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Prevalence of ESBL-Producing Gram-Negative Bacteria Among Isolates Obtained from Fecal Samples of Outpatients of Nablus Area, West Bank-Palestine

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Abstract

Extended-spectrum β-lactamase (ESBL)-producing bacteria are responsible for a considerable burden of difficult to treat infections in different regions of the world. This study was conducted to assess the prevalence, characterize the isolates, and assess the antibiotic susceptibility profiles of ESBL-producing bacteria in fecal samples of outpatients in Nablus, Palestine. The design of this study was a retrospective cross-sectional design, during which 161 Gram-negative bacterial isolates were obtained from the fecal samples of 268 outpatients et al.-Rahma Center, Rafidia Surgical Hospital, and Al-Watani Hospital. These bacterial isolates were identified previously as potential ESBL-producers and then were stored at − 8 0 °C. Out of these isolates 112 (41.7%) were phenotypically confirmed to be ESBL producers and their antibiotic-resistance profile were examined using the disk diffusion method. Female patients were 2.21-times more likely to test positive for ESBL-producing bacteria compared to male patients (95% CI 1.08–4.52) among the tested isolates. *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were the most prevalent ESBL-producing bacteria.

Keywords *Escherichia coli* · *Klebsiella* · Combination-disc · *Stenotrophomonas maltophilia* · Extended-spectrum β-lactamase · ESBL-producing gram-negative bacteria · Antibiotic susceptibility

1 Introduction

Enterobacteriaceae is a large family of Gram-negative bacteria that includes 51 genera and 238 species [1, 2]. Many members of this family inhabit the gut of humans and animals and some members are free living [3]. Although some members are primary human pathogens, while others are opportunistic pathogens such as *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and *Klebsiella* spp. [4, 5].

The primary pathogens of this family are transmitted from human to human through fecal oral route which involves contaminated hands, drinking water, raw milk, as well as contaminated fruits and vegetables [6, 7]. Infections with the opportunistic pathogens of this family may occur as either endogenous infections or exogenous infections. Endogenous infections occur mainly due to fecal contamination, for examples of wounds and the urinary tract, due poor personal hygiene. On the other hand, exogenous infections with these pathogens may occur from animal feces or the surrounding environment [8, 9].

The transmission of these pathogens through various routes is what makes prompt and effective treatment an important clinical priority, which is where antibiotics play a crucial role. Antibiotics are chemical substances that exhibit a selective toxicity to bacteria. They do so by inhibiting essential components of the bacterial cell such as essential enzymes or ribosomes, therefore interfering with their viability and resulting in either death of the bacterial cells

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or inhibition of their replication [10, 11]. Unfortunately, bacterial pathogens have developed several strategies that mediate resistance to antibiotics, among these is the production of enzymes that chemically modify or hydrolyze antibiotics thus inactivating them [12–14]. Emergence of these antibiotic-resistant bacterial pathogens was initially recognized at health care setting, where antibiotics are utilized on a daily basis, thus promoting the emergence of resistant bacteria. Accordingly, infections with these resistant bacteria were most commonly associated with nosocomial infections [15]. However, antibiotic resistant bacteria are being increasingly reported in community acquired infections as well, indicating the spread of these resistant bacteria throughout the communities [16].

Beta-lactams (β-lactams) are among the most commonly used antibiotics world-wide [17], as they are used for the treatment of various types of bacterial infections. Antibiotics that belong to this family share the presence of a β-lactam ring in their chemical structure, hence the name, β-lactam antibiotics. This family includes four subfamilies, which are, Penicillins, Cephalosporins, Carbapenems and Monobactam [18].

In general, β-lactam antibiotics are bactericidal antibiotics that kill the bacterial cell by inhibiting Penicillin binding proteins (PBPs). PBPs are transpeptidases that mediate cross-linking of peptidoglycan, which is the most important component of the bacterial cell wall. Interfering with this cross-linking of peptidoglycan weakens the cell wall, which consequently results in osmotic lyses of the bacterial cells [18]. Unfortunately, many bacteria have developed enzymes called β-lactamases, which are a large family of bacterial enzymes including many subfamilies that can inactivate β-lactam antibiotics by hydrolyzing their β-lactam ring (Fig. 1) [19].

