ORIGINAL ARTICLE

Correlation between Extended Spectrum Beta-lactamase Production and Biofilm Formation in Gram-negative Bacteria: A Laboratory-based Pilot Study

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Abstract

Introduction: Gram-negative bacteria are among the most common causes of community-acquired, nosocomial, and opportunistic infections. The recent increase in biofilm formation and extended-spectrum beta-lactamase (ESBL) production in bacteria has led to widespread multidrug resistance, creating significant treatment challenges. This study aimed to investigate the antibiotic susceptibility profile, biofilm formation, and molecular detection of ESBL-encoding genes in clinical gram-negative isolates. **Materials and Methods:** Thirty Gram-negative isolates were collected from a tertiary care hospital, and the Kirby-Bauer disk diffusion method was used to assess antibiotic susceptibility. The double-disk synergy test phenotypically confirmed the production of ESBL. Biofilm formation was evaluated using the crystal violet microtiter plate method. Molecular characterization of *ESBL* genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and $bla_{CTX-M-15}$) was performed using polymerase chain reaction. **Results:** Of the 30 isolates, 8 (26.7%) were phenotypically confirmed as ESBL producers. All isolates (100%) carried the bla_{TEM} gene, while bla_{SHV}, bla_{CTX-M}, and $bla_{CTX-M-15}$ were detected in 30%, 33.3%, and 50% of isolates, respectively. Notably, bla_{CTX-M} was absent in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Resistance to gentamicin was observed in 100% of isolates, whereas *Escherichia coli* and *K. pneumoniae* showed lower resistance rates to amoxicillin-clavulanate. Regarding biofilm formation, 15 isolates (50%) were identified as biofilm producers. **Conclusion:** This study concluded that isolates were extensively drug resistant, and biofilm producers tended to be less drug-resistant.

Key words: Beta-lactamases, biofilm, gram-negative bacteria, multidrug resistance

INTRODUCTION

Micro-organisms show complex and diverse social behaviors, enabling quick and dynamic phenotypic adaptations to changing environmental conditions.[1] The microbes show communal behaviors, especially extensive biofilm formation, which improves cellular resilience, nutrient gathering, and tolerance to abiotic stresses.[2] Such collective traits require coordinated regulatory mechanisms and are usually triggered when they provide a selective advantage, thus enhancing synchronized and efficient population-level functions.[1]

Gram-negative bacteria are often significant pathogens in both hospital-acquired and community-acquired infections. A major global health concern is the increasing emergence and spread of antibiotic resistance among bacterial populations. Among various resistance strategies, biofilm formation is a key virulence factor that promotes persistent infections and reduces the effectiveness of antimicrobial treatments. The growth and dissemination of antibiotic resistance mechanisms present an urgent challenge to public health worldwide.[3]

In clinical settings, sustained selective pressures promote the evolution, persistence, and clonal expansion of resistant strains, including multidrug-resistant (MDR) and

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pan-drug-resistant phenotypes resistant to all clinically used antibiotic classes). This leads to therapeutic failures, increased patient morbidity and mortality, and higher healthcare costs. The prevalence of antibiotic resistance continues to grow in both community and healthcare environments.[4] Microbial biofilms consist of structurally complex, surface-adherent communities embedded within an extracellular polymeric substance matrix produced by the microorganisms. This matrix provides protection against environmental threats and antimicrobial agents, supporting the chronicity and recurrence of biofilm-related infections.

Acinetobacter baumannii, *Escherichia coli*, and *Klebsiella pneumoniae* are clinically significant Gram-negative bacteria frequently cause both hospital-acquired and community-acquired infections. *A. baumannii* is a major nosocomial pathogen with high levels of MDR and extensively drug-resistant. It is listed among the top six MDR pathogens by the Infectious Diseases Society of America.[5,6] *E. coli*, although commonly used as a model organism in microbiological research, it includes strains capable of causing various human infections.[7] *K. pneumoniae* is an encapsulated bacterium responsible for a wide range of opportunistic and severe infections. It is categorized into opportunistic, hypervirulent, and MDR types based on its accessory genome.[8] All these organisms produce extended-spectrum β-lactamases (ESBLs) that hydrolyze β-lactam antibiotics such as extended-spectrum cephalosporins and monobactams, leading to enhanced resistance.[9] Despite their differences in morphology and ecology, these pathogens share the ability to form biofilms and exhibit antimicrobial resistance, thereby posing considerable challenges to patient care and infection control efforts. Hence, this study was conducted to detect biofilm formation by Gram-negative bacteria and to determine their antimicrobial resistance patterns.

MATERIALS AND METHODS

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the Central Ethics Committee at Nitte (Deemed to be) University (NU/CEC/2020/0337) on September 9, 2020. No patient consent was required because the study involved anonymized bacterial isolates collected during routine diagnostics.

