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# Correlation of Biofilm and Extended Spectrum Beta-Lactamase Production among Gram-negative Bacteria in a Pediatric Hospital – Gaza Strip, Palestine

Nabil Abdullah El Aila[1*], Ahmed Mohamed-Saed Al Afifi[2], Nahed Ali Al Laham[3]

[1]Department of Medical Laboratory Sciences Faculty of Medical Sciences, Al-Aqsa University Gaza Strip, Palestine.

[2]Microbiology Department, Al Nasser Hospital, Ministry of Health-Gaza.

[3]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al Azhar University

*** Corresponding author**
e-mail address::na.elaila@alaqsa.edu.ps

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## Abstract:

**The Background:** Gram-negative bacilli (GNB) are the most frequent cause of community-acquired infections, health care associated infections, and opportunistic infections. The combined effects of biofilm formation and beta-lactamase production play an important role in facilitating the widespread spread of MDR GNB strains.

**Objectives:** Detection of biofilm formation among GNB, evaluation of their antibiotic susceptibility profile, and assessment of extended spectrum beta lactamase (ESBL) production. Furthermore, to investigate the relationship between ESBL production and biofilm formation in the pediatric patient population at Al Nasser Hospital.

**Methods:** A total of 89 GNB isolates were isolated from different clinical samples collected at Al Naser referral pediatric hospital. The isolates were tested for ESBL production using the double disk synergy test (DDS). Antimicrobial susceptibility testing was done using the Kirby-Bauer disk diffusion method. Quantitative detection of biofilm production was performed using the microtiter plate method.

**Results:** Out of the 89 isolates, 65 (73%) demonstrated the ability to produce biofilm. Among these, 47 isolates (52.8%) were identified as ESBL producers. A statistically significant correlation was observedwas observed between drug resistance (ESBL production) and biofilm formation, with a p-value of 0.0016. Meropenem and amikacin had the highest rates of overall susceptibility, with susceptibility rates of 69.9% and 69.7%, respectively. The frequencies of P. aeruginosa, E. coli, K.pneumoniae, A. spp., Proteus mirabilis, and Serratia marcescens were 34.8%, 31.4%, 29.2%, 2.2%, 1.1%, and 1.1% respectively. Biofilm formation in the previous bacteria was 80.6%, 64.3%, 73.1%, 100%, 100%, and 0% respectively.

**Conclusion:** Antibacterial resistance was correlated with the production of biofilm and ESBL. The coexistence of ESBL and biofilm in GNB is of great public health concern. These factors contribute to the development of chronic, persistent, and recurrent infections, leading to significant morbidity and mortality rates. They therefore represent a serious health crisis.

**Keywords:**
Gram-negative bacilli; Extended-spectrum beta-lactamases; Biofilm; Antibiotic resistance; Pediatric; Gaza Strip.

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## Introduction:

The global spread of bacteria resistant to antimicrobial drugs has reached a critical state. This worldwide crisis of antibiotic resistance has resulted in approximately 700,000 deaths each year (CDC, 2019). Antimicrobial resistance has recently become a major issue for the economy and the world's health. The increase in infections caused by antimicrobial-resistant pathogens or MDR microorganisms impacts healthcare costs, patient well-being, and the capacity to control the spread of diseases (Cepas et al., 2019). Bacteria acquire resistance to antibiotics through a variety of mechanisms, including uptake restriction, alteration of targets, enzyme deactivation, and the utilization of efflux pumps (Munita and Arias, 2019).

The emergence of beta-lactam antibiotic resistance has become a growing problem in the management of healthcare-associated and community-acquired infections caused by GNB. This resistance is mainly attributed to the acquisition and expression of ESBL. ESBLs are a diverse, complex, and plasmid-mediated group of rapidly evolving enzymes that pose significant challenges in patient treatment (Bradford, 2001). GNB is capable of inactivating beta-lactam antibiotics that contain an oxyimino group, such as oxyimino cephalosporins (e.g. ceftriaxone, cefotaxime, ceftazidime), oxyimino-monobactam (e.g. aztreonam) and penicillins (Azekhume et al., 2015). The combination of ESBL production and biofilm formation in GNB can pose a formidable public health challenge, leading to increased antibiotic resistance and difficulty in eradicating infections.

