following criteria *V* = 0.1: Small effect, V= 0.3: Medium effect, V= 0.5: Large effect [23].

2.6 Software and Tools: Statistical analyses were conducted using SPSS (v26) and R program (v4.0.2). Graphs for confidence intervals and effect size distributions were generated in R using the ggplot2 package [24].

2.7 Molecular Characterization of Isolates: To detect the presence of **blaTEM** and **blaSHV genes** (which indicate **ESBL resistance**), genomic DNA **was extracted** from bacterial isolates using a boiling method. A single colony **was suspended** in 100 µL sterile water, **boiled**, and **centrifuged**; the supernatant **was then used** for PCR. blaTEM (F: AGTGGGTGCACGAGTGGGTT, R: TGCTTAATCAGTGAGGCACC, 860 bp) and blaSHV (F: ATGCGTTATATTCGCCTGTG, R: TGCTTTGTTAGTGTTGCCAG, 713 bp) primers **were used** to amplify the **blaTEM** and **blaSHV genes**, and the PCR reaction **included** PCR buffer, $MgCl_2$, dNTP mix, primers, and Taq DNA polymerase. Thermal cycling conditions **involved** initial denaturation at 95°C, followed by 35 cycles of denaturation, annealing, and extension, with a final extension at 72°C. The amplified PCR products **were analyzed** using gel electrophoresis on agarose gel, stained with ethidium bromide, and run at 100V. A DNA ladder **was used** as a molecular weight marker, and the bands **were visualized** under UV transillumination. *K. pneumoniae* ATCC 700603 (ESBL producer) and *E. coli* ATCC 25922 (non-ESBL strain) **served** as positive and negative controls, respectively.

3. Results and Discussion

A total of 444 clinical isolates were put through antimicrobial susceptibility testing. The analysis covered six antibiotics (CTX, CAZ, CTR, IPM, GEN, S) across various clinical isolates. The data was categorized into Resistant (R), Intermediate (I), and Sensitive (S**)** responses based on the measurement of diameter of zone of inhibition. (Fig.1-3) GEN (Gentamicin) and S (Streptomycin) showed the highest sensitivity, with 444 samples categorized as Sensitive (S). IPM (Imipenem) showed a concerning resistance rate, with 201 isolates marked as Resistant (R). CTR (Ceftriaxone) had the highest resistance among cephalosporins, with 110 Resistant isolates. CAZ (Ceftazidime) and CTX (Cefotaxime) also exhibited significant resistance, with 95-96 resistant isolates each. CTX, CAZ, and IPM exhibited moderate levels of intermediate resistance, with CTX (33), CAZ (34), and IPM (42) isolates categorized as Intermediate (I). 54.93% of samples (245) were fully sensitive (no resistance detected to any antibiotic). 16.59% of samples (74) showed resistance to a

single antibiotic. 2.02% of samples (9) exhibited resistance to two antibiotics. 13.90% of samples (62) were resistant to three antibiotics, suggesting increasing multidrug resistance (MDR). 12.56% of samples (56) demonstrated resistance to four antibiotics, which is a high-risk MDR group that may pose significant treatment challenges. Some specimen types, such as CSF, ET Secretion, and Pleural Fluid, have a higher proportion of multidrug-resistant (MDR) samples (resistance to 3 or more antibiotics) (Fig.4). CSF (Cerebrospinal Fluid) and ET Secretion exhibit a substantial number of MDR cases (resistance to 3+ antibiotics), highlighting a serious concern for treatment challenges in meningitis and respiratory infections. A total of 136 samples tested positive for ESBL (Extended-Spectrum Beta-Lactamase) (Table 1). ESBL-positive samples were found across multiple specimen types, with varying proportions. ET secretion (38.00%) had the highest ESBL prevalence, followed closely by Urine (35.00%), Sputum (34.29%), Pleural fluid (32.73%), Blood (28.33%), Pus (27.50%), CSF (Cerebro Spinal Fluid) (25.00%), Aspirates (24.44) and Other Fluids (22.86%) exhibited moderate prevalence (Fig.5-6). Some specimen types exhibited significantly higher ESBL presence, particularly ET secretion and urine specimens, indicating a potential risk for hospital-acquired or severe systemic infections. ESBL prevalence fluctuates rather than remaining uniform across age groups. The highest ESBL prevalence is observed in 71-80 years (67.65%) and 81–90 years (55.56%). The lowest ESBL prevalence is found in 11–20 years (18.33%) and 31–40 years (19.23%). Under statistical analysis chi-square analysis of the categorical variables were done (Table 3) between categorical variables Antibiotic Resistance vs. Susceptibility Chi-Square Value: 546.68, p-Value: 4.59×10^{-111}, df: 10), between ESBL Status vs. Specimen Type (Chi-Square Value: 11.23, p-Value: 0.2605 (higher than 0.05), df: 9), between ESBL Status vs. Gender (Chi-Square Value: 0.0048, p-Value: 0.9445, df : 1) and between ESBL Positivity vs. Age Group (Chi-Square Value: 47.98740506 , p-Value: 2.57E-07 , df: 9). Cramer's V analysis was done on the same categorical variables (Table 4) resulting in following values Antibiotic Resistance vs. Susceptibility (Cramer's V: 0.143252157, Effect Size: Moderate/Small), ESBL Status vs. Specimen Type (Cramer's V: 0.037478869, , Effect Size: Small Effect), ESBL Status vs. Age Group (Cramer's V: 0.077488258,, Effect Size: Moderate/Small), ESBL Status vs. Gender (Cramer's V : 0.003295158, Effect Size: Negligible Effect). Out of the 444 bacterial isolates obtained from different specimens, colony PCR of random 10 isolates were done to check the presence of the lactamase gene blaTEM and blaSHV. Among them 08 of the isolates showed a band of 860 bp when subjected to colony PCR amplification using blaTEM primer (Fig. 9), while similar number of isolates showed band of 713 bp when subjected to colony PCR amplification using blaSHV primer (Fig. 10). When the same isolates were subjected to multiplex PCR using both the primers 02 isolates showed 860 bp single band, 05 isolates showed 713 bp single band while 02 isolates showed presence of both the bands (Table 5 & Fig. 11).