Inclusion criteria

All non-repetitive Gram-negative bacterial isolates were included in the study.

Exclusion criteria

Gram-positive bacterial isolates were excluded from the study.

Study design and bacterial isolates

This is a descriptive cross-sectional study. Thirty non-repetitive isolates of Gram-negative bacteria were collected from various clinical specimens at a tertiary care hospital in Mangalore (NU/CEC/2020/0337). The strains included *A. baumannii* (n = 10), *E. coli* (n = 10), and *K. pneumoniae* (n = 10), which were identified and analyzed using the VITEK2 Compact system (bioMérieux VITEK, USA).

Antibiotic susceptibility testing

The antibiotic susceptibility of the bacterial isolates was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (HiMedia, Mumbai, India), as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2023). A total of 15 antibiotic disks were used, including Amikacin (30µg), Amoxicillin/clavulanic acid (30 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Cefuroxime (30µg), Cefoperazone-Sulbactam (75/30 µg), Ceftazidime (30µg), Cotrimoxazole (25 µg), Ertapenem (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Meropenem (10µg), and Piperacillin/Tazobactam (100/10 µg). The zone of inhibition was measured, isolates were classified as susceptible, intermediate, or resistant, and results were interpreted using CLSI, 2023 guidelines. Isolates resistant to three or more different classes of antimicrobials were classified as MDR.

Phenotypic detection of ESBL production by double disk diffusion test

A phenotypic confirmatory test for ESBL producers was conducted using the double disk synergy test. The organism to be tested was spread onto a Mueller-Hinton agar plate. The antibiotics ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), and amoxicillin/clavulanic acid (20/10 µg) were placed at a distance of 20 mm (edge to edge) from the amoxicillin/clavulanic acid disk, which was placed in the middle of the plate. After 24 h of incubation, if an enhanced zone of inhibition appeared between any of the cephalosporin antibiotics and the amoxicillin/clavulanic acid disk, the test was considered positive. This indicated synergistic activity with clavulanic acid and the presence of an ESBL.[10] Quality assurance was performed using ESBL-producing isolates of *A. baumannii* ATCC-MCC2076, *E. coli* ATCC-35218, and *K. pneumoniae* ATCC-700603 as positive controls. Negative controls, such as *Pseudomonas aeruginosa* ATCC-27853 and *E. coli* ATCC-25922, were also used.

Biofilm formation assay

Overnight inoculum for isolates grown in BHI broth was taken for this experiment. 180 µL and 20 µL of culture grown in BHI were added to a 96-well sterile microtiter plate and

incubated at 35–37°C for 24 h. The biofilm formation ability was quantified using a crystal violet assay. The planktonic cells were removed and washed 3 times with 125–200 µL of phosphate-buffered saline (PBS). 150 µL of methanol was added and incubated at room temperature for 20 min to fix the cells. The contents were emptied by inverting the plate, and 150 µL of 0.1% Crystal violet stain was added. The plate was then incubated at room temperature for 15 min, washed with PBS until it was stain-free, and air-dried for 5 min. 150 µL of 33% glacial acetic acid was added for elution. The ability to form biofilm was quantified by measuring optical density (OD) at 570 nm using a microplate reader (TECAN, Switzerland).[11] The isolates were further classified as non-biofilm formers (OD≤ODc), weak biofilm formers (ODc≥OD≤2ODc), moderate biofilm formers (2ODc≥OD≤4ODc), and strong biofilm formers.

Molecular detection of β-lactam genes

All ESBL-producing bacteria identified by phenotypic methods were analyzed by polymerase chain reaction (PCR) to identify genes associated with these phenotypes. DNA was extracted from cultured isolates using the boiled cell suspension method. Briefly, culture suspensions of isolates in LB broth were centrifuged, and pellets were resuspended in 100 µL sterile 1X TAE buffer. The suspension was heated at 96°C for 10 min in a dry bath to lyse the cells and release DNA. Suspensions were cooled for 5 min on ice, followed by centrifugation at a lower speed, around 3000 rpm for 5 min. The purity and concentration of DNA were assessed using a bio-spectrophotometer (Eppendorf, Hamburg, Germany). The supernatant was used as template DNA for amplification. The reactions were performed in 20 µL final volumes with 0.5 µM of each primer, 2 µL DNA, and 10 µL of the HiChrom Master Mix (HiMedia, India). Primer sequences used in the study of the detection of *ESBL* genes are listed in Table 1. The beta-lactam genes bla_{TEM}, bla_{CTX-M}, bla_{SHV}, and $bla_{CTX-M15}$ were amplified with an expected fragment of 329 bp, 503 bp, 656 bp, and 584 bp, respectively. The amplified products were resolved on a 1.8% agarose gel. The fragments were stained with ethidium bromide and visualized and photographed using a gel documentation system (G Box, Syngene). A 100 bp ladder was run as a molecular weight marker. A water sample was run as a blank negative amplification control in each run to exclude contamination.