Extended-spectrum β-lactamases (ESBLs) are a group of enzymes that mediate inactivation of penicillins, oxyiminocephalosporins, and the oxyimino-monobactam (Aztreonam) [20]. These enzymes are most commonly found in *Escherichia coli*, *Klebsiella pneumoniae* and other members of the *Enterobacteriaceae* family, as well as in other Gram-negative species that belong to different families [20, 21]. Extended-spectrum β-lactamases (ESBLs) are derivatives of plasmid-encoded β-lactamases that belong to the TEM, Sulfhydryl variable (SHV), and Oxacillin (OXA) enzyme families of β-lactamases [22]. They emerged due to certain genetic mutation(s) that altered their active sites, enabling them to accommodate the large molecular size of the oxyimino side chains of Oxyimino Cephalosporins and Aztreonam, thereby facilitating their hydrolysis [23]. Interestingly, ESBLs can be inhibited in vitro by β-lactamase inhibitors such as Clavulanate, Sulbactam, or Tazobactam. However, inhibition is not effective in vivo [19].

Carbapenems are commonly used for the treatments of infections caused by ESBLs-producing Gram-negative bacteria [24]. However, the emergence of Carbapenem-resistant Gram-negative bacteria has jeopardized the clinical effectiveness of these antibiotics [20, 24]. In the Gaza Strip, the rate of Carbapenem-resistance among 247 clinical and environmental isolates of Gram-negative bacteria was found to reach up to 12.1% [21].

Fecal carriage of ESBLs- and Carbapenemase- producing Gram-negative bacteria may cause endogenous infections that can be either community-acquired or nosocomial [25–27]. In 2021, a meta-analysis study revealed that the average global prevalence of fecal carriage of ESBL-producing *E. coli* in the community was about 16.5% [28].

In Palestine, most of the studies that have investigated the prevalence of ESBL-producing and Carbapenem-resistant Gram-negative bacteria were mainly based on isolates obtained from clinical outpatients [29, 30]. However, a recent study conducted in the Gaza strip area of Palestine in 2023 has found that the prevalence ESBLs-producers among Gram-negative bacteria isolated from fecal samples obtained from both hospitalized patients and out patients was about 37% [31]. No previous studies have been conducted before in the West-Bank area of Palestine to investigate the prevalence of fecal carriage of ESBL-producing and Carbapenem-resistant Gram-negative bacteria in the community. Therefore, the main goal of this study was to assess the prevalence of ESBLs-producing and Carbapenem-resistant Gram-negative bacteria isolated from fecal samples of outpatients in Nablus area -Palestine, as well as to identify these isolates and determine their antibiotic susceptibility profiles.

2 Methodology

2.1 Study Design and Settings

This study was conducted using a retrospective cross-sectional design. This design was chosen to achieve the study objectives as to determine the prevalence of ESBL-producing and Carbapenem-resistant Gram-negative bacteria among isolates that were previously obtained from fecal samples of outpatients in Nablus area (Al-Rahma Center, Rafidia Surgical Hospital, and Al-Watani Hospital), which were stored at − 80 °C until use.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria: Gram-negative bacterial isolates were obtained from fecal samples of outpatients in Nablus area (Al-Rahma Center, Rafidia Surgical Hospital, and Al-Watani Hospital) and stored at − 80 °C.

Exclusion criteria: Gram-positive bacterial isolates.