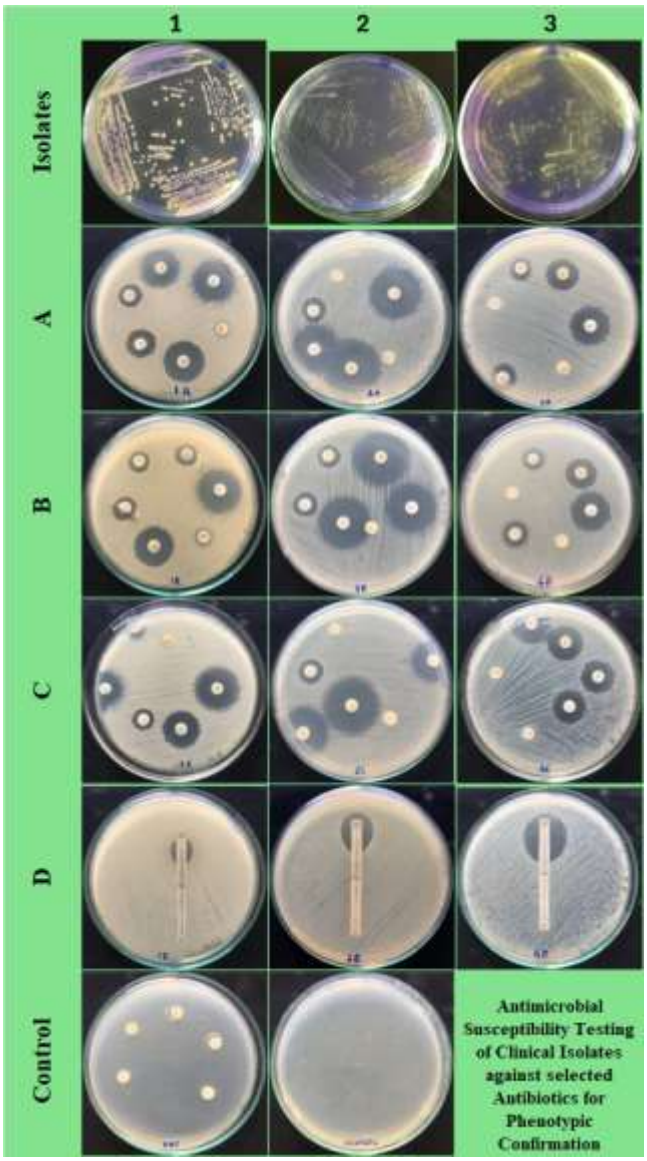

Figure 1: Representative plates showing zone of Inhibition obtained as a result of Antimicrobial Susceptibility Testing of Clinical Isolates against specific Antibiotics for Phenotypic Confirmation- Top row left to right clinical isolates grown on MacConkey Agar and assigned unique nos. **Bottom row** negative controls. **Second to third row** (A-B) phenotypic characterization of resistance of each isolate against a battery of antibiotics tested by spreading 0.5 McFarland units of culture on Muller-Hilton agar and followed by placement of different antibiotic discs. **D row** characterization of ESBLs and MICs of each isolate. **Antibiotics Tested:** CTX, CAZ, CTR, IMP, GEN, S, (with standard abbreviations) ESBL detection through EzyMIC™ (HiMedia)

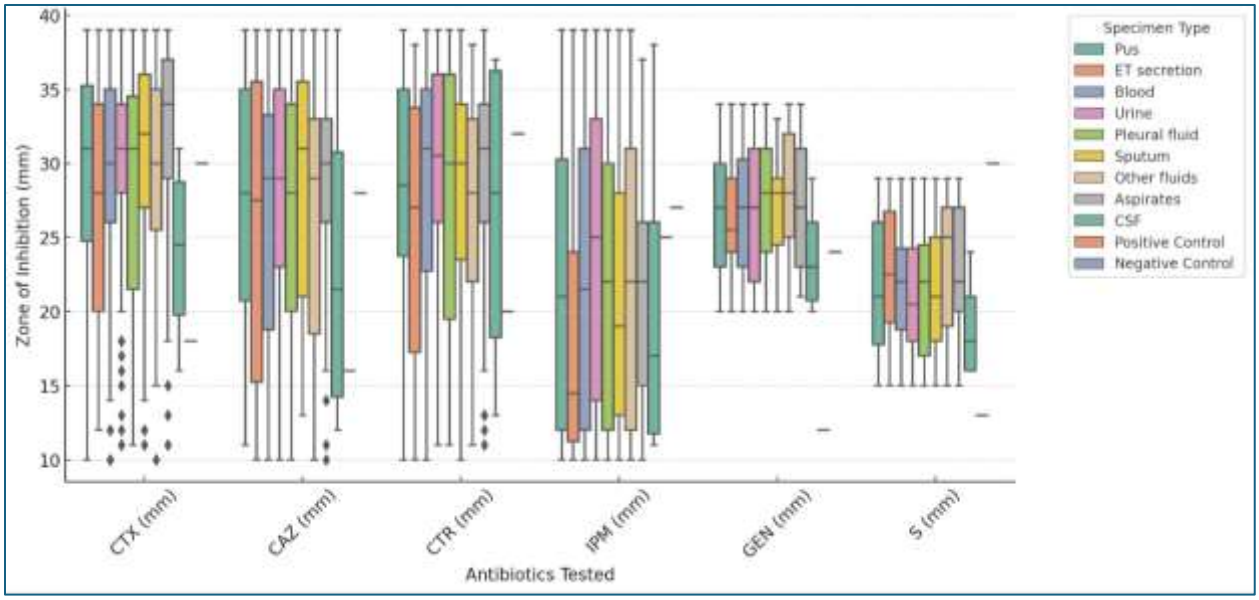

Figure 2: Distribution of zone of inhibition against six different antibiotics tested on the samples across various specimen types.

