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ORIGINAL ARTICLE

Prevalence and characterization of quinolone resistance and integrons in clinical Gram-negative isolates from Gaza strip, Palestine

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Abstract

Background Gram-negative bacteria with quinolone resistance and extended-spectrum beta-lactamases (ESBLs) present significant treatment challenges. This study evaluated the prevalence and characteristics of quinolone resistance in Gram-negative strains, investigating the relationship between plasmid-mediated quinolone resistance (PMQR), ESBLs, and integrons.

Methods and results We collected 146 Gram-negative isolates from patients in three Palestinian hospitals. For quinolone resistance isolates, the presence and characterization of PMQR, β-lactamase genes and integrons were studied by PCR and sequencing. Out of 146 clinical isolates, 64 (43.8%) were resistant to quinolones, with 62 (97%) being multidrug-resistant (MDR) and 33 (51.5%) ESBL-producers. PMQR-encoding genes were present in 45 (70.3%) isolates, including *aac(6')-Ib-cr* (26.6%), *qnrA* (18.8%), *qnrS1* (20.8%), and *qnrB* (6.4%). $Bla_{\mathrm{CTX-M}}$ genes were detected in 50% (32/64) of isolates, with $bla_{\mathrm{CTX-M-15}}$ being the most common. Bla_{TEM-1}, $bla_{\mathrm{SHV-1}}$ and bla_{VIM} genes were found in 13, 6, and 4 isolates, respectively. Class I integrons were found in 31/64 (48%) of isolates, with 14 containing gene cassettes conferring resistance to trimethoprim (*dhfr17, dfrA12, dfrA1*) and aminoglycosides resistance genes (*aadA1*, *aadA2*, *aadA5*, and *aadA6*).

Conclusions This study found a high rate of quinolone resistance, ESBL and integrons in clinical Gram-negative isolates from our hospitals. Urgent measures are crucial, including implementing an antimicrobial resistance surveillance system, to control and continuously monitor the development of antimicrobial resistance.

Keywords Quinolone resistance genes · Qnr genes · Extended-spectrum-beta-lactamase (ESBL) · Integrons · Multi-drug resistant (MDR) · Gram negative bacteria

Introduction

Gram-negative bacteria pose a pervasive threat as the leading causes of a spectrum of severe infections, including pneumonia, bloodstream infections, wound infections, and meningitis. These infections are primarily caused by *Klebsiella*, *Pseudomonas aeruginosa*, and *E. coli* [1]. Gram-negative bacteria are a major public health concern worldwide due to their high resistance to most available antibiotics, leading them to become multidrug-resistant (MDR). The prevalence of infections caused by MDR Gram-negative bacteria has witnessed a concerning increase [2]. MDR Gram-negative strains hold pivotal clinical importance, instigating a spectrum of high-risk infections that contribute to elevated morbidity and mortality rates. The global impact of these bacteria is exacerbated by the limited treatment options available for such infections [3].

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Quinolone and fluoroquinolone are synthetic antimicrobials that disrupt bacterial DNA synthesis by inhibiting bacterial topoisomerase type II and topoisomerase IV. They are broad-spectrum agents used to treat infections caused by both Gram-positive and Gram-negative bacteria [4]. However, the prolonged and improper use of quinolone drugs has led to a significant increase in quinolone-resistant isolates [2]. Several mechanisms of quinolone resistance have been reported. These include mutations in chromosomal genes encoding the target enzymes DNA gyrase and topoisomerase IV, as well as a decrease in the intracellular concentration of fluoroquinolones due to efflux pump activity mediated by genes such as *qepA*, *qepA2*, and *oqxAB* [5]. Additionally, plasmid-mediated quinolone resistance (PMQR) determinants play a role in resistance mechanisms. These include *qnr* genes and *aac(6')-Ib-cr*, which protect the targets of quinolones through qnr proteins and hydrolyze quinolones through the aac(6′)-Ib-cr protein [4]. There are five main family groups of *qnr* genes discovered on plasmids or chromosomes in bacteria, namely *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* [2]. Plasmids carrying quinolone resistance genes can spread horizontally, leading to the accumulation of chromosomal mutations that increase the resistance rate and contribute to therapeutic failure [5].

The global incidence of extended-spectrum-beta-lactamase (ESBL) producing Gram-negative bacteria, coupled with quinolone resistance, is on the rise. Infections caused by these strains have been associated with reduced treatment efficacy, increased mortality and morbidity rates, prolonged hospitalization and higher healthcare costs [5].