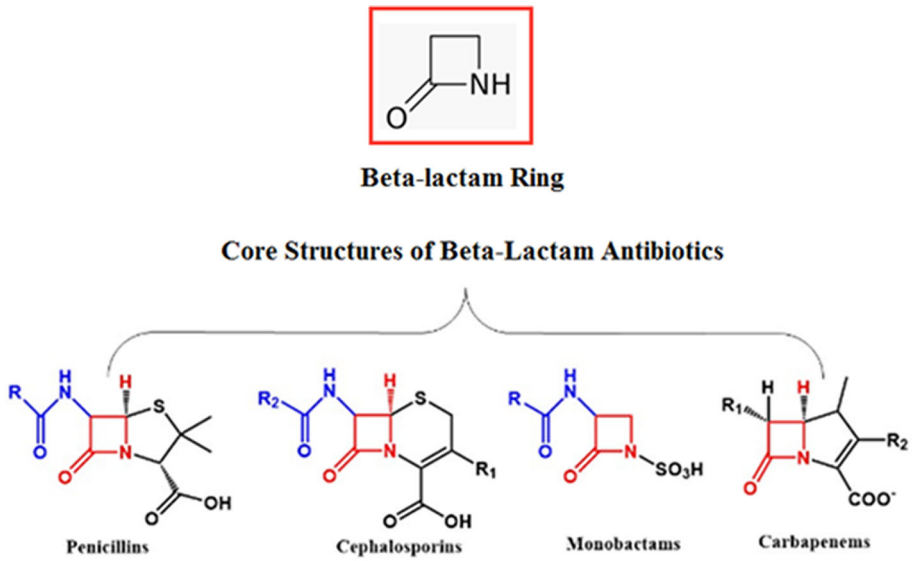

Fig. 1 The β-lactam ring and the core structures of β-lactam antibiotics (18)

2.3 Sample Size

161 Gram-negative bacterial isolates were obtained from fecal samples of 268 outpatients in Nablus area (Al-Rahma Center, Rafidia Surgical Hospital, and Al-Watani Hospital). These isolates were selected based on the basis of their ESBL production upon their screening using freshly-prepared MacConkey agar plates supplemented with Ceftazidime (1 μg/ml). Then the selected isolates were stored at − 80 °C until use.

2.4 Variables

Independent variables: Isolates obtained from fecal samples of outpatients of Nablus Area, West Bank-Palestine.

Dependent variable: The Prevalence of ESBL-producing Gram-negative bacteria and their antibiotic resistance profile.

Background variables: male and female.

2.5 Identification of Gram-Negative Bacterial Isolates

Identification was conducted using the API 20E system according to the manufacturer instruction (BioMerieux, Marcyl' Etoile, France) as well as by using standard microbiological diagnostic tests including Gram stain and growth on MacConkey [32].

2.6 Identification of ESBL-Producers and Investigating Their Antibiotics Resistance Profiles

Phenotypic confirmatory tests were done to examine the production of ESBLs by the bacterial isolates. The phenotypic confirmatory test for the production of ESBLs was conducted using the combination-disc method on Mueller–Hinton agar using Ceftazidime, Ceftazidime-Clavulanic acid, Cefotaxime, and Cefotaxime-Clavulanic acid in accordance with the clinical & laboratory standard institute CLSI guidelines [33, 34]. Any of the obtained isolates was considered as an ESBL-producer whenever it exhibited ≥ 5-mm increase in diameter of the inhibition zone for any of the two antibiotics mentioned above in combination with Clavulanic acid in comparison to the diameter of the inhibition zone when each of these two antibiotics were tested alone [34].

The antibiotic susceptibility/resistance profiles for all of the obtained ESBLs-producers were assessed using the disk diffusion method as recommended by CLSI [34]. The antibiotics used were: Cefepime 30 μg, Aztreonam 30 μg, Amoxicillin 10 μg, Gentamicin 10 μg, Amikacin 30 μg, Tetracycline 30 μg, Ciprofloxacin 5 μg, Trimethoprim-Sulfamethoxazole 12.5/23.75 μg, Imipenem 10 μg, Meropenem 10 μg, Chloramphenicol 30 μg, Ceftazidime 30 μg, Cefotaxime 30 μg. *E. coli* ATCC 25922 was included as a negative control.

2.7 Data Analysis

Data was entered into Excel Sheets and SPSS v.21.0. Age was a continuous variable and was presented as mean ± standard deviation (SD). Additionally, the median with the interquartile range was also presented. Categorical variables were presented as numbers with their corresponding percentages. They were compared using Chi-square tests. Odds ratios with their 95% confidence intervals were calculated using logistic regression. A p value < 0.05 was considered statistically significant.