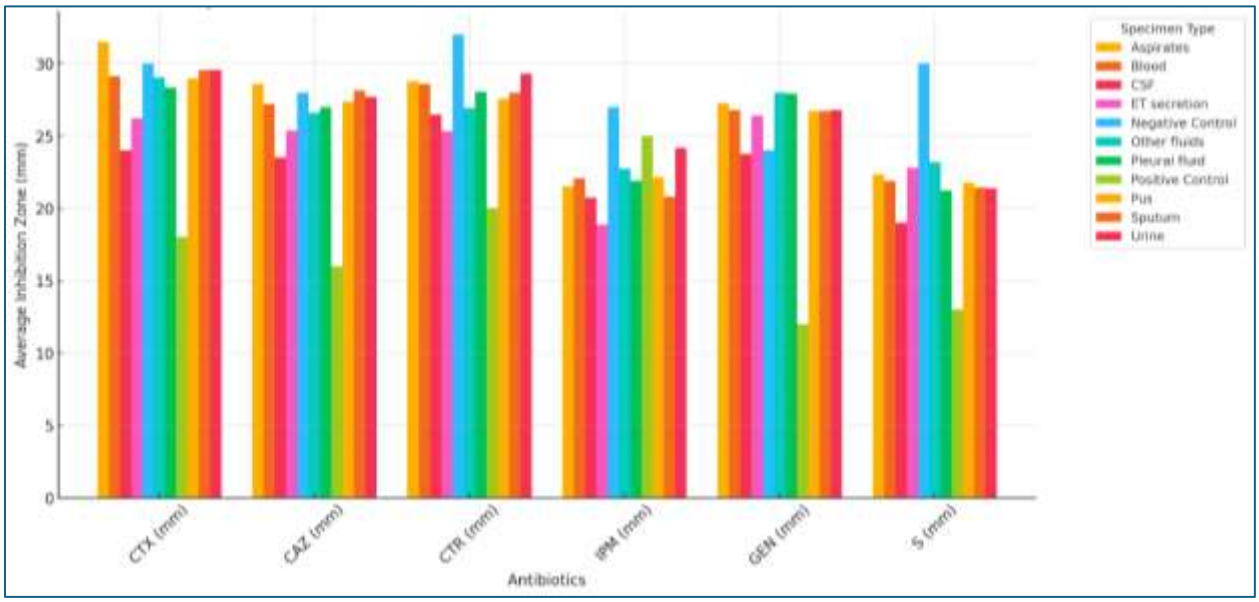

Figure 3: Average diameter (mm) of zone of inhibition across specimen

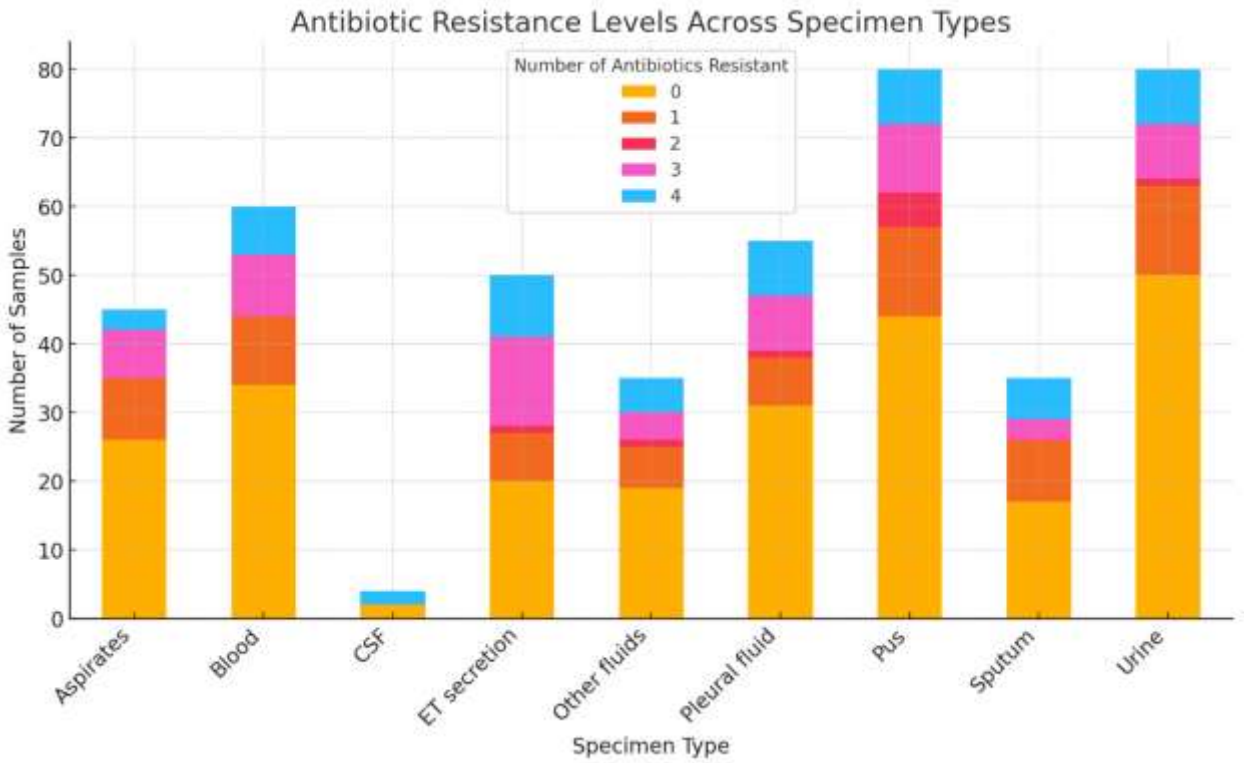

Figure 4: Resistance levels against multiple antibiotics displayed by samples across specimen.

Specimen Type	E	N	Total	Percentage of ESBL Positive
Aspirates	11	34	45	24.44
Blood	17	43	60	28.33
CSF	1	3	4	25.00
ET secretion	19	31	50	38.00
Other fluids	8	27	35	22.86
Pleural fluid	18	37	55	32.73
Pus	22	58	80	27.50
Sputum	12	23	35	34.29
Urine	28	52	80	35.00
Total	136	308	444	30.63

Table 1: ESBL Proportions Across Specimen Types Abbreviations used in the table: E: ESBL-positive — Extended-Spectrum Beta-Lactamase producing organism. **N**: Non-ESBL — The organism does not produce ESBL. **PC**, Positive Control (*K. pneumoniae* ATCC 700603, ESBL) **NC,** Negative Control (*E. coli* ATCC 25922, Non-ESBL)

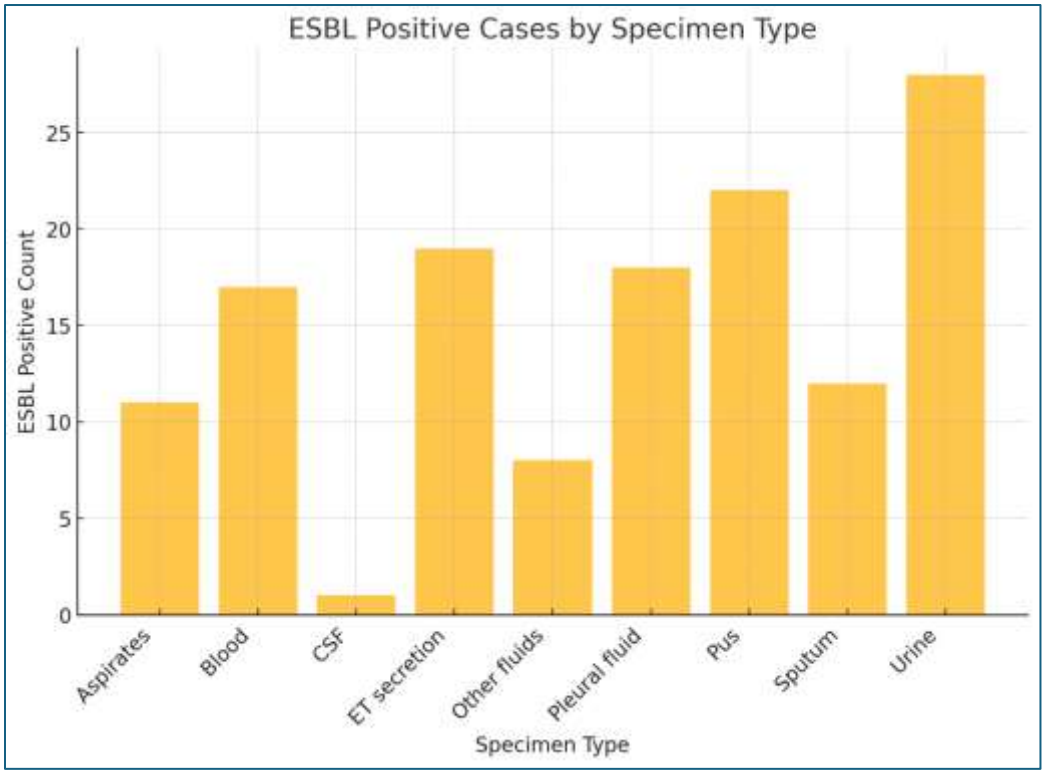

Figure 5: ESBL Proportions Across Specimen Types.