Several studies have confirmed a significant association between ESBL-producing isolates and the presence of plasmid-mediated quinolone resistance (PMQR) determinants. These studies have reported the presence of *qnr* genes and *aac (6')-Ib-cr* in ESBL-producing isolates [4–8].

Integrons are genetic elements responsible for carrying antibiotic resistance genes and facilitating their dissemination among different bacterial species. These elements are transferred via mobile genetic elements, such as transposons and plasmids [9]. Integrons consist of an integrase gene (*intI*), an integration site (attI) and an attachment site (attC) for gene cassettes. The attI serves as the target site for cassette integration and contains a promoter [10]. Antimicrobial resistance genes are carried within integrated cassettes in integrons, and these genes can spread through the transfer of plasmids or transposons that contain the integrons. Integrons are classified into different classes based on the *intI* genes, with class I and II being the most predominant in resistant clinical isolates [9].

Quinolone resistance has been reported to be increasing in Palestine [11, 12], though few studies have investigated the molecular mechanisms of quinolone resistance, and the association between PMQR with ESBL and integrons [13, 14]. This study aims to evaluate the prevalence and characteristics of quinolone resistance in Gram-negative strains, as well as explore the association between PMQR with ESBL and integrons.

Materials and methods

Bacterial collection and identification

Over a 3 month period from March to June, 2013, a total of 146 non-repetitive Gram-negative bacterial isolates were collected from two Palestinian hospitals (Military Balsam Hospital and Al- Shifa Hospital) and the AL-Remal Martyrs' Health Center in Gaza strip, Palestine. These isolates were isolated from various sources such as urine, wound infections, enteric infections, blood, ear infections, and sputum. The collected samples were transported to the Laboratory of Microorganisms and Active Biomolecules at the Faculty of Sciences of Tunis, University of Tunis El Manar (Tunisia) following standard procedures for bacterial isolation and identification. All isolates were plated on Brain Heart Infusion Agar and MacConkey Agar. Bacterial identification of the Gram-negative isolates was carried out using conventional biochemical tests and the API 20E system (BioMérieux, France). The interpretation of the results interpretation was performed using the API web database. To confirm the identity of the Gram-negative isolates, PCR amplification and sequencing of the 16 S rRNA gene were performed. After bacterial identification, the isolates were stored in skim milk broth at -20 °C and − 80 °C for further analysis.

Antimicrobial susceptibility test

The susceptibility of the Gram-negative isolates to quinolones (nalidixic acid, NAL; 30 µg) and fluoroquinolones (ciprofloxacin, CIP; 5 µg)) was determined using the Kirby-Bauer disk diffusion test. This test was performed following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). In addition to quinolones, the sensitivity of the isolates to other antibiotic agents was assessed. This included β-lactam antibiotics such as ampicillin (AMP; 10 µg), cefoxitin (FOX; 30 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), and amoxicillin–clavulanic acid (AMC; 20/10 µg) (20/10). The sensitivity to imipenem (IMP; 10 µg), a carbapenem antibiotic, was also tested. Aminoglycosides including gentamicin (GM; 10 µg), tobramycin (TOB; 10 µg), and kanamycin (GM; 30 µg), were included in the susceptibility testing. Other antibiotics assessed were trimethoprim–sulfamethoxazole (SXT;

1.25/23.75 µg) and tetracycline (TET; 30 µg). Isolates that exhibited low sensitivity to third-generation cephalosporins were further screened for ESBL production using the double-disk synergy method. This method involved testing the isolates with ceftazidime and cefotaxime disks in the presence of a disk containing amoxicillin/ clavulanic acid [15].

DNA extraction and polymerase chain reaction (PCR)

Genomic extraction was performed using the boiling method. Bacterial colonies were suspended in 500 µl of sterile distilled water and heated for 10 min at 100 °C. After centrifugation for 10 min at 12,000 rpm, the supernatant containing the genomic DNA was collected and stored at -20 °C for later use in PCR [16]. The concentration and purity of the extracted genomic DNA were evaluated using a NanoDrop™ spectophotometer and UV light at 260/280 nm. The acceptable ratio value for all samples was within the range of 1.7–1.9.