Treatment failure cannot be solely attributed to ESBL-producing bacteria. According to Oslen, some bacteria have the capacity to form biofilms, which significantly increase their resistance to antibiotics up to 1,000 times greater resistance. (Oslen, 2015). Biofilms consist of communities of microorganisms enclosed in a self-produced exopolysaccharide matrix containing a variety of substances including polysaccharides, proteins, and DNA (Cepas et al., 2019; Wang et al., 2020). Around 65% of infectious diseases and 80% of chronic infections are linked to biofilm formation on medical devices and biological surfaces, respectively(Jamal et al., 2018). GNB have a special ability to form biofilms in comparison to Gram-positive bacteria, mainly due to their unique features. The most significant GNB with biofilm-forming ability are *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* (Diekema et al., 2019). These four species are classified as critical priorities by the World Health Organization (WHO) in terms of MDR pathogen concerns (WHO, 2021). To improve protection against infections caused by MDR or , extensively drug resistant (XDR) bacteria, it is essential to accurately understand the interactions between ESBL production and biofilm formation, which is critical to develop effective strategies to combat these drug-resistant infections. Therefore, the aim of this study was to investigate the correlation between biofilm production and ESBL in GNB isolated from a pediatric patient population at Al Nasser Hospital, Gaza Strip.

# Methods:

## Study design

A cross-sectional study was conducted at Al-Nasser Hospital, involving 89 clinical isolates of GNB collected from pediatric patients. The isolates were obtained from different sources, including urine, pus, sputum, blood, and ear discharges. These clinical isolates were collected from August – December 2021. The study received approval from the Department of Human Resources and Development, Ministry of Health – Gaza, and from the local Helsinki Committee of the Palestinian Health Research Council in the Gaza Strip. The study approval number is PHRC/HC/766/20.

## Culture of clinical isolates

The clinical isolates were subcultured on MacConkey agar (HI Media, India) and then incubated aerobically overnight at 37°C. Identification of bacterial isolates was performed by evaluating their culture characteristics and relevant biochemical reactions including API 20E (API, bioMérieux, Marcy- L'Etoile, France).

## Antimicrobial susceptibility testing

Antibiotic susceptibility testing using the modified Kirby-Bauer disk diffusion method was performed on Mueller-Hinton agar according to instructions provided by the Clinical and Laboratory Standards Institute (CLSI, 2017). Prior to inoculation, a swab was dipped in a bacterial suspension with a visual turbidity meeting McFarland standard of 0.5 and fourteen antibiotics were tested; amoxicillin-clavulanic acid (20/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), amoxicillin (30 µg), cephalexin (30 µg), cefuroxime (30 µg), co-trimoxazole (25 µg), meropenem (10 µg), and doxycycline (30 µg).

The zone of inhibition of each antimicrobial agent was evaluated, and the results were reported as resistant, intermediate, or susceptible, indicating the organism's susceptibility to the respective antibiotic.

## Phenotypic detection of ESBLs using Double Disk Synergy test

The DDS test was used to confirm the presence of ESBL producers. In this test, the organism under study was plated on a Mueller–Hinton agar plate (HiMedia, India). Four antibiotics, namely ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), and amoxicillin/clavulanic acid (20/10 µg), were placed at a distance of 20 mm from the amoxicillin/clavulanic acid disc, which was positioned in the middle of the plate. An enhanced zone of inhibition between any of the cephalosporin antibiotics and the amoxicillin/clavulanic acid disc after a 24-hour incubation period indicated a positive result (Niumsup et al., 2008). To ensure quality assurance, *K. pneumoniae*, ATCC number 700603 (known to be an ESBL-producing isolate), and *E. coli* ATCC number 25922 (known to be a susceptible isolate) were used as positive and negative controls, respectively, and this process was performed on a weekly basis.