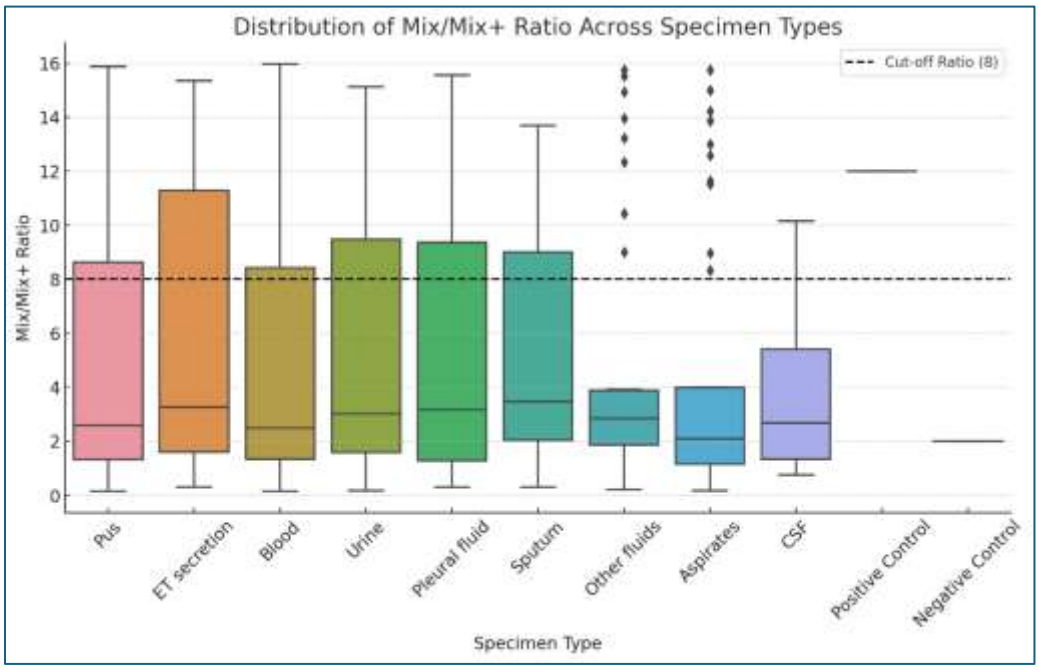

Figure 6: Box plot representing the MIC Distribution (Ceftazidime & Cefotaxime-Mix / Ceftazidime, Cefotaxime & Clavulanic acid -Mix+ Ratio) across different specimen types. The red dotted line indicates the threshold ratio ≥ 8 above which ESBL positive samples are shown.

Age Group (Years)	Number of Isolates (n)	ESBL-Positive Isolates	Prevalence (%)
0–10	49	10	20.41
11–20	60	11	18.33
21–30	52	13	25.00
31–40	52	10	19.23
41–50	48	12	25.00
51–60	55	14	25.45
61–70	49	18	36.73
71–80	34	23	67.65
81–90	45	25	55.56
Total	444	136	30.63

Table 2: Age-Wise ESBL Prevalence

Statistical Analysis

- The association between age groups and ESBL prevalence was evaluated using a chi-square test (p=0.11), suggesting no statistically significant relationship.
- However, **effect size analysis** using Cramér's V (V=0.0878) indicated a small effect, pointing to potential clinical relevance despite the lack of statistical significance.

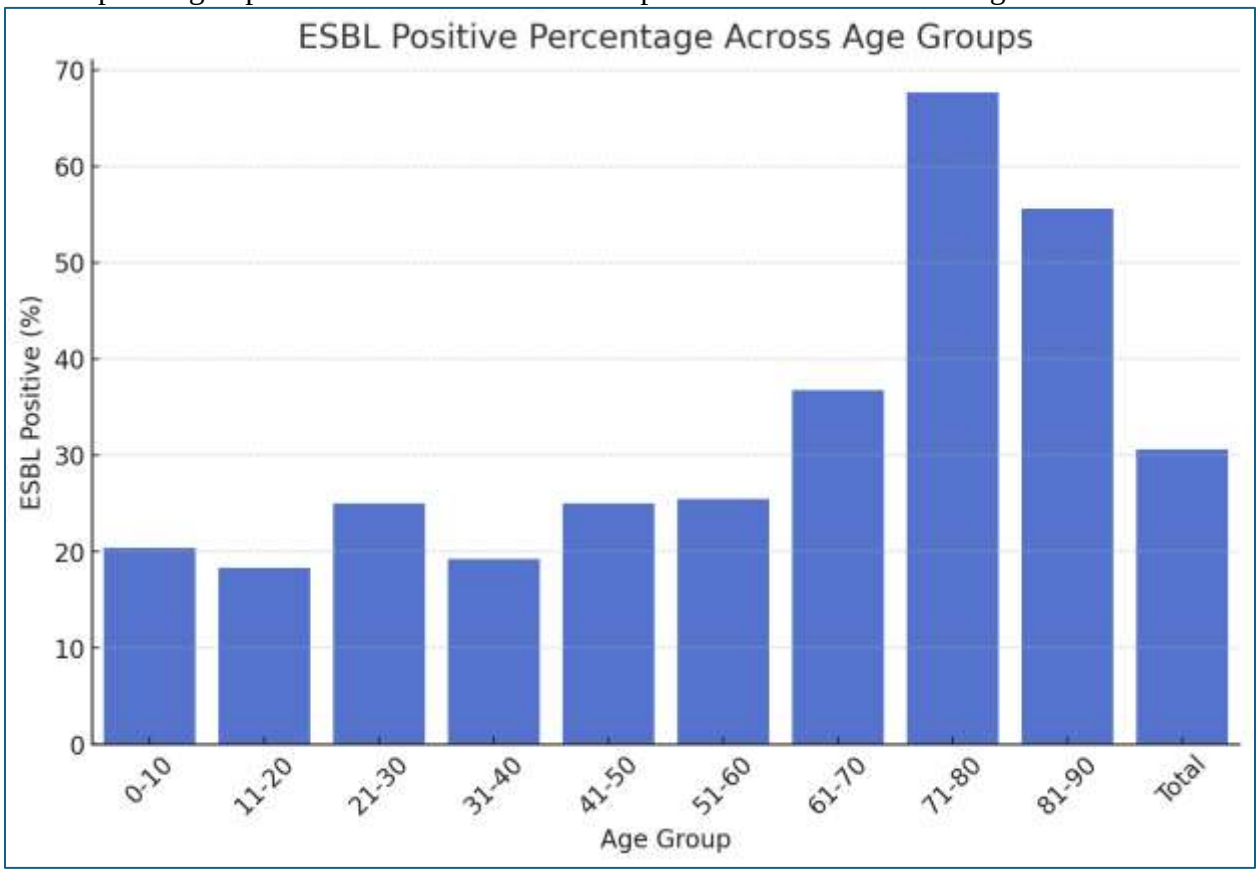

Figure 7: ESBL Prevalence based across different age groups

Test	Chi-Square Value	p-Value	Degrees of Freedom
Antibiotic Resistance vs. Susceptibility	546.6842464	4.59E-111	10
ESBL Status vs. Specimen Type	11.22608779	0.260529624	9
ESBL Status vs. Gender	0.004842697	0.944520405	1
ESBL Positivity vs. Age Group	47.98740506	2.57E-07	9

Table 3: Chi square Analysis to examine the relationship between two or more categorical variables

Test	Cramer's V	Effect Size Interpretation
Antibiotic Resistance vs. Susceptibility	0.143252157	Moderate/Small
ESBL Status vs. Specimen Type	0.037478869	Small Effect
ESBL Status vs. Age Group	0.077488258	Moderate/Small
ESBL Status vs. Gender	0.003295158	Negligible Effect

Table 4: Effect Size Analysis (Cramér's V) to measure the strength of association between two categorical variables