Statistical analysis

The statistical analyses were performed using GraphPad Prism v9.4.1 software (http://www.graphpad.com, accessed on 25th June 2025).

RESULTS

A total of 30 Gram-negative isolates, including *A. baumannii* (n = 10), *E. coli* (n = 10), and *K. pneumoniae* (n = 10), were evaluated for antibiotic susceptibility, ESBL production, biofilm formation, and genotypic characterization.

Antibiotic susceptibility profile

The resistance pattern among all ten isolates of *A. baumannii* showed 100% resistance to antibiotics such as Amikacin, Cefepime, Ceftazidime, Ciprofloxacin, Gentamicin, Imipenem, Levofloxacin, Meropenem, and Piperacillin-tazobactam. Only one isolate was intermediate to Cefoperazone/sulbactam. All *A. baumannii* isolates tested were found to be MDR, indicating a high prevalence of MDR and highlighting the serious treatment challenge posed by this organism.

The resistance pattern in *E. coli* varied. Seventy percent of isolates were resistant to Ceftriaxone and Ciprofloxacin, 60% showed resistance to Co-trimoxazole, Meropenem, and Clavulanic acid. Resistance to Piperacillin-tazobactam and Amikacin was observed in 50% of the isolates, followed by lower resistance to Amoxicillin/clavulanic acid (20%). Compared to *A. baumannii*, *E. coli* demonstrated a somewhat more favorable susceptibility profile.

The resistance pattern in *K. pneumoniae* isolates showed high resistance to twelve antibiotics. All isolates (100%) were resistant to Ceftriaxone, and a large proportion exhibited resistance to Gentamicin (90%), Cefepime (80%), and

Table 1: The list of primers used for the polymerase chain reaction reactions to identify β-lactam genes

Gene	Primer sequence (5'- 3')	Product size (bp)	Reference
bla_{SHV}	F- AAAGCGAAAGCCAGCTGTCG	656	Kaftandzieva *et al.* (2012)[12]
	R- GTTATTCGGGCCAAGCAGGG		
bla_{TEM}	F- CGCCCCGAAGAACGTTTTCC	329	
	R-CGTTGGGAACCGGAGCTG		
bla_{CTX-M}	F-CGGTGCAACAAAAGCTGGCG	503	
	R- GCGGCTGGGTAAAATAGGTC		
$bla_{CTX-M15}$	F-ATCACTGCGCCAGTTCACGCT	584	
	R-GGCTGGGTGAAGTAAGTGACC		

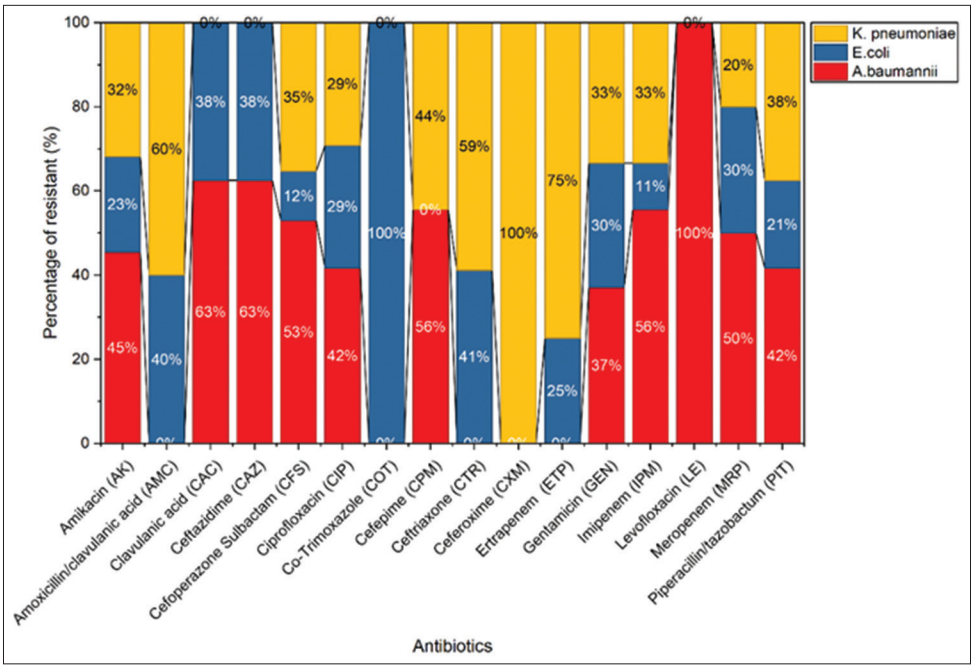

Figure 1: Antibiotic resistance pattern of Gram-negative isolates (*Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumonia*) Bar graph depicting the percentage of resistance against 16 different antibiotics