## Investigation of biofilm production:

The method described by Christensen et al. (1995) is widely regarded as the benchmark for detecting biofilms (Mathur et al., 2006). The samples were tested in duplicates. Briefly, 10 µl cell suspension with optical density of 0.5 at 600 nm was added to each 96 microtiter plate well containing 190 µl of Tryptic Soy Broth (TSB) medium (HiMedia, India). Additionally, 200 µl of autoclaved distilled water was introduced into the peripheral wells to minimize water loss. Then, the microplate was incubated for 24 h at 37°C. After the removal of planktonic cells, the biofilm was fixed with 99% methanol. Following this, the plates underwent two washes with either phosphate buffer saline (PBS) or sterile saline water and were allowed to air-dry. Next, 200µl of a 0.2% crystal violet solution was applied to all wells. After a 15-minute incubation, excess crystal violet was eliminated, and the plates

underwent two more PBS washes and air-drying. Finally, 33% acetic acid was used to dissolve the crystal violet that was attached to the cells. Using an ELISA Microplate Reader (MediaPharm, Italy), the optical density at 570 nm was measured in a flat-bottom 96-well plate to gauge the growth of the biofilm (Stepanović et al., 2000). Biofilm formation of each isolate was classified as weak, moderate, or strong based on the recorded OD values. The intensity of biofilm formation for GNB isolates was determined according to the following criteria:

OD $\leqslant$ 0.1 non-adherent

OD 0.1 < to $\leqslant$ 0.2 weakly adherent

OD 0.2 to $\leq$ 0.4 moderately adherent

OD 0.4 < strongly adherent.

**Statistical Analysis**

The collected data were structured and analyzed using the Statistical Package for the Social Sciences (SPSS) version 20. To assess statistical significance at a significance level below 0.05, various statistical methods were used, including frequency analysis, cross-tabulation, and the use of the chi-square test.

## Results

**Phenotypic ESBL detection**

Out of the 89 isolates, 42 (47.2%) were non-ESBL and 47 (52.8%) were positive for ESBL production. The frequency of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. mirabilis*, and *S. marcescens* was 34.8%, 31.5%, 29.2%, 2.24%, 1.12%, and 1.12%, respectively (Table 1).

**Table 1. Distribution of ESBL and non-ESBL by type of bacteria.**

| | ESBL (%) | Non-ESBL (%) | Total (%) |
|---|---|---|---|
| *Escherichia coli* | 17 (60.7) | 11 (39.3) | 28 (31.5) |
| *Klebsiella pneumoniae* | 17 (65.4) | 9 (34.6) | 26 (29.2) |
| *Pseudomonas aeruginosa* | 10 (32.3) | 21 (67.7) | 31 (34.8) |
| *Acinetobacter baumannii* | 2 (100) | 0 (0.0) | 2 (2.2) |
| *Proteus mirabilis* | 1 (100) | 0 (0.0 | 1(1.1) |
| *Serratia marcescens* | 0 (0.0) | 1 (100) | 1(1.1) |
| Total | 47 (52.8) | 42 (47.2) | 89 (100) |

**Biofilm formation**

Out of the 89 clinical isolates of GNB, 65(73%) were determined to be capable of producing biofilm. In the group of biofilm-producing isolates (65), 27 isolates (41.5%) were classified as strong biofilm producers, 20 isolates (30.8%) as moderate biofilm producers and 18 strains (27.7%) were weak biofilm producers. On the other hand, 24 of the 89 strains (27%) were found to be a non-biofilm producers (Table 2).

**Table 2. Biofilm production among the different isolates of GNB**

| Gram Negative Bacteria | Biofilm (%) | Non-biofilm (%) | Weak (%) | Moderate (%) | Strong (%) |
|---|---|---|---|---|---|
| *Escherichia coli* | 18 (64.3) | 10 (35.7) | 5 | 7 | 6 |
| *Klebsiella pneumoniae* | 19 (73.1) | 7 (26.9) | 5 | 4 | 10 |
| *Pseudomonas aeruginosa* | 25 (80.6) | 6 (19.4) | 8 | 8 | 9 |

| Gram Negative Bacteria | Biofilm (%) | Non-biofilm (%) | Weak (%) | Moderate (%) | Strong (%) |
|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 2 (100) | 0 (0.0) | 0 | 1 | 1 |
| *Proteus mirabilis* | 1(100) | 0 (0.0) | 0 | 0 | 1 |
| *Serratia marcescens* | 0 (0.0) | 1 (100) | 0 | 0 | 0 |
| Total | 65 (73.0) | 24 (27.0) | 18 | 20 | 27 |

The prevalence of biofilm formation among *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. mirabilis* was 80.6%, 64.3%, 73.1%, 100%, and 100% respectively (Table 2).