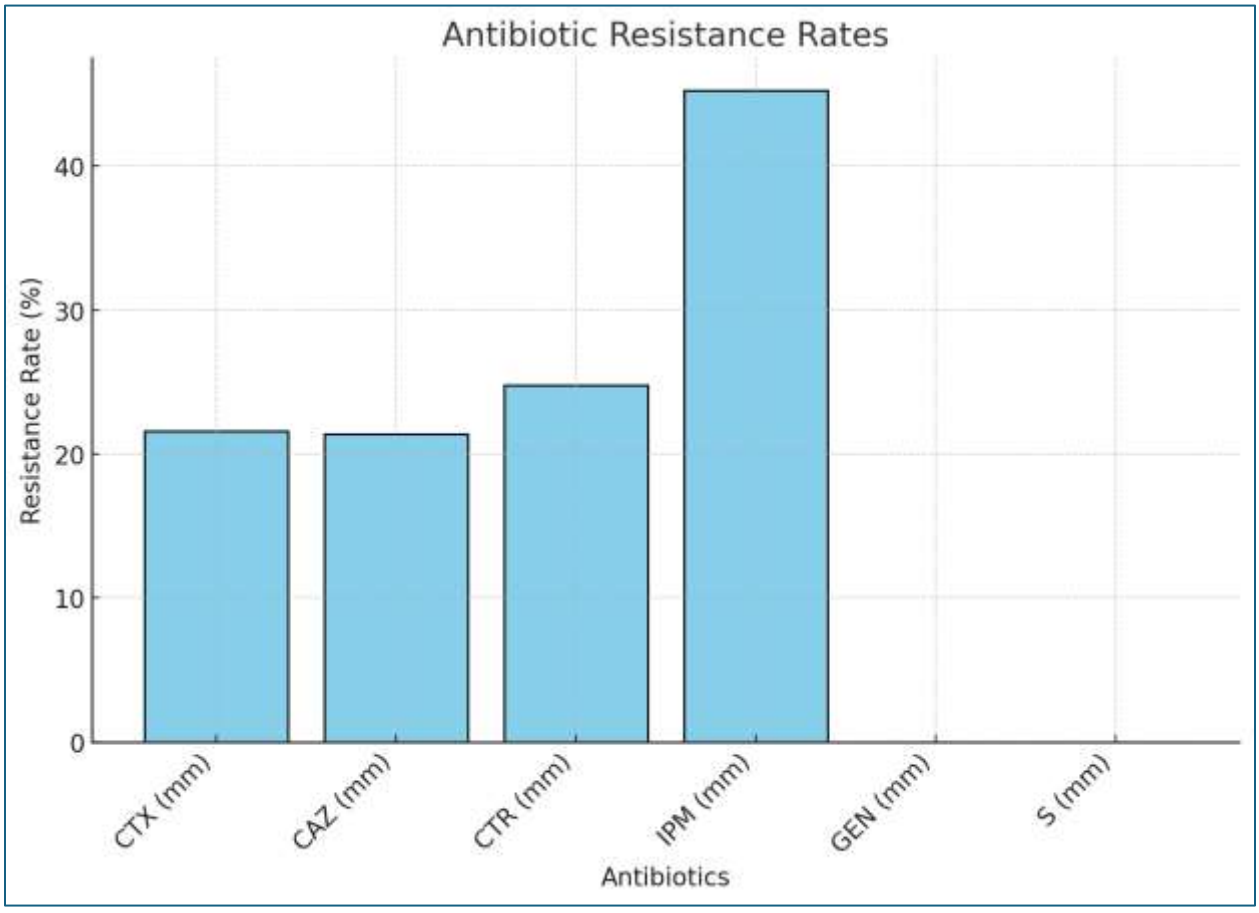

Figure 8: Resistance rates of isolates across different antibiotics tested

Isolate ID	ESBL Phenotypic Confirmation Status	blaTEM (860 bp)	blaSHV (713 bp)	Multiplex PCR	
				blaTEM (860 bp)	blaSHV (713 bp)
PU-001	+	+	+	+	-
ET-081	+	+	+	-	+
BL-131	+	+	+	+	+
UR-191	+	+	+	+	+
PL-271	+	+	-	-	+
SP-326	+	-	+	-	+
OT-361	+	+	+	-	+
AS-396	+	+	+	-	+
CS-441	-	-	-	-	-
CS-442	+	+	+	+	-

Table 5: PCR amplification of blaTEM & blaSHV genes among the random few ESBL positive clinical isolates

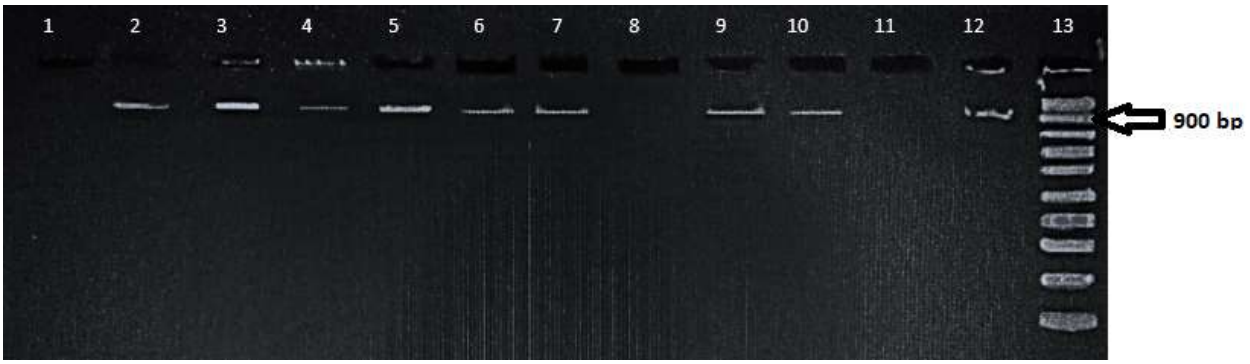

Figure 9: Agarose Gel Electrophoresis image of PCR amplification of isolates using blaTEM primer Shown below Lane 1: negative control, lane 2: positive control, lane 3-12: samples, lane13: 100 bp ladder

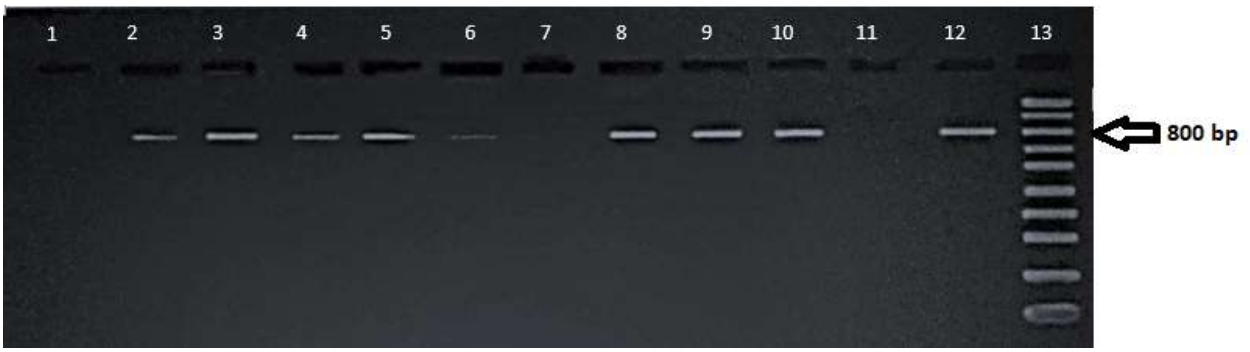

Figure 10: Agarose Gel Electrophoresis image of PCR amplification of isolates using blaSHV primer Shown below Lane 1: negative control, lane 2: positive control, lane 3-12: samples, lane13: 100 bp ladder