Table 1 Demographic characteristics of the patients

Variable	*n*	%
Gender		
Male	88	54.7
Female	73	45.3
Age group		
< 18	42	26.1
18–40	66	41
40–60	35	21.7
> 60	18	11.2

Table 2 Association between age and sex with fecal colonization with ESBL-Producing Gram-negative bacteria*

Variable	Category	Number	% within category	*p*
Gender	Male	55	39	0.028
	Female	57	44.9	
Age groups	< 18	26	37.1	0.096
	18–40	50	48.5	
	40–60	22	33.8	
	> 60	14	46.6	

*$n = 112$

3 Results

3.1 Demographic Characteristics of the Patients

Two hundred and sixty eight (268) patients were screened for fecal carriage of ESBL-producing Gram-negative bacteria. The mean age of the 268 patients was 32.3 ± 19.6 years (the median was 28.0, IQR = 17.0, 46.0 years). Of the patients, 70 (26.1%) were younger than 18 years, and 30 (11.2%) were older than 60 years. More than half of the patients (52.6%) were males (males to females' ratio was 1.0:1.1). These detailed characteristics of the patients are shown in Table 1. On the basis of this screening, 161 of the enrolled patients were found to have fecal carriage of Gram-negative bacteria that are potentially ESBL-producers.

3.2 Phenotypic Identification of ESBL-Production

The obtained 268 isolated were subjected for phenotypic identification of ESBL-production using the combination-disc method. Based on this test, 112 isolates were found to be ESBL-producer (Table 2). This implied that 112 patients (41.8%) out of the total patients (268) enrolled in the study were having fecal carriage/intestinal colonization with ESBL-producing Gram-negative bacteria.

Table 3 ESBL-producing gram-negative bacteria*

Strain	Number	% (out of the ESBL producer isolates)	% (out of the total patients colonized with this isolate)
Escherichia coli	87	77.6	32.5
Klebsiella pneumoniae	9	8	3.4
Klebsiella oxytoca	4	3.5	1.5
Stenotrophomonas maltophilia	1	0.8	0.4
Kluyvera spp.	2	1.6	0.8
Providencia rettgeri	1	0.8	0.4
Other species	8	7.1	3

*$n = 112$

3.3 Association between Age and Gender and Fecal Colonization with ESBL-Producing Gram-Negative *Bacteria*

Chi-square tests showed that there was a significant association ($p = 0.028$) between the gender of the patient and testing positive for ESBL-producing Gram-negative bacteria (Table 3). When the odds ratios were calculated, female patients were 2.21-times (95% CI 1.08–4.52) more likely to test positive for ESBL-producing Gram-negative bacteria compared to male patients. On the other hand, age showed no significant association ($p = 0.096$) with testing positive for ESBL-producing Gram-negative bacteria.

3.4 Sensitivity/Resistance to Antibiotics

The sensitivity/resistance profiles of the identified bacteria are shown in Table 4. Of the 112 isolates, 1 (0.9%) was resistant and 111 (99.1%) were sensitive to Imipenem, 1 (0.9%) was resistant and 111 (99.1%) were sensitive to Meropenem, 14 (12.5%) were resistant and 63 (56.3%) were sensitive to Aztreonam, 112 (100%) were resistant and zero sensitive to Amoxicillin, 75 (67%) were resistant and 12 (10.7%) were intermediate resistant to Cefotaxime, 4 (3.6%) were resistant and 74 (66.1%) were sensitive to Ceftazidime. 19 (17.0%) were resistant and 59 (52.7%) were sensitive to Cefepime, 1 (0.9%) was resistant and 111 (99.1%) were sensitive to Amikacin, 24 (21.4%) were resistant and 63 (56.3%) were sensitive to Gentamicin, 64 (57.1%) were resistant and 23 (20.5%) were sensitive to Tetracycline, 57 (50.9%) were resistant and 24 (21.4%) were sensitive to Trimethoprim/Sulfamethoxazole, 17 (15.2%) were resistant and 69 (61.6%) were sensitive to Ciprofloxacin, and