**Correlation between biofilm production and ESBL production**

Among the 47 isolates that produced ESBL, 11 (23.4%) were classified as moderate biofilm producers, while 24 (51.1%) were categorized as strong biofilm producers. In contrast, out of the 42 non-ESBL-producing isolates, 3 (7.1%) were identified as strong biofilm producers, 9 (21.4%) as moderate producers, and 14 (33.3%) as weak biofilm producers. The correlation between strong biofilm formation and ESBL production was found to be highly statistically significant with a *p*-value of 0.0016 (Table 3, Figure 1).

**Table 3: Biofilm production among ESBL and non-ESBL producers.**

| ESBL detection | Strong (%) | Moderate (%) | Weak (%) | Non-Biofilm (%) | Total Isolates (%) | P-value |
|---|---|---|---|---|---|---|
| **ESBL Producer** | 24 (51%) | 11 (23.4%) | 4 (8.5%) | 11 (23.4%) | 47 (52.8%) | 0.00016 |
| **Non-ESBL Producer** | 3 (7.1%) | 9 (21.4%) | 14 (33.3%) | 16 (38%) | 42 (47.2%) | |
| **Total** | 27 (30.3%) | 20 (22.4%) | 18 (20.2%) | 27 (30.3%) | 89 (100%) | |

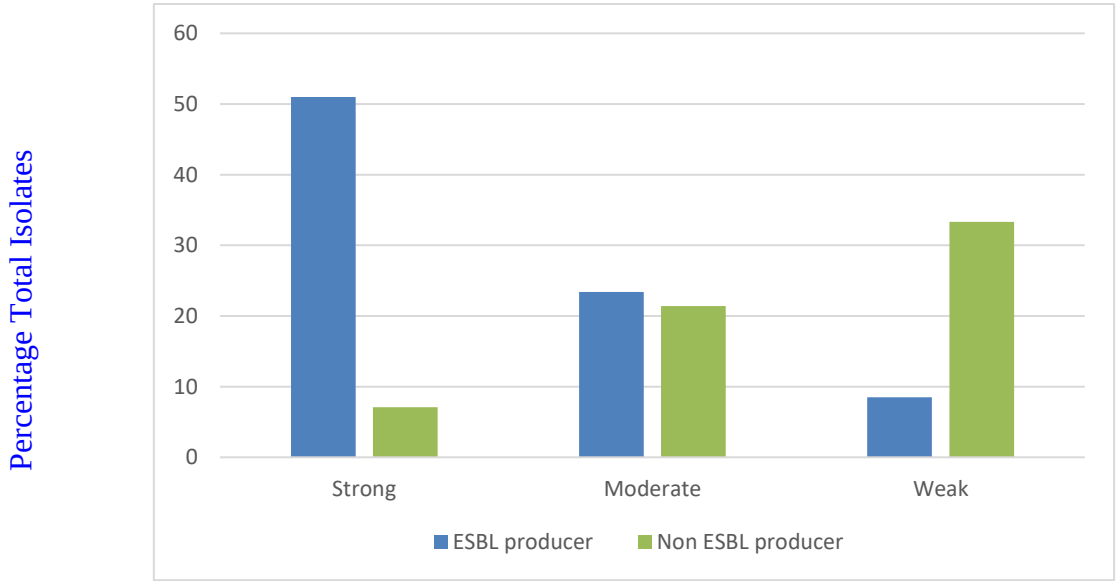


**Figure 1: Correlation between biofilm production among ESBL and non-ESBL producers.**

**Antibiotic susceptibility profile**

Antibiotic susceptibility patterns are presented in Table 4. Among both ESBL and non-ESBL-producing microorganisms, amikacin, carbapenems including meropenem and imipenem, and

piperacillin/tazobactam showed the highest efficacy. In contrast, cefuroxime, cefotaxime, ceftriaxone, piperacillin, and amoxicillin showed notable resistance, especially in ESBL isolates. Compared with non-ESBL-producing bacteria, ESBL-producing bacteria had significantly higher resistance rates to all antibiotics tested in this study, as shown in (Table 4).