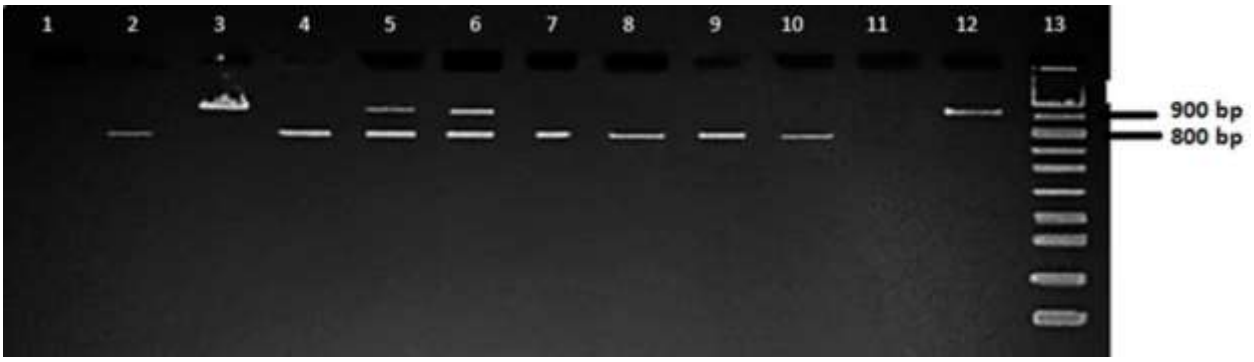

Figure 11: Agarose Gel Electrophoresis image of Multiplex-PCR amplification of isolates using blaTEM & blaSHV primers Shown below Lane 1: negative control, lane 2: positive control, lane 3-12: samples, lane13: 100 bp ladder

ESBL Prevalence in Clinical Isolates and Demographic Patterns: Our study found that 30.63% of the clinical isolates collected were ESBL-producing organisms, with notably higher rates in older patients and certain critical-care specimen types. The highest ESBL positivity was observed in elderly groups (71–80 and 81–90 years) and in specimens like endotracheal secretions and cerebrospinal fluid, suggesting that advanced age and intensive care interventions are associated with greater risk of ESBL infection. This trend aligns with recent epidemiological data showing increased ESBL infection rates among older patients (Centers for Disease Control and Prevention 2023) [25]. For instance, a large CDC surveillance report in 2021 noted that the incidence of ESBL-producing Enterobacterales infections rose progressively with age (Centers for Disease Control and Prevention 2023). Similarly, a 2024 study in Iceland identified higher age as a significant risk factor for UTIs caused by ESBL-producing *E. coli*, alongside factors like recent hospitalization [25]. Our findings reinforce these observations – elderly patients, often with comorbidities and repeated healthcare exposures, are particularly vulnerable to ESBL-related infections. In terms of specimen source, the prominence of ESBL isolates in respiratory and invasive samples is consistent with patterns reported in critical care settings. Ventilator-associated pneumonias and bloodstream infections in ICUs have increasingly been linked to ESBL-producing bacteria [26]. For example, one hospital study found that carbapenem-resistant K. pneumoniae (often also ESBL producers) were significantly more frequent in endotracheal aspirates of ICU patients [26], underlining the convergence of ventilator support and multidrug-resistant infections. On the other hand, our moderate ESBL rates in urine and wound isolates parallel the endemic levels seen in many hospitals. Compared to other regions, our overall ESBL prevalence (~30%) is within a mid-range: lower than some reports from South Asia, yet much higher than those in certain high-income settings. Some hospitals in India have documented extremely high ESBL rates (70–90% of Enterobacteriaceae) [27], reflecting a severe endemic problem. In contrast, Scandinavian studies a decade ago reported ESBL prevalence below 5%, though rising [26]. For instance, Sweden saw an increase in ESBL-producing Gram-negative infections from 1.9% in 2008 to 5% by 2010, and up to 13.5% by 2009 in one region [26]. Our finding of ~30% ESBL positivity thus represents a serious burden that is consistent with global trends: a recent meta-analysis observed a pooled ESBL-*E. coli* prevalence around 25–26% worldwide [27], and the WHO's GLASS surveillance indicates a median ~42% resistance rate to third-generation cephalosporins in *E. coli* across 76 countries [28]. In summary, the prevalence we observed underscores that ESBL-producing pathogens are firmly established in our healthcare setting, particularly affecting elderly and critically ill patients, much as has been reported in other hospitals globally. This convergence of evidence highlights the need for heightened vigilance in these high-risk groups.

Antibiotic Resistance Patterns and Comparison with Recent Studies: Our ESBL-producing isolates exhibited broad resistance to β-lactam antibiotics, especially third-generation cephalosporins, alongside notable carbapenem resistance – a profile that mirrors findings from numerous recent studies. In our dataset, resistance rates to ceftriaxone, cefotaxime, and ceftazidime were approximately 21–25%, which, given a 30% ESBL prevalence, indicates most ESBL-producers were non-susceptible to these cephalosporins. This is expected, as ESBL enzymes hydrolyze oxyimino-cephalosporins, rendering drugs like ceftriaxone ineffective. Comparable resistance patterns are reported globally. For instance, a systematic review of hospital-acquired infections in low- and middle-income countries found third-generation cephalosporin resistance rates of 75–77% in *E. coli* and *K. pneumoniae* – reflecting the high impact of ESBLs [26]. Our somewhat lower percentages likely reflect that our denominator included all isolates (ESBL-producers and non-producers), yet the trend remains consistent: third-generation cephalosporins can no longer be relied on for empirical therapy in many settings [26]. This aligns with WHO reports of widespread cephalosporin resistance, with a median of 42% of *E. coli* globally now resistant to these drugs [28]. Notably, our data showed imipenem resistance in 45.27% of isolates, a finding of grave concern. Carbapenems are often last-resort antibiotics for ESBL infections, so nearly half of our isolates being carbapenem-resistant suggests the emergence of carbapenem-resistant Enterobacterales (CRE) in our hospital. Recent regional and global studies similarly warn of rising CRE rates. A 2023 review noted that Asia and Africa now lead in CRE prevalence among Enterobacterales [29]. In South Asia, up to 66% of *K. pneumoniae* isolates from hospital infections are carbapenem-resistant [30], far exceeding the global average CRKP rate (~29%) [30]. Our observed 45% carbapenem resistance is in line with these high rates, underscoring that our locale is not isolated from this alarming global trend of CRE proliferation. The presence of carbapenem resistance alongside ESBL production has also been observed elsewhere – for example, pooled data from LMIC hospitals showed ~39% of *K. pneumoniae* were resistant to carbapenems [26], and such strains have been associated with up to 50% mortality in infected patients [26]. Encouragingly, not all antibiotic options are exhausted in our isolates. We found 0% resistance to gentamicin and streptomycin, indicating that all tested strains were susceptible to these aminoglycoside agents. This finding suggests that aminoglycosides may retain activity as potential therapy adjuvants in our setting. Our result is somewhat unusual when compared to other reports – many hospitals still report some resistance to aminoglycosides among ESBL producers. For instance, an Omani study of ESBL-producing *E. coli* and *K. pneumoniae* found only 38% of isolates remained gentamicin-susceptible, whereas nearly 94% were susceptible to amikacin [31]. In our case, the complete susceptibility to gentamicin could reflect differences in local antibiotic usage patterns or the genetic backgrounds of our strains. While this is a positive finding, cautious interpretation is needed. Aminoglycosides have pharmacologic limitations: they achieve bactericidal concentrations in certain compartments (e.g. urine) but have toxicity concerns and are seldom used as monotherapy for systemic infections. In practice, they are often used in combination with β-lactams for synergistic effect, particularly in severe infections, rather than as standalone therapy. Nonetheless, our data hint that agents like gentamicin could be effective partners in combination regimens against our ESBL-producing organisms – a strategy echoed by recent treatment guidance that recommends carbapenem-sparing approaches (such as pairing a β-lactam with an aminoglycoside) when appropriate [27,31]. Apart from β-lactams and aminoglycosides, it is worth noting that ESBL producers frequently carry additional resistances – for example, co-resistance to fluoroquinolones and trimethoprim-sulfamethoxazole is common [26]. Although our study focused on key β-lactams and a few others, the multidrug-resistant (MDR) profile of ESBL organisms reported worldwide suggests clinicians must be alert: an organism found ESBL-positive often implies that drugs like ciprofloxacin or cotrimoxazole may