Ciprofloxacin (70%). In addition, 60% were resistant to Cefoperazone-sulbactam, Ertapenem, and Imipenem, while 40% showed resistance to Meropenem. *K. pneumoniae* also exhibited lower resistance to Amoxicillin/clavulanic acid (30%) compared to *E. coli*. Figure 1 summarizes the percentage of antibiotic resistance. The resistance pattern of *A. baumannii* was significantly higher ($P < 0.0001$) than that of *E. coli* and *K. pneumoniae* ($P = 0.0018$). Similarly, the percentage of drug resistance in *E. coli* was significantly higher than in *K. pneumoniae* ($P = 0.0235$).

Phenotypic detection of ESBL production

Out of 30 strains, eight (26.7%) were ESBL producers, while twenty-two (73.3%) were phenotypically non-ESBL producers. The combination disk diffusion tests showed that none of the *A. baumannii* isolates were ESBL producers.

Biofilm detection

A total of 15 (49.9%) isolates were identified as biofilm producers using the microtiter plate method. In *A. baumannii*, biofilm formation was minimal. Out of ten isolates, two (20%) were weak biofilm producers, while the remaining eight (80%) were non-producers. This suggests that the *A. baumannii* isolates used in this study have limited ability to form biofilms. Four of the ten *E. coli* isolates (40%) produced weak biofilms, while six (60%) did not produce any biofilms. None of these isolates formed moderate or strong biofilms, indicating that *E. coli* strains have a relatively low capacity for biofilm formation. Among the species examined, *K. pneumoniae* showed the highest capacity for biofilm production. Of ten isolates, three (30%) were strong biofilm producers, five (50%) were moderate, one (10%) was weak, and one (10%) was a non-producer. This indicates a higher level of virulence associated with biofilm development in *K. pneumoniae*. Figure 2 presents a bar graph illustrating biofilm formation (none, weak, moderate, and strong) across the three bacterial species. Out of 30 isolates, 15 (50%) did not produce biofilms, 7 (23.3%) were weak producers, 5 (16.6%) were moderate producers, and 3 (10%) were strong biofilm producers. *K. pneumoniae* accounted for most of the strong and moderate biofilm producers, while *E. coli* and *A. baumannii* were the most common weak and non-biofilm producers.

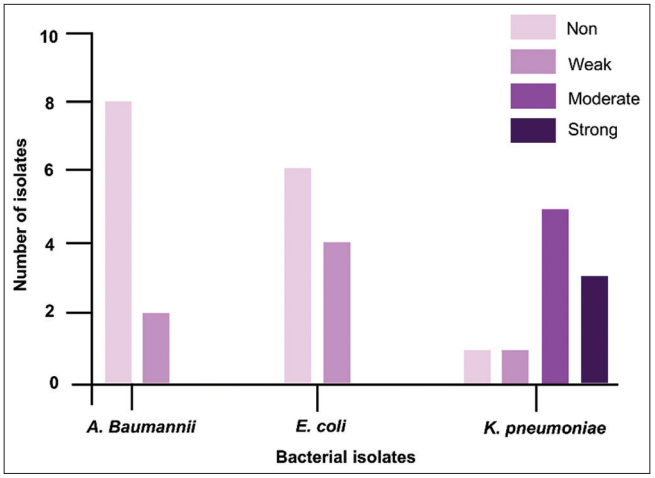

Figure 2: Distribution of biofilm-forming ability among *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumonia* isolates a bar graph shows biofilm production levels (none, weak, moderate, strong) across the three bacterial species

Production of ESBL genes

All 30 isolates were tested for the presence ofbla_{TEM}, bla_{SHV}, bla_{CTX-M}, *and* $bla_{CTX-M-15}$. The isolates showed positive results for ESBL production using phenotypic methods; however, all *A. baumannii* isolates tested negative phenotypically. When screening the 30 isolates for *ESBL* genes via PCR, all were positive for bla_{TEM} (100%), 10 (33.3%) were positive for bla_{SHV} only, 10 (33.3%) were positive for bla_{CTX-M} only, and 15 (50%) were positive for both $_{bla}$CTX-M and $bla_{CTX-M15}$. The most common variant among the ten *A. baumannii* isolates was bla_{TEM} (100%), followed by $bla_{CTX-M15}$ (40%) and bla_{SHV}(20%). Among ten *E. coli* isolates, bla_{TEM} and bla_{CTX-M} were the most common *ESBL* genes (100%), with $bla_{CTX-M15}$ present in 30%. In contrast, bla_{TEM}, bla_{SHV}, and $bla_{CTX-M15}$ were the most prevalent in *K. pneumoniae* isolates (100%, 80%, and 80%, respectively). However, bla_{CTX-M} was not detected in either *A. baumannii* or *K. pneumoniae*, despite nine isolates (30%) testing positive for at least one of the three genes screened (bla_{TEM}, bla_{SHV}, and $bla_{CTX-M-15}$). The distribution of ESBL genes among Gram-negative bacterial isolates is shown in Figure 3, while Figure 4 presents a Venn Diagram illustrating the distribution and overlap of ESBL-encoding genes among clinical isolates.