Table 4 Antibiotic resistance patterns of isolated bacteria

Antibiotic		*E. coli* ($n = 87$)	*K. pneumoniae* ($n = 9$)	*K. oxytoca* ($n = 4$)	*S. maltophilia* ($n = 1$)	*Kluyvera spp.* ($n = 2$)	*P. rettgeri* ($n = 1$)	*Other species* ($n = 8$)
Imipenem	*S*	86 (98.9)	7 (77.8)	4 (100)	1 (100)	2 (100)	0 (0)	8 (100)
	I	0 (0)	2 (22.2)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
	R	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	*S*	68 (78.7)	6 (66.7)	4 (100)	1 (100)	1 (50)	1 (100)	3 (37.5)
	I	1 (1.1)	3 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	18 (20.2)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	5 (62.5)
Trimethoprim/Sulfamethoxazole	*S*	24 (27)	2 (22.2)	0 (0)	0 (0)	1 (50)	0 (0)	3 (37.5)
	I	6 (6.7)	1 (11.1)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
	R	59 (66.3)	6 (66.7)	3 (75)	1 (100)	1 (50)	1 (100)	5 (62.5)
Amoxicillin	*S*	–	–	–	–	–	–	–
	I	–	–	–	–	–	–	–
	R	87 (100)	9 (100)	4 (100)	1 (100)	2 (100)	1 (100)	8 (100)
Chloramphenicol	*S*	67 (75.3)	8 (88.9)	2 (50)	0 (0)	2 (100)	1 (100)	7 (87.5)
	I	3 (3.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	19 (21.3)	1 (11.1)	2 (50)	1 (100)	0 (0)	0 (0)	1 (12.5)
Amikacin	*S*	86 (98.9)	9 (100)	4 (100)	1 (100)	2 (100)	1 (100)	8 (100)
	I	–	–	–	–	–	–	–
	R	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	*S*	23 (27)	2 (22.2)	1 (25)	0 (0)	1 (50)	0 (0)	4 (50)
	I	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	64 (73)	6 (66.7)	3 (75)	1 (100)	1 (50)	1 (100)	4 (50)

Table 4 (continued)

Antibiotic		*E. coli* ($n = 87$)	*K. pneumoniae* ($n = 9$)	*K. oxytoca* ($n = 4$)	*S. maltophilia* ($n = 1$)	*Kluyvera spp.* ($n = 2$)	*P. rettgeri* ($n = 1$)	*Other species* ($n = 8$)
Aztreonam	*S*	63 (71.9)	8 (88.9)	3 (75)	1 (100)	2 (100)	1 (100)	6 (75)
	I	10 (11.2)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)
	R	14 (16.9)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	1 (12.5)
Gentamicin	*S*	63 (71.9)	7 (77.8)	2 (50)	0 (0)	2 (100)	1 (100)	7 (87.5)
	I	–	–	–	–	–	–	–
	R	24 (28.1)	2 (22.2)	2 (50)	1 (100)	0 (0)	0 (0)	1 (12.5)
Meropenem	*S*	86 (98.9)	9 (100)	4 (100)	1 (100)	2 (100)	1 (100)	8 (100)
	I	–	–	–	–	–	–	–
	R	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefepime	*S*	60 (67.4)	7 (77.8)	3 (75)	1 (100)	2 (100)	1 (100)	6 (75)
	I	7 (10.1)	2 (22.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	20 (22.5)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	2 (25)
Cefotaxime	*S*	2 (2.2)	0 (0)	0 (0)	0 (0)	1 (50)	1 (100)	0 (0)
	I	10 (13.5)	3 (33.3)	1 (25)	0 (0)	0 (0)	0 (0)	2 (25)
	R	75 (84.3)	6 (66.7)	3 (75)	1 (100)	1 (50)	0 (0)	6 (75)
Ceftazidime	*S*	76 (85.4)	8 (88.9)	4 (100)	1 (100)	2 (100)	0 (0)	8 (100)
	I	7 (10.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	4 (4.5)	1 (11.1)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)

S, sensitive; *I*, intermediate resistance; *R*, resistant

Table 5 Ranking for different bacterial isolates for the resistance/sensitivity of antibiotics