For PCR amplification, a final reaction volume of 25uL was used in a DNA thermal cycle (Applied Biosystems Thermal Cycler). The PCR conditions consisted of three steps: denaturation at 94 °C for 5 min, followed by 30–40 cycles of denaturation at 94 °C for 30s, annealing step at a specific temperature listed in Supplementary file S1 (delete table 1 here and add Supplementary file S1) for 30s and extension at 72 °C for 1 min. A final extension step at 72 °C for 5 min was performed. To visualize the amplified PCR fragments, agarose gel electrophoresis was performed. A 1.5% agarose gel in 1× TBE (Tris-borate-EDTA) buffer was used, and the gel was run for 45–60 min at room temperature at 100 volts. The individual bands representing the amplified gene fragments were visualized under UV light after staining with ethidium bromide, which acts as a fluorescent DNA stain. Positive and negative controls were included in all PCR amplifications conducted in the laboratory.

Quinolone resistance elements

To investigate the presence of plasmid-mediated quinolone resistance (PMQR) genes, namely *qnrA, qnrB, qnrS, qepA* and *aac(6')*-Ib, PCR amplification and sequencing were performed. The quinolone resistance isolates were screened for these genes using the methodology described in a previous study by [17] (Supplementary file S1).

Identification of β-lactamase genes and other resistance genes

PCR assays were performed on β-lactam resistance isolates for the detection of $bla_{\text{CTX-M}}$, bla_{TEM}, $bla_{\text{OXA-1}}$, bla_{SHV}, $bla_{\text{CMY-2}}$, bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{SIM} and bla_{GIM} (Supplementary file 1) [18].

Positive PCR reactions were sequenced to confirm the specific variant of β-lactamase genes. The sequences obtained were compared to the blast database of GenBank (www.ncbi.nlm.nih.gov) for identification and characterization purposes. In addition to β-lactamase genes, the presence of antibiotic resistance genes for sulfamethoxazole [*sul*1, *sul*3 and *sul*2], tetracycline [*tet*(A), *tet*(B), and *tet*(C)] and aminoglycosides [*aac*(3)-IV, *aac*(3)-I, and *aac*(3)-II] was detected using PCR [18].

Identification and characterization of integrons

In order to identify and characterize integrons, several PCR assays were performed. The presence of *intI1* and *intI2* genes, which are associated with integrons, was investigated in quinolone resistance isolates. PCR was used to screen for the presence of *qacEΔ1* + *sul1* genes in 3'-conserved regions of class 1 integrons. To detect the gene contents within the variable region of integrons, PCR amplification was performed, and the resulting amplicons were sequenced. Detailed information regarding the primer sequences and PCR conditions used for these experiments are listed in Supplementary file S1 [19].

Results

Bacterial isolates

A total of 146 samples were collected. Of these, 76 (52.1%) were obtained from patients admitted to Al-Shifa Hospital, 47 (32.2%) from Balsam Hospital and 23 (15.7%) from Al-Remal Clinic. The frequency of microbial agents in the infections in the present study was as follows: *E. coli* accounted for 69 (47.3%) isolates, *Pseudomonas* spp. for 35 (24%) isolates, *Klebsiella pneumoniae* for 27 (18.5%) isolates, *Enterobacter cloacae* for 4 (2.8%) isolates, *Proteus mirabilis* for 3 (2.1%) isolates, *Salmonella* spp. for 3 (2.1%) isolates, *Serratia liquefacients* for 2 (1.4%) isolates, *Morganella morganii* for 1 (0.7%) isolate, *providencia rettegeri* for 1 (0.7%) isolate, and *Pasteurella pneumotropica* for 1 (0.7%) isolate. The frequency of microorganisms in terms of the infection site is shown in Table 1. The most common specimen sources were urine, accounting for 86 (58.9%) samples, and wound infections, accounting for 44 (30.1%) samples. Among the urine samples, *E. coli* was the most prevalent strain, accounting for 60 (70%) isolates, followed by *K. pneumoniae* with 13(15.1%) isolates, and *Pseudomonas* spp. with 8 (9.3%) isolates. In the case of wound infections, *Pseudomonas* spp. was the most frequently isolated

Table 1 Frequency of Gram-negative isolates according to the origins of infections in the present study

	Urinary Tract Infections	Wound infections	Enteric infections	Blood stream infections	Ear infection	Sputum	Total
E. coli	60	9					69
Pseudomonas spp.	8	19	2	2	3	1	35
K. pneumoniae	13	10		3	1		27
S. liquefaciens	1		1				2
E. cloacae		4					4
P. mirabilis	3						3
Salmonella spp.			3				3
P. rettegeri		1					1
P. Pneumotropica	1						1
M. morganii		1					1
Total	86	44	6	5	4	1	146