Association of beta-lactamase production and biofilm formation

Out of 7 ESBL-producing Gram-negative bacteria, 1 (14.3%) was a strong biofilm producer, 1 (14.3%) was a moderate biofilm producer, and 2 (28.6%) were weak biofilm producers. In contrast, among the 23 non-ESBL-producing isolates, 2 (8.7%) were identified as strong biofilm producers, 4 (17.4%) as moderate producers, and 5 (21.7%) as weak biofilm producers. Finally, we statistically analyzed the correlation and found a significant negative correlation between biofilm formation and ESBL production, with a $P = 0.0657$ [Table 2; Figures 5 and 6].

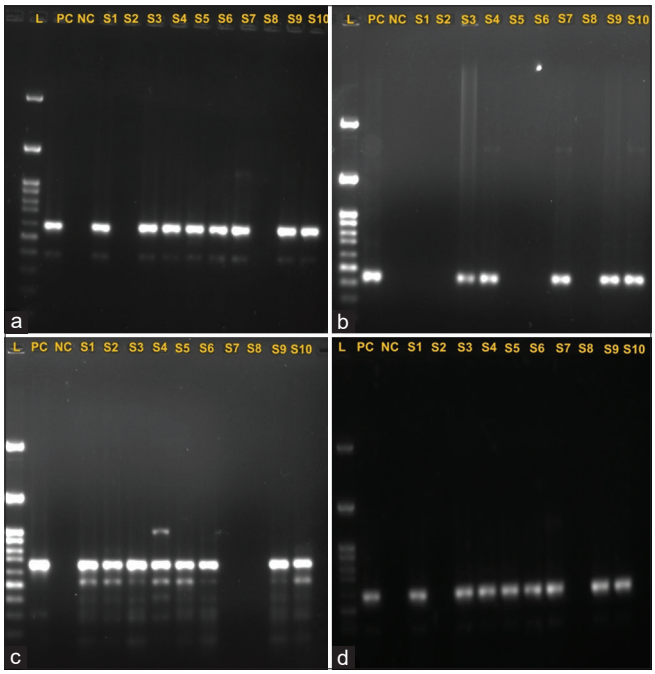

Figure 3: Prevalence of *extended-spectrum beta-lactamase* genes (a) $bla_{CTX-M15}$ (b) bla_{TEM} (c) bla_{SHV} (d) bla_{CTX-M}, among gram-negative isolates Key. L: Ladder, PC: Positive control, NC: Negative control, S1-S10: Samples

DISCUSSION

Bacterial colonization, which forms biofilm, is associated with a wide range of infections. The bacterial biofilm is characterised by cell aggregation, horizontal gene transfer (via plasmids), and increased antimicrobial resistance compared to planktonic bacteria. These structures also enable bacteria to survive in harsh environmental conditions. Organisms such as *A. Baumannii*, *E. coli*, and *K. pneumoniae* are particularly concerning due to their ability to form biofilms.[13] Due to their MDR characteristics, these organisms have been causing infections at an alarming rate in recent years.[14] Before the spread of ESBL-producing bacteria was discovered, beta-lactams were considered miracle drugs. However, the widespread presence of ESBL genes has undermined the effectiveness of beta-lactam antibiotics.[10] This study analyzed 30 clinical isolates of *A. baumannii*, *E. coli*, and *K. pneumoniae* to explore the connections between antibiotic resistance, biofilm formation, and related genes.[15]

In our study, 90% *A. baumannii* strains were found MDR, *w*hich is extremely high. This finding is comparable to