also be ineffective [26]. Indeed, many of our isolates showed MDR traits in phenotypic testing (as reflected in the overall high resistance rates across multiple antibiotic classes). In summary, our resistance pattern findings – moderate to high resistance to cephalosporins, emerging carbapenem resistance, and retained aminoglycoside susceptibility – are largely in accord with recent literature. They paint a concerning picture of dwindling antibiotic options for ESBL infections, consistent with global surveillance data, but also leave a small window for alternative therapies that could be leveraged in our setting.

Molecular Characterization of ESBL Genes: blaTEM, blaSHV, and Global Context: We performed molecular characterization on a subset of ESBL-producing isolates, targeting common β-lactamase genes. Our PCR analysis detected the blaTEM gene in 80% of tested isolates and blaSHV in 80%, with some isolates carrying both genes simultaneously. This indicates that the TEM and SHV families of ESBL enzymes are prevalent among our organisms. Such a finding is in line with the historical distribution of ESBL types: TEM- and SHV-type β-lactamases were among the earliest ESBLs described and have been widely reported in hospital isolates for decades [31,32]. For instance, a recent study from Iraq in patients with UTIs found blaTEM in 81% of ESBL-producing *E. coli* (similar to our 80% figure), often alongside other genes [32]. In that study, blaSHV was present in a smaller fraction of *E. coli* (16%) and [32] more frequently in *K. pneumoniae* (35%) [32], highlighting that the distribution of specific ESBL genes can vary by bacterial species and setting. Our detection of several isolates carrying both TEM and SHV genes underscores the potential for co-occurrence of multiple ESBL genes in a single strain, a phenomenon noted in prior research. Co-harboring of ESBL genes can occur via plasmids that carry multiple resistance determinants, and other authors have documented strains with two or more ESBL gene families present [33]. This co-existence may broaden the spectrum of β-lactam hydrolysis and potentially enhance resistance levels, complicating treatment choices further. It is important to note, however, that ESBL genetic landscapes have evolved over time. While TEM and SHV enzymes remain important, especially in Klebsiella and some *E. coli*, numerous recent studies have shown that CTX-M type ESBLs have become the dominant family globally [27,34]. CTX-M enzymes (notably CTX-M-15) emerged in the early 2000s and rapidly spread worldwide, often displacing TEM/SHV in prevalence [34]. Our study did not include PCR for blaCTX-M (a limitation of our molecular analysis), so we cannot directly comment on its presence. However, given global trends, it is very likely that CTX-M producers are present in our setting. In fact, regional data strongly suggest this: for example, CTX-M-15 is reported as the predominant ESBL gene in India, Bangladesh, and Pakistan in recent years [27]. One surveillance report from a neighboring country found blaCTX-M to be the most common genotype among ESBL-producing Enterobacteriaceae, whereas blaTEM was detected less frequently [27]. Thus, our findings of prevalent TEM/SHV should be interpreted in context – they confirm the circulation of these classic ESBL genes in our hospital, but do not rule out co-existing CTX-M or other ESBL types that we did not test for. The persistence of blaTEM and blaSHV in our isolates is noteworthy because it suggests a mix of older ESBL lineages alongside newer ones. TEM and SHV genes have often been associated with hospital outbreaks in the 1990s and early 2000s, before CTX-M became dominant. Their continued presence (often together) may reflect the clonal persistence of certain resistant strains or plasmids in our institution. Moreover, the detection of both genes in some isolates could indicate either multiple plasmids or integrons in those bacteria, a sign of highly drug-resistant phenotypes that accumulate resistance mechanisms. Comparable multi-gene occurrences have been documented – for instance, researchers in Egypt and other countries have identified E. coli isolates co-producing TEM, SHV, and even CTX-M or other β-lactamases [35]. In summary, our molecular characterization reveals that blaTEM and blaSHV type ESBL genes are widespread among the ESBL-producing strains in our study, often in combination. This finding is in general

agreement with earlier studies of ESBL genetics, though the global dominance of CTX-M enzymes suggests that future work should confirm whether CTX-M variants are also present in our isolates. Understanding the specific ESBL genes circulating locally has practical implications: TEM and SHV producers may differ slightly in antibiotic susceptibility patterns (for example, some SHV variants preferentially hydrolyze certain cephalosporins), and the presence of particular genes can inform infection control tracking of plasmid-mediated outbreaks. Our results contribute to the growing body of literature mapping the molecular epidemiology of ESBLs, indicating that our locale harbors a diverse assortment of ESBL determinants, consistent with the heterogeneous mix of ESBL genes reported worldwide [27,32].

Clinical Implications and Expert Perspectives: The convergence of our findings – a substantial ESBL prevalence, high multidrug resistance rates (including worrisome carbapenem resistance), and diverse β-lactamase genes – carries significant clinical implications for healthcare settings. Infections caused by ESBL-producing organisms are more difficult to treat, often requiring alternative antibiotics and longer hospital stays, and they have been associated with increased morbidity and mortality [26,31]. When ESBL production is combined with carbapenem resistance, as indicated by our 45% imipenem resistance rate, the challenge becomes critical. The CDC classifies CRE as an "Urgent Threat" to public health, with mortality rates for serious CRE infections reported as high as 40–50% [26]. Our data suggest that such highly resistant organisms are already present in our facility, which raises concern for potential outbreaks and adverse patient outcomes if aggressive measures are not taken. From a treatment standpoint, the high rate of cephalosporin resistance means that standard empirical therapies for common infections may fail if ESBL producers are involved [26]. Clinicians must consider the local prevalence of ESBLs when choosing antibiotics – for example, urinary or intra-abdominal infections in our hospital may require empiric coverage with a carbapenem or beta-lactam/beta-lactamase inhibitor combination, rather than a third-generation cephalosporin. However, the concomitant rise in carbapenem resistance, as we observed, complicates this approach and illustrates a perilous "arms race" in antimicrobial therapy. Infectious disease experts have been warning that over-reliance on carbapenems in response to ESBLs can speed the selection of CRE [36]. Our findings echo this concern – the substantial use of carbapenems in our setting (likely a reaction to high ESBL rates) may be driving the emergence of carbapenemase-producing strains (e.g., KPC, NDM, OXA-48 producers). This creates a vicious cycle: broader resistance begets use of even more potent or toxic drugs. Expert commentary and current guidelines recommend breaking this cycle through stewardship and newer therapies. One strategy is to employ "carbapenem-sparing" regimens whenever feasible [27]. For instance, piperacillin-tazobactam or cefepime (if the isolate is susceptible), or newer beta-lactamase inhibitor combinations (such as ceftazidime-avibactam or meropenem-vaborbactam) can be used to treat ESBL infections, reserving carbapenems for only the most resistant cases [27]. Our observation that all isolates were gentamicin-susceptible hints at the possible utility of aminoglycosides as adjunct therapy, which is supported by clinical practice guidance in certain scenarios (e.g. an aminoglycoside added to a beta-lactam for severe infections) [31]. Nonetheless, such approaches must be balanced against toxicity and the availability of oral options for step-down therapy. The implications of our study extend beyond individual patient treatment to hospital infection control and public health. The presence of multiple ESBL genes (TEM, SHV, and likely others) in our isolates suggests that resistant plasmids are circulating; thus, robust infection control measures are needed to prevent horizontal spread. Rigorous hand hygiene, contact precautions for patients colonized or infected with ESBL-producing organisms, and environmental cleaning are critical to containing these pathogens. Additionally, routine surveillance cultures and molecular typing in outbreak situations could help track the spread of specific clones or plasmids. The fact that certain ESBL genes (like CTX-M-15) have achieved