**Table 4: Antibiotic susceptibility profile of ESBL and non-ESBL producers.**

| Antibiotic | ESBL = 47 | | | | | | Non-ESBL = 42 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | | I | | R | | S | | I | | R | |
| | % | N | % | N | % | N | % | N | % | N | % | N |
| Amoxicillin | 2.1 | 1 | 4.3 | 2 | 93.6 | 44 | 7.1 | 3 | 9.5 | 4 | 83.3 | 35 |
| Amoxicillin/ Clavulanic acid | 6.4 | 3 | 17 | 8 | 76.6 | 36 | 16.6 | 7 | 11.9 | 5 | 71.4 | 30 |
| Amikacin | 68.1 | 32 | 8.5 | 4 | 23.4 | 11 | 71.4 | 30 | 11.9 | 5 | 16.6 | 7 |
| Aztreonam | 29.7 | 14 | 2.1 | 1 | 70.2 | 33 | 40.4 | 17 | 2.3 | 1 | 59.5 | 25 |
| Cephalexin | 19.2 | 9 | 2.1 | 1 | 78.7 | 37 | 26.1 | 11 | 0 | 0 | 73.8 | 31 |
| Cefuroxime | 0 | 0 | 0 | 0 | 100 | 47 | 2.3 | 1 | 4.7 | 2 | 92.8 | 39 |
| Cefotaxime | 0 | 0 | 0 | 0 | 100 | 47 | 0 | 0 | 23.8 | 10 | 76.1 | 32 |
| Ceftriaxone | 0 | 0 | 2.1 | 1 | 97.9 | 46 | 4.7 | 2 | 7.1 | 3 | 88 | 37 |
| Ceftazidime | 19.2 | 9 | 2.1 | 1 | 78.7 | 37 | 47.6 | 20 | 21.4 | 9 | 30.9 | 13 |
| Chloramphenicol | 29.7 | 14 | 2.1 | 1 | 68 | 32 | 23.8 | 10 | 14.2 | 6 | 61.9 | 26 |
| Ciprofloxacin | 31.9 | 15 | 2.1 | 1 | 66 | 31 | 42.8 | 18 | 11.9 | 5 | 45.2 | 19 |
| Co-trimoxazole | 8.5 | 4 | 4.3 | 2 | 87.2 | 41 | 16.6 | 7 | 0 | 0 | 83.3 | 35 |
| Imipenem | 63.8 | 30 | 10.7 | 5 | 25.5 | 12 | 71.4 | 30 | 7.1 | 3 | 21.4 | 9 |
| Meropenem | 66 | 31 | 6.4 | 3 | 27.6 | 13 | 73.8 | 31 | 4.7 | 2 | 23.8 | 10 |
| Piperacillin | 4.3 | 2 | 2.1 | 1 | 93.6 | 44 | 14.2 | 6 | 4.7 | 2 | 80.9 | 34 |
| Piperacillin/ tazobactam | 59.6 | 28 | 6.4 | 3 | 34 | 16 | 69 | 29 | 9.5 | 4 | 30.9 | 13 |

## Discussion

WHO has identified bacteria classified as critical, high, and medium priority, necessitating urgent antibiotic development, with many belonging to GNB (Breijyeh et al., 2020). Biofilm formation is a key microbial defense mechanism that contributes to antimicrobial resistance, chronic infection persistence, and increased sepsis recurrence (Ghanbarzadeh et al., 2015; Dumaru et al., 2019; Minayan, 2019). Biofilm-associated infections can be both device-related and non-device-related, affecting various medical devices such as contact lenses, heart valves, and central venous catheters (Jamal et al., 2018).