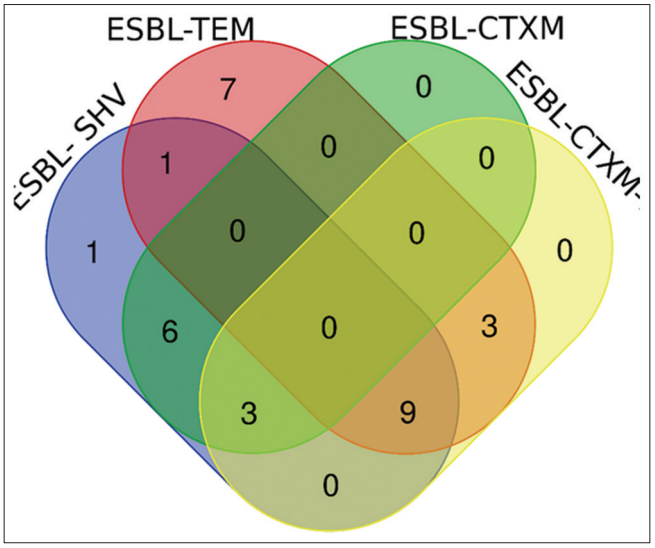

Figure 4: Venn diagram representing the distribution and overlap of extended-spectrum beta-lactamase-encoding genes among clinical isolates. Venn diagram showing the number of isolates positive for bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and $bla_{CTX-M-15}$ genes as detected by polymerase chain reaction. Overlapping regions indicate isolates harboring multiple genes

Table 2: Biofilm production among ESBL and Non-ESBL producers

ESBL detection	Strong biofilm producers (%)	Moderate biofilm producers (%)	Weak biofilm producers (%)	Non-biofilm producers (%)	Total isolates (%)	*P*-value
ESBL producers	1 (14.3)	1 (14.3)	2 (28.6)	3 (42.0)	7 (23.3)	0.0657
Non-ESBL producers	2 (8.7)	4 (17.4)	5 (21.7)	12 (52.17)	23 (76.6)	
Total	3 (10)	5 (16.6)	7 (23.3)	15 (50)	30 (100)	

ESBL: Extended-spectrum beta-lactamase

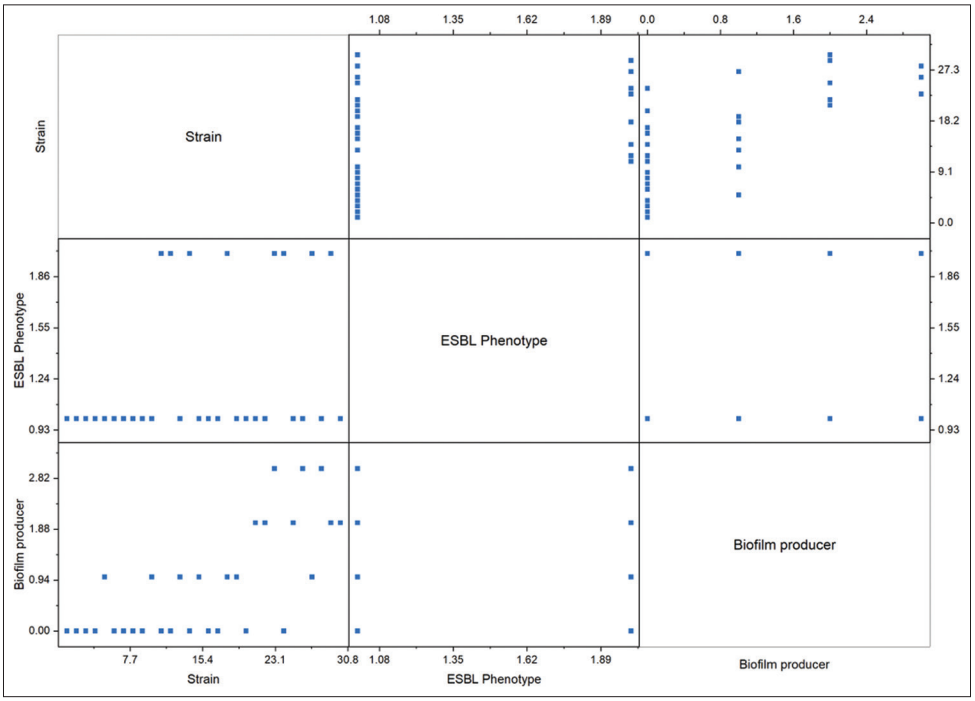

Figure 5: Scatter matrix depicting the association between extended-spectrum beta-lactamase phenotypes and biofilm production