Antibiotic	*E. coli*			*K. pneumoniae*			*K. oxytoca*			*S. maltophilia*			*Kluyvera spp.*			*P. rettgeri*			Other species		
	S	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*
Amikacin	8	1	1	6	1	1	5	1	1	2	1	1	3	1	1	2	1	1	6	1	1
Amoxicillin	1	1	11	1	1	5	1	1	5	1	1	2	1	1	3	1	1	2	1	1	6
Aztreonam	4	6	3	5	2	1	4	1	2	2	1	1	3	1	1	2	1	1	4	2	2
Cefepime	3	5	6	4	3	1	4	1	2	2	1	1	3	1	1	2	1	1	4	1	3
Cefotaxime	2	7	10	1	4	4	1	2	4	1	1	2	2	1	2	2	1	1	1	3	5
Ceftazidime	7	5	2	5	1	2	5	1	1	2	1	1	3	1	1	1	1	2	6	1	1
Chloramphenicol	5	3	5	5	1	2	3	1	3	1	1	2	3	1	1	2	1	1	5	1	2
Ciprofloxacin	6	2	4	3	4	1	5	1	1	2	1	1	2	1	2	2	1	1	2	1	4
Gentamicin	4	1	7	4	1	3	3	1	3	1	1	2	3	1	1	2	1	1	5	1	2
Imipenem	8	1	1	4	3	1	5	1	1	2	1	1	3	1	1	1	2	1	6	1	1
Meropenem	8	1	1	6	1	1	5	1	1	2	1	1	3	1	1	2	1	1	6	1	1
Tetracycline	2	1	9	2	2	4	2	1	4	1	1	2	2	1	2	1	1	2	3	1	3
Trimethoprim/Sulfamethoxazole	2	4	8	2	2	4	1	2	4	1	1	2	2	1	2	1	1	2	2	1	4

S, sensitive; *I*, intermediate resistance; *R*, resistant

finally19 (17.0%) were resistant and 65 (58.0%) were sensitive to Chloramphenicol. The ranking of bacterial resistance to antibiotics is shown in Table 5. The negative control *E. coli* strain exhibited resistance only to Amoxicillin.

3.5 The Prevalence of Multi-Drug Resistance Among the Obtained ESBL-Producing Isolates

The sensitivity profiles for several bacterial isolates are shown in Table 6. Sixty four percent (64.1%) of the isolates were multidrug resistant (resistant to 3 or more antibiotic different families) (24). It's worth mentioning that ESBLs are predicted to be resistant to Penicillins, Monobactam and Oxyiminocephalosporins (except Cephamycin). Although most of the isolates were sensitive in vitro to these antibiotics as shown in Table 6, they are expected to exhibit resistance in vivo [35, 36]. Two isolates showed resistance to Carbapenems, while four isolates showed intermediate resistance, as shown in Table 6.

4 Discussion

ESBL-producing Gram-negative bacteria are responsible for a considerable burden of difficult to treat infections in different regions of the world [20, 21, and 24]. Up to our knowledge, this study is the first to assess the prevalence of these bacteria in fecal samples obtained from outpatients in the Nablus area of Palestine. The findings of this study showed a high prevalence of ESBL-producing bacteria, with 112 isolates among the 268 (41.7%) identified as ESBL-producers. There was also a significant association between the gender of the patient and the likelihood of testing positive for ESBL-producing bacteria. Specifically, female patients were 2.21 times more likely to test positive for ESBL-producing bacteria compared to male patients. The findings of this study also showed variabilities in the antibiotic susceptibility/resistance profiles of the bacterial strains isolated. The results of this study are considered noteworthy and may be beneficial to microbiologists, medical laboratory scientists, infectious disease specialists, gastroenterologists, and other healthcare providers involved in designing measures to treat and prevent the potential infections caused by these or similar strains.

In this study, the prevalence of ESBL-producing bacteria was considerably high. These findings are consistent with previous studies which have reported high prevalence of ESBL-producing bacteria [32]. Specifically, several studies have reported variable prevalence rates of ESBL-producing bacteria in the range of 15%–80% [34, 37, and 38]. It is worth mentioning that this variability in the prevalence rates can be explained by the population of patients included the history of antibiotic intake, settings in which the studies were conducted, nature of the samples obtained, sampling methods, and various variabilities in the analytical/testing methods used [39]. The findings reported in this study indicate the need for healthcare providers to consider local policies and measures to treat and prevent the infections that could be caused by these ESBL-producing bacteria.