In this study, it was found that 73% of the isolates had the ability to produce biofilm. In comparison, a study conducted by Shrestha et al. in Nepal reported 71.8% of *E. coli* isolates are biofilm producers (Shrestha et al., 2018). Our study identified a higher prevalence of biofilm-forming isolates compared to earlier research conducted by Di Domenico et al. in Italy (64.28%) (Di Domenico et al., 2016), Dumaru et al. in Nepal (62.73%) (Dumaru et al., 2019), and Juliana et al. in India (45.63%) (Juliana et al., 2022).Compared to our results, Shanmugam et al. reported higher biofilm detection in 79 (84%) of the Gram-negative and positive isolates, in addition to a high rate of heavy biofilm production observed in 50 (63.2%) of the isolates (Shanmugam et al., 2017). Furthermore, Swarna et al. and Zubair et al documented a biofilm formation rate of 91% and 80% in their respective studies (Swarna et al., 2012; Zubair et al., 2011).

The biofilm-forming rates of the GNB isolates studied were as follows: *E. coli* (64.3%), *K. pneumoniae* (73.1%), P. aeruginosa (80.6%), *A. baumannii* (100%), and *P. mirabilis* (100%). Notably, *S. marcescens* did not produce any biofilm. Our results were comparable with another study

in Egypt where they reported biofilm formation in a percentage of 60.33%, 77.55%, and 73.68% among *E. coli*, *K. pneumoniae*, and *P. aeruginosa* respectively (Allam, 2017). Our results showed that *E. coli* exhibited the lowest capacity for biofilm formation, while *P. aeruginosa* demonstrated higher capability for biofilm formation, these results were comparable with another study by Syaiful et al (Syaiful et al., 2023). Biofilm forming ability of the isolates was detected in (80.6%) of *P. aeruginosa* isolates which coincides with the finding of Elmanama et al. (80.9%) (Elmanama et al., 2019). Our findings revealed a higher biofilm production rate in K. pneumoniae strains (73%) compared to a study by Thiyagarajan et al., which reported a biofilm formation rate of 50% (Thiyagarajan et al., 2014). On the other hand, the study by Tayal et al. reported a much lower rate of biofilm formation, with only 18% of *K. pneumoniae* strains exhibiting biofilm production (Tayal, Baveja, & De, 2015). This variability may be due to several factors, including the specific hospital where the research was conducted, local antibiotic prescribing habits, the techniques used for biofilm testing, the origin of GNB from outpatients or hospitalized patients, and the nature of the antibiotics used clinical samples. The increasing occurrence of ESBL-producing GNB in various regions of the world, especially in developing countries, is worrisome. Infections caused by ESBL-producing GNB are associated with serious conditions, largely because these strains express virulence factors that contribute significantly to their pathogenicity (Sahly et al., 2008). According to the results of this study, the overall frequency of ESBL in GNB is 52.8%. This is consistent with our recent results (51.6%) from an investigation conducted in four pediatric hospitals within the Gaza Strip (El Aila et al., 2023). Our results showed higher figures in comparison to earlier reports from both regions of Palestine, including the Gaza Strip and the West Bank (Adwan and Abu Jaber 2016; Al-Masri and Jouhari 2016; El Astal and Ramadan 2008; El Aila, 2017). *K. pneumoniae* had the highest prevalence among ESBL-producing GNB, with a rate of 65.4%, closely followed by *E. coli* at 60.7%. This finding was consistent with our most recent findings from pediatric patients in the Gaza Strip, where the prevalence rates of *K. pneumoniae* and *E. coli* were 63.4% and 55.3%, respectively. (El Aila et al., 2023).