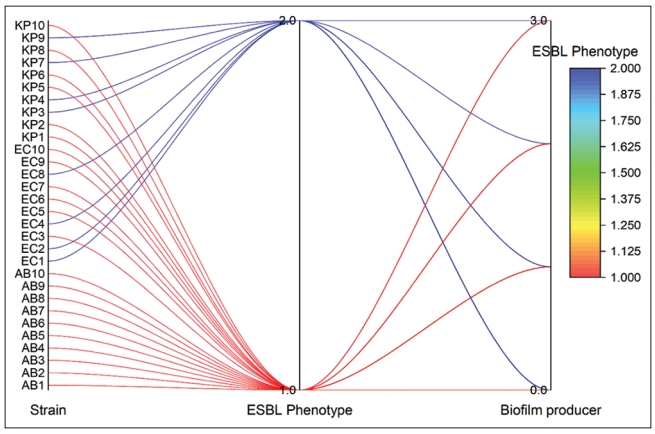

Figure 6: Alluvial chart to describe the interrelation between extended-spectrum beta-lactamase producers and biofilm producers among gram-negative isolates

previous studies reported by Shrestha *et al*., 2024 (96%) and Shrestha *et al*., 2024 (95%).[16] Similarly, Al-Sheboul *et al*. reported a comparable prevalence of 90.3% among *A. baumannii*.[17] The aspartate aminotransferase results demonstrated a high level of resistance against the majority of the antibiotics tested. Approximately 95% of the isolates were resistant to three or more antibiotics and thus can be classified as MDR. Also, *A. baumannii* isolates from Iraq showed high resistance to antibiotics, especially β-lactams, carbapenems, and aminoglycosides.[18] For *E. coli*, ESBL-producing isolates showed the highest susceptibility to amoxicillin-clavulanic acid (80%), ertapenem (70%), and imipenem (70%). On the other hand, gentamicin, ceftriaxone, and ciprofloxacin exhibited high resistance rates (80%, 70%, and 70%, respectively). In contrast, another study reported meropenem (83.1%) and amikacin (82.5%) as the most effective agents, while cefotaxime, ceftriaxone, and ceftazidime showed high resistance rates (79.5%, 78.9%, and 79.5%, respectively).[9] Study from North India on uropathogenic *E. coli* showed the highest resistance to cephalosporins and fluoroquinolones, consistent with our findings.[19]

According to a study by Nirwati *et al*., the majority of *K. pneumoniae* strains were resistant to different antibiotics, with ampicillin, cefazolin, and cefuroxime being the least effective.[8] Amikacin, piperacillin-tazobactam, and meropenem had the best profiles for *K. pneumoniae*.

According to our study, *K. pneumoniae* isolates were equally susceptible to amikacin and completely resistant to ampicillin. Amoxicillin, clavulanic acid, and ciprofloxacin demonstrated resistance rates of 38.75% and 36.69%, respectively. Jomehzadeh *et al.* observed extreme drug resistance in *K. pneumoniae* isolates with AmpC producers, consistent with our findings.[20] Resistance to carbapenems in MDR isolates is often linked to the overuse of the last-resort drug carbapenem. Our study observed 100% resistance to carbapenems in *A. baumannii* isolates, while 60% resistance was noted in *K. pneumoniae*. A similar pattern was observed in studies from Iraq with *Klebsiella* spp.[21,22]

Biofilm formation is a major virulence factor of *A. baumannii*, enhancing its survival and persistence. In our study, 80% of *A. baumannii* isolates were non-biofilm producers, while 20% were weak biofilm producers. A similar study reported 75 MDR *Acinetobacter* isolates categorized as 16% weak, 12% moderate, 40% strong, and 32% non-biofilm producers using the microtiter plate method.[23] In our previous study, we found that bacterial isolates of *A. baumannii* and *K. pneumoniae* were primarily extremely drug-resistant, with positive ESBL phenotypes and strong biofilm producers at 24 h, whereas the current study shows an inverse trend with ESBL phenotypes and strong biofilm producers.[24]