Table 6 The antibiotics-resistance profiles of the majority of the ESBL-producing *E. coli* isolates

	Amox	Cefo	Tet	Tri/Sul	Chlo	Gent	Cefe	Aztr	Cip	Imi	Mero	Ceft	Amik
N.C	R	S	S	S	S	S	S	S	S	S	S	S	S
Profile-1													
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	S	S	S	S	S
Profile-2													
E.coli	R	R	R	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	S	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	S	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	S	S	S	S	S	S	S	S	S	S
Profile-3													
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	R	R	S	S	S	S	S	S	S	S	S
Profile-4													
E.coli	R	R	R	R	R	R	S	S	S	S	S	S	S
E.coli	R	R	R	R	R	R	S	S	S	S	S	S	S
E.coli	R	R	R	R	R	R	S	S	S	S	S	S	S
Profile-5													
E.coli	R	R	R	R	R	R	R	R	S	S	S	S	S
E.coli	R	R	R	R	R	R	R	R	S	S	S	S	S
E.coli	R	R	R	R	R	R	R	R	S	S	S	S	S
E.coli	R	R	R	R	I	R	R	R	S	S	S	S	S

Table 6 (continued)

Profile-6													
E.coli	R	R	R	R	I	R	R	R	S	S	S	I	S
E.coli	R	R	R	R	R	R	R	R	S	S	S	I	S
E.coli	R	R	R	R	R	R	R	R	S	S	S	I	S
E.coli	R	R	R	R	R	R	R	R	S	S	S	I	S
Profile-7													
E.coli	R	R	R	R	R	R	R	R	R	S	S	R	S
E.coli	R	R	R	R	R	R	R	R	R	S	S	R	S
E.coli	R	R	R	R	R	R	R	I	R	S	S	R	S
Profile-8													
E.coli	R	R	R	R	R	R	I	R	S	S	R	I	S
E.coli	R	R	R	R	R	R	R	R	S	R	S	I	S
Profile-9													
E.coli	R	R	S	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	R	S	S	S	S	S	S	S	S	S
E.coli	R	R	S	I	S	S	S	S	S	S	S	S	S
E.coli	R	I	S	R	S	S	S	S	S	S	S	S	S
E.coli	R	I	S	R	S	S	S	S	S	S	S	S	S
Profile-10													
E.coli	R	R	S	S	S	S	S	S	R	S	S	S	S
E.coli	R	R	S	S	S	S	S	S	R	S	S	S	S
E.coli	R	I	S	S	S	S	S	S	R	S	S	S	S
Profile-11													
E.coli	R	R	S	S	S	R	S	S	R	S	S	S	S
E.coli	R	R	S	S	S	R	S	S	R	S	S	S	S
Profile-12													
E.coli	R	R	R	R	S	R	S	S	S	S	S	S	S
E.coli	R	R	R	R	S	R	S	S	S	S	S	S	S
Profile-13													
E.coli	R	R	R	I	S	S	R	S	S	S	S	S	S
E.coli	R	R	R	I	S	S	R	S	S	S	S	S	S
E.coli	R	R	R	I	S	S	I	S	S	S	S	S	S
E.coli	R	R	R	I	S	S	I	S	S	S	S	S	S
Profile-14													
E.coli	R	R	R	R	S	S	S	I	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	I	S	S	S	S	S
E.coli	R	R	R	R	S	S	S	I	S	S	S	S	S
Profile-15													
E.coli	R	R	R	R	S	S	S	S	R	S	S	S	S
E.coli	R	R	R	R	S	S	S	S	R	S	S	S	S
Profile-16													

Table 6 (continued)

E.coli	R	R	R	R	S	S	R	R	S	S	S	S	S
E.coli	R	R	R	R	S	S	R	I	S	S	S	S	S
E.coli	R	R	R	R	S	S	I	I	S	S	S	S	S
E.coli	R	R	R	R	S	S	I	I	S	S	S	S	S
Profile-17													
E.coli	R	R	R	R	R	S	S	S	S	S	S	I	S
E.coli	R	I	R	R	I	S	S	S	S	S	S	R	S