global dissemination via travel and food chains [27] underscores that our local battle is part of a larger, One Health issue – resistant bacteria move between hospitals, communities, and even agriculture. Experts emphasize coordinated efforts across healthcare and community settings to address this, including antimicrobial stewardship programs to minimize unnecessary antibiotic use (thereby slowing the selection of ESBLs/CRE) [36]. Our findings provide local evidence to support such efforts, showing the consequences of antibiotic pressure in our patient population. In summary, this study's results reinforce the urgent messages from infectious disease specialists and organizations worldwide: ESBL-producing and carbapenem-resistant bacteria represent a pressing clinical threat that demands proactive management. The high prevalence of ESBLs we observed, particularly in vulnerable patient groups, means clinicians must remain vigilant and update empiric therapy protocols in our hospital to ensure adequate coverage. At the same time, the rise in resistance to even last-resort agents call for judicious antibiotic use – every effort should be made to use targeted narrow-spectrum therapy when possible and to implement stewardship interventions that preserve the efficacy of our remaining drugs [27,36]. As noted in recent expert guidelines, innovative treatments (such as novel β-lactamase inhibitors and bacteriophage or antibody therapies in development) and infection control strategies will be pivotal in mitigating this crisis [27,37]. Our study adds to the growing body of evidence that the epidemiology of ESBL and CRE is worsening, and it offers a data-driven impetus for clinicians and policymakers in our region to intensify efforts in surveillance, prevention, and prudent antibiotic management. Through comparisons with contemporary research, we show that our local scenario is a microcosm of a global problem – one that will require concerted, multidisciplinary action to protect patients and public health.[38][19][4][13]

4. Conclusions

Imipenem (IPM) has the highest resistance (45.27%), indicating serious concerns for carbapenem-resistant strains. Cefotaxime (CTX), Ceftazidime (CAZ), and Ceftriaxone (CTR) all show moderate resistance (~21-25%), confirming ESBL-related resistance patterns. Gentamicin (GEN) and Streptomycin (S) show 0% resistance. The high sensitivity of GEN and S suggests that these antibiotics may still be effective for treating infections caused by the studied pathogens. The high resistance to IPM (Imipenem) is a concerning trend, as carbapenem resistance is often linked to multidrug-resistant (MDR) organisms. The significant resistance to third-generation cephalosporins (CTX, CAZ, CTR) aligns with global trends of ESBL-producing Enterobacteriaceae, emphasizing the need for antibiotic stewardship programs. The data highlights rising resistance to key antibiotics, particularly beta-lactams and carbapenems, which could limit treatment options. Gentamicin and Sulfonamides remain viable options for treatment, but continuous surveillance is essential to track resistance trends. There is a pressing need for antimicrobial stewardship programs and alternative therapeutic strategies to combat growing resistance, especially to cephalosporins and carbapenems. The majority (54.93%) of isolates remain sensitive, which is promising for antimicrobial therapy. However, 27.46% of samples (resistant to 3 or more antibiotics) are classified as multidrug-resistant (MDR), signaling a critical need for enhanced antibiotic stewardship and surveillance. The 12.56% highly resistant isolates may indicate carbapenem-resistant or ESBL-producing strains, necessitating alternative treatment approaches and infection control measures. While a significant proportion of isolates remain treatable, the presence of MDR strains emphasizes the need for careful antibiotic selection and resistance monitoring. Antimicrobial resistance (AMR) mitigation strategies, including antibiotic stewardship and rapid diagnostics, should be prioritized. Empirical antibiotic treatments may need to be reconsidered, with an emphasis on combination therapy and last-resort antibiotics for MDR cases. The high ESBL positivity in ET secretion and urine specimens highlights critical risks in

severe infections. ESBL prevalence is fluctuating across different age groups. The age group with the highest ESBL positivity is 71-80 years, with an ESBL positivity rate of 67.65%. The age group with the lowest ESBL positivity is 11-20 years, with a rate of 18.33%. This suggests that elderly individuals (71-80) are at the highest risk, possibly due to increased antibiotic exposure, weakened immunity, or more frequent hospital visits. Conversely, younger individuals (11-20) have the lowest ESBL positivity, indicating lower exposure or better resistance to infections. There is a strong association between age group and ESBL positivity. Older individuals are at a much higher risk of ESBL infections, possibly due to increased antibiotic exposure, weakened immunity, or hospitalization. Resistance varies across antibiotics, but the effect size is not overwhelmingly strong. Specimen type has very little influence on ESBL positivity. Therefore, while the chi-square test does not provide strong evidence for a statistically significant association between ESBL status and specimen type, the observed differences in ESBL prevalence may still be clinically relevant. These findings can inform targeted infection control measures and empirical antibiotic therapy, particularly in high-risk specimen types such as ET secretion and urine. Further investigation, potentially with a larger sample size or more detailed analysis of confounding factors, may be warranted to fully elucidate the relationship between specimen type and ESBL prevalence. Antibiotic resistance patterns exist, but the effect size is moderate. There is a highly significant association between antibiotic type and resistance pattern. This means that resistance is not random. antibiotics face significantly higher resistance than others, emphasizing the need for careful antibiotic selection. Specimen types show minor influences on ESBL positivity. This suggests that ESBL-producing bacteria are evenly distributed across different infection sites, rather than being concentrated in specific specimen types. Gender has almost no impact on ESBL positivity. This suggests that males and females are equally likely to be ESBL-positive or negative. Gender is not a determining factor in ESBL infections. The results obtained underscores the need for antimicrobial stewardship and targeted antibiotic therapy. The widespread presence of ESBL bacteria emphasizes the need for broader infection control measures. There is a need for age-specific infection control and antibiotic prescribing policies. It also indicates that gender is not a risk factor for ESBL infections. Carbapenem resistance (IPM ~45.27%) is a serious issue, requiring urgent clinical intervention. Surveillance programs should continue monitoring AMR trends and guide evidence-based antibiotic use. Rapid diagnostic tools should be utilized to detect resistance mechanisms early and optimize treatment decisions. Surveillance programs should continue monitoring AMR trends and guide evidence-based antibiotic use. Rapid diagnostic tools should be utilized to detect resistance mechanisms early and optimize treatment decisions. This study highlights the high prevalence of blaTEM and blaSHV genes among bacterial isolates, emphasizing the urgent need for enhanced molecular surveillance and antibiotic stewardship programs. The increasing co-occurrence of resistance genes necessitates novel treatment strategies and robust infection control measures to curb the spread of ESBL-producing bacteria.

5. References

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