E. coli isolates, 40% were weak biofilm producers and 60% were non-biofilm producers. Subramanian *et al.* found that 80% of biofilm-forming *E. coli* strains also exhibited MDR phenotypes, highlighting the correlation between biofilm formation and drug resistance.[25] A study in Bali, Indonesia, showed similar results wherein *E. coli* and *K. pneumoniae* had higher antibiotic resistance patterns. Still, biofilm production was not significantly associated with the antibiotic phenotypes.[26] According to Nirwati *et al*., out of the 167 *K. pneumoniae* isolates examined, 143 (85.63%) were biofilm producers and 24 (14.37%) were not, out of the total of 167 isolates.[8] Biofilm formation in *K. pneumoniae* was observed in larger amounts.[27] In our study of *K. pneumoniae*, 30% of the participants were strong biofilm producers, 50% were moderate biofilm producers, and 10% were non-biofilm producers. When a phenotypic profile was studied using both of these qualities, it was shown that antibiotic resistance was closely connected with the capacity for biofilm formation. 73% (22/30) isolates had produced biofilms in ESBL–producing *K. pneumoniae*.[28] Bacteria with higher antibiotic resistance lacked or had reduced potential to form biofilms.[29] According to Smiline *et al*., higher rates of bla_{TEM} were found in numerous investigations, with decreased or no prevalence of bla_{SHV} and $bla_{\text{CTX-M}}$.[30] We discovered that more than half of the resistant isolates had bla_{TEM}, less than a tenth had *bla* SHV, and none had $bla_{\text{CTX-M 15}}$. In the current study, bla_{TEM} detection alone confirmed all ESBL-generating *A. baumannii* isolates. In contrast, only two isolates were confirmed by bla_{SHV} detection and four isolates by $bla_{\text{CTX-M 15}}$ detection. When tested for ESBL using phenotypic techniques, all of these isolates tested negative, which is similar to our study. Study on clinical Gram-negative bacteria isolates revealed that 95.0% of *Enterobacterales* and 100% of non-fermenting Gram-negative bacteria were biofilm producers. Further, 16 (5.3%) isolates were phenotypically positive for ESBL, three (50%) carried the *TEM* gene, and one (16.7%) was positive for the SHV.[31] Their study contradicted our findings, where *Enterobacterales* showed average biofilm production, while most non-fermenting Gram-negative bacteria were strong producers. Dirar *et al.* reported prevalent ESBL genotypes were bla_{TEM} (86%), $bla_{\text{CTX-M}}$ (78%), and bla_{SHV} (28%); these genes were found in *K. pneumoniae* (34%, 31%, 26.1%), respectively.[32] In our study, isolates were evaluated for the presence of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{CTX-M15}}$ genes in agarose gel electrophoresis; among them, bla_{TEM} 10(100%), were bla_{SHV} 08 (80%), and $bla_{\text{CTX-M15}}$ 09 (90%), and $bla_{\text{CTX-M}}$ was absent for *K. pneumoniae* isolates.[32] A study from Iran identified 25 (25.5%) *E. coli* isolates as ESBL producers. The $bla_{\text{CTX-M}}$ gene was the most prevalent (44%), followed by bla_{TEM} (24%) and bla_{SHV} (8%), aligning with our findings.[33]

Overall, our findings identified an inverse correlation between ESBL production and biofilm formation, thereby emphasizing on the importance of assessing biofilm formation ability in clinical settings rather than relying solely on AST. This explains the mystery of antibiotic therapy failure and the persistence of infections despite *in vitro* susceptibility.

CONCLUSION

In this study, among the three MDR pathogens screened for biofilm production, *K. pneumoniae* produced significantly more than *A. baumannii*, and *E. coli* and *A. baumannii* were the least biofilm producers. However, when screened for *ESBL* genes, *A. baumannii* carried major *ESBL* genes, followed by *E. coli* and *K. pneumoniae*. Biofilm-forming pathogens pose a significant clinical problem, causing difficult-to-treat infections in hospitalized and comorbid patients. Removing catheters and other implants is essential for controlling biofilm-related infections, but it can be an invasive procedure that negatively affects patients' quality of life. The primary focus is on targeting biofilms. The relationship between drug-resistant Gram-negative isolates and their biofilm-forming potential remains unclear. This may be influenced by the origin of the isolates and their phylogroup distribution. More research is warranted to fully understand the association between the ESBL phenotype and biofilm formation. This will help develop new therapeutic options for mitigating MDR Gram-negative infections.

Outcomes of study

The study showed that all tested Gram-negative isolates were multidrug-resistant, with few showing ESBL production and biofilm formation. However, there was no direct link between ESBL production and biofilm development.

Rationale of study

Thereby reducing antibiotic efficacy and complicating the treatment of infections. They cause the chronicity, persistence, and recurrence of infections, leading to high morbidity and mortality, and pose a major health concern. The clear link between these traits has not been fully explained. Therefore, this study aimed to examine the relationship between beta-lactamase and biofilm formation in Gram-negative isolates.

Limitations

The study's limitations include a small sample size and the lack of screening for biofilm-producing genes in the isolates. Detecting genes related to biofilm formation can aid in the exploration and treatment of biofilm-associated infections.

AUTHOR'S CONTRIBUTIONS

Shriya C Shetty (SCS): Conceptualization, Data Curation, Methodology, Visualization, Writing – Original Draft, Apoorva Sherigar (AS): Data Curation, Formal Analysis, Methodology, Writing – Original Draft, Arun Magdum (AM): Data Curation, Formal Analysis, Methodology, Writing – Original Draft, Sharanya Naik (SN): Data Curation, Formal Analysis, Methodology, Writing – Original Draft, Mohammed Anees TM (MATM): Data Curation, Formal Analysis, Methodology, A Veena Shetty (AVS): Conceptualization, Project Administration, Resources, Supervision, Visualization, and Writing – Review & Editing.

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