N.C, negative control (*E. coli* ATCC 25922); *S*, sensitive; *I*, intermediate resistant; *R*, resistant; Amox, Amoxicillin; Cefo, Cefotaxime; Tet, Tetracycline; Tri/Sul, Trimethoprim/Sulfamethoxazole; Chlo, Chloramphenicol; Gent, Gentamicin Cefe; Cefepime; Aztr, Aztreonam Cip, Ciprofloxacin; Imi, Imipenem; Mero, Meronem; Ceft, Ceftazidime; Amik, Amikacin

As mentioned above, there was a significant association between the gender of the patients and the likelihood of testing positive for ESBL-producing bacteria potentially increasing the likelihood of colonization by such resistant bacterial strains. This could be attributed to several factors, such as the differences in healthcare-seeking behaviors among males and females in different cultures and geographical regions. Although this can also be influenced by many demographic, social, and economic factors. Women typically seek healthcare more often than men, notably for urinary and reproductive health issues. This results in more women being exposed to antibiotics and therefore a higher likelihood of testing positive for antibiotic-resistant bacteria.

In this study, there were no statistically significant differences between the prevalence rates among different age groups including children (younger than 18 years). These findings might encourage larger future studies with the aim of assessing the prevalence of fecal carriage of ESBL-producing bacteria among children, adults, and the elderly. Additionally, our findings might also encourage future studies to compare the prevalence of ESBL-producing bacteria among males and females. These studies should help further understand the underlying reasons for this sex-related disparity.

The results also indicate that different ESBL-producing bacterial strains showed variable sensitivity/resistance profile to the tested antibiotics. Our findings were consistent with other studies that reported such variability in the sensitivity/resistance to different antibiotics [40]. Such high resistance rates are alarming due to the limited treatment options for these bacterial infections. Therefore, these findings are beneficial to healthcare providers involved in recommending treatments and preventive strategies to reduce the prevalence of ESBL-producing bacteria. They are also informative to those involved in setting antimicrobial stewardships and use of antibiotics guidelines. Our findings should help reduce the use of ineffective empirical treatments. In this study, all ESBL-producing bacteria strains were resistant to Amoxicillin and almost all ESBL-producing bacteria strains were sensitive to Meropenem and Imipenem with the exception of 5 isolates out of 112 isolates (4.5%) of the obtained ESBL-producing isolates that exhibited either resistance or intermediate resistance to Carbapenems (Table 4). This implies that about 1.9% of the enrolled patients have fecal colonization with Carbapenem-resistant or intermediate resistant Gram-negative bacteria.

Based on the findings of this study, it is important to educate the general community on the importance of rationale use of antibiotics. Moreover, surveillance programs should be implemented to continuously monitor the spread of antibiotic-resistant bacteria throughout the community as well as to investigate their antibiotics resistance profiles in order to guide empirical treatments. More studies should be conducted to understand the disparities and the contributing factors in the prevalence of ESBL-producing bacterial strains among males and females.

4.1 Strengths and Limitations

One of the most important strengths of this study is the focus on assessing the prevalence rates of ESBL-producing bacteria. The prevalence of these difficult to treat infections was not assessed before in the West Bank, notably among the outpatient population in Nablus area. Therefore, the findings of this study provide important local data that can inform regional policies. In addition, the sensitivity/resistance profiles to antibiotics profiles were established, which should improve the empirical treatments of such infections. Moreover, the well-defined laboratory methods used in this study to identify and characterize the ESBL-producing bacteria add to the robustness of this study and the reported findings.

On the other hand, the Carbapenemase-producing lactose-fermenting gram-negative bacteria were not characterized in this study due to time and financial constraints. In addition, the limited number of samples included in this study was one of the main limitations. Future studies should consider the inclusion of a larger number of samples. Moreover, the

samples were obtained from patients in Nablus region only, and therefore inclusion of samples from different regions in the West Bank should improve the representativeness and external validity of these findings.

5 Conclusion

ESBL-producing Gram-negative bacteria were identified in fecal samples that were obtained from outpatients in the Nablus area. The prevalence of ESBL-producing Gram-negative bacteria was high. *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were the most prevalent ESBL-producing Gram-negative bacteria. Female patients were significantly more likely to test positive for ESBL-producing Gram-negative bacteria. The findings of this study indicate a need for robust antimicrobial stewardship.

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Declarations

Conflict of interest All authors declare no conflict of interest.

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