The profile of ESBL-producing organisms may vary by location, particularly in cases where isolates change rapidly over time. This is primarily due to the complicated distribution of ESBLs, variations in detection techniques, and various other influencing factors in their epidemiology (Al-Jasser, 2006; Azekhueme et al., 2015). High rates of ESBL infection may be due to a variety of factors, such as inappropriate use of antibiotics to treat febrile infections and inadequate implementation of infection control measures to prevent the spread of multidrug-resistant strains. The results of this study showed a significant difference in biofilm formation, with ESBL producers showing a significantly stronger biofilm formation (51% vs. 7.1%; P = 0.0016). The results are consistent with other studies (Gharrah et al., 2017; Sa'id et al., 2020). Conversely, Dumaru et al reported no significant association between ESBL and biofilm production (Dumaru et al., 2019). Moreover, Shrestha et al, reported a weak positive correlation relationship between biofilm production and ESBL production (Shreshta et al., 2019). Various research studies have pointed out a notable correlation between biofilm formation and ESBL-producing strains in *E. coli* isolates. (Poovendran et al., 2013; Neupane et al., 2016; Tadepalli et al., 2016; Shanmugam et al., 2017), *P. aeruginosa* (Heydari & Eftekhar, 2015) and *A. baumannii* (Roa et al, 2008). In this study, a positive correlation was reported between biofilm formation and ESBL-producing *E. coli*. This agrees with a study reported by (Neupane et al., 2016). This suggests that the biofilm promotes the transmission of the ESBL gene between *E. coli* and other microorganisms. This is due to the matrix of the biofilm, which stabilizes and improves the horizontal transfer of genetic material. In addition, this mechanism helps to bypass immune defenses (Flemming et al., 2016; Maheshwari et al., 2016; Neupane et al., 2016). Compared to other bacteria that do not form biofilms, bacteria in biofilms have the ability to protect themselves from the effects of

antimicrobial agents (Bellifa et al., 2013). Bacteria that form biofilms are frequently linked to drug resistance, relapses, and the emergence of chronic infections (Deotale et al., 2015; Tajbakhsh et al., 2015). This represents a significant problem when initiating therapy (Panda et al., 2016).
Biofilm cells have the ability to detach from their current location and initiate infection at another location. Therefore, it is important to initiate treatment with effective and powerful antibiotics to eliminate biofilm and prevent its formation (Jamal et al., 2018). Bacteria that form biofilms are of great concern because they are associated with hospital-acquired infections and pose a challenge to infection control. This is because drug-resistant bacteria can spread in the biofilm in the hospital area (Deotale, Attal, Joshi, & Bankar, 2015).

**Antibiotic Susceptibility profile**

All ESBL-producing isolates had the highest level of resistance to cefuroxime and cefotaxime, with a resistance rate of 100%. In contrast, the lowest resistance was observed in meropenem (27.6%) and imipenem (25.5%). This is consistent with the studies by Sa'id et al. (Sa'id et al., 2020) and Yazgan et al. (Yazgan et al., 2018), both of which reported resistance rates of 100% for third-generation cephalosporins. This may be due to the extensive use of third-generation cephalosporins without a comprehensive understanding of the severity of the infection (Mshana et al., 2009). Carbapenems are widely used and highly effective antibiotics against GNB. They have a broad ability to fight bacterial infections by targeting penicillin-binding proteins. In addition, they exhibit remarkable resistance to degradation by most lactamases, enzymes that can break down antibiotics (El-Gamal et al., 2017).
Inappropriate use of antibiotics contributes significantly to the emergence of multidrug-resistant bacteria. Therefore, it is essential to monitor the antibiotic sensitivity trends of bacteria commonly found in community settings. This information will guide the initiation of initial treatment, to limit the rise of resistant strains.

## Conclusion

The present study indicates a potential link between the production of ESBL enzymes and the formation of biofilms, which may contribute to antibiotic resistance in GNB isolates. All identified ESBL producers showed resistance to third-generation cephalosporins, but they remain susceptible to carbapenems such as imipenem and meropenem. There is a worrying increase in resistance rates to several commonly used antibiotics. Nevertheless, increased awareness among healthcare professionals and improved diagnostic evaluations in laboratories are crucial to minimize treatment ineffectiveness and prevent the spread of GNB isolates that produce ESBLs. Furthermore, the introduction of a strict antibiotic protocol in hospitals is essential to effectively contain the level of resistance.

## Permissions and Ethical Consideration

The study received approval from the department of human resources and Development, Ministry of Health – Gaza and by the local Helsinki Committee of the Palestinian health research council in the Gaza Strip. The study approval number is PHRC/HC/766/20.

## Competing interests

The authors declare that they have no competing interests

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