Table 2 The frequency of quinolones resistance among qram-negative isolates

Bacterial isolates	Quinolones susceptible	Quinolones resistance	Total
E. coli	41 (59.4%)	28 (40.6%)	69
E. cloacae	3 (75%)	1 (25%)	4
K. pneumoniae	18 (66.7%)	9 (33.3%)	27
M. morganii	1 (100%)	0	1
P. pneumotropica	0	1 (100%)	1
P. mirabilis	3 (100%)	0	3
P. rettegeri	1 (100%)	0	1
Pseudomonas spp.	11 (31.4%)	24 (68.6%)	35
Salmonella spp.	3 (100%)	0	3
S. liquefaciens	1 (50%)	1 (50%)	2
Total	82 (56.2%)	64 (43.8%)	146

pathogen, accounting for 19 (43.2%) isolates, followed by *K. pneumoniae* with 19 (22.7%) isolates.

Resistance to quinolones

Among the 146 clinical isolates analyzed in the study, 64 (43.8%) showed resistance to quinolones and/or fluoroquinolones, specifically nalidixic acid and/or ciprofloxacin. The prevalence of quinolones resistance varied among different bacterial species. In *E. coli*, the resistance rate was 40.6% (28 out of 69 isolates). *Pseudomonas* spp. exhibited a higher resistance rate of 68.6% (24 out of 35 isolates), while *K. pneumoniae* had a resistance rate of 33.3% (9 out of 27 isolates). Among the smaller sample sizes, *E. cloacae* showed a resistance rate of 25% (1 out of 4 isolates), *P. pneumotropica* had a 100% resistance rate (1 out of 1 isolate), and *S. liquefaciens* displayed a resistance rate of 50% (1 out of 2 isolates) (Table 2).

Antibiotic susceptibility pattern

Sixty-two (97%) of the quinolones resistance isolates were identified as multidrug-resistant (MDR) which exhibited resistant to three or more classes of antibiotics. The majority of the quinolone-resistant isolates exhibited resistance to ampicillin, cefotaxime, trimethoprim-sulfamethoxazole, and Kanamycin. Half of the isolates showed resistance to sulfamethoxazole/trimethoprim, ceftazidime, gentamycin, and cefoxitin. The susceptibility of the isolates to imipenem was 70% (Table 3).

Table 3 Antimicrobial resistance of 64 quinolones resistance isolates

Antibiotics	Susceptible No. (%)	Intermediate No. (%)	Resistance No. (%)
Amoxicillin–clavulanic acid	25 (39.1%)	9 (14.0%)	30 (46.9%)
Ceftazidime	29 (45.3%)	9 (14.0%)	26 (40.7%)
Cefotaxime	8 (12.4%)	1 (1.6%)	55 (86%)
Gentamycin	37 (57.8%)	0	27 (42.2%)
Ampicillin	1 (1.6%)	0	63 (98.4%)
Imipenem	40 (62.5%)	3 (4.7%)	21 (32.8%)
Kanamycin	12 (18.8%)	7 (10.9%)	45 (70.3%)
Nalidixic acid	0	2 (3.1%)	62 (96.9%)
Trimethoprim–sulfamethoxazole	14 (21.9%)	2 (3.1%)	48 (75%)
Cefoxitin	24 (37.5%)	6 (9.4%)	34 (53.1%)
Tobramycin	20 (31.3%)	5 (7.8%)	39 (60.9%)
Ciprofloxacin	19 (29.7%)	3 (4.7%)	42 (65.6%)
Tetracycline	20 (31.3%)	2 (3.1%)	42 (65.6%)

Detection of ESBLs

Among the 64 quinolone-resistant isolates, 51.5% (33 out of 64 isolates) were confirmed phenotypically as ESBL-producers. These 33 ESBL-producers are composed of 22 strains of *E. coli* with a rate of 35.5% (22 out of 60), 9 strains of *K. pneumoniae* (9/27; 33.3%), one strain *E. cloacae* (1/4; 25%), and one strains of *S. liquefaciens* (1/2; 50%).

Quinolone resistance elements

The presence of plasmid-mediated quinolone resistance (PMQR) genes, including *qnrA, qnrB, qnrS, qepA* and *aac(6')*-Ib, was investigated in the quinolone-resistant

isolates using PCR amplification and sequencing, following the methodology described by Rocha-Gracia et al. (2010). Among the quinolone-resistant isolates, 39% (25 out of 64) were found to harbor PMQR genes. The most prevalent gene was *qnrA*, detected in 18.8% (12 out of 64) of the isolates. *qnrS1* was identified in 14.1% (9 out of 64) of the isolates, while *qnrB* was detected in two variants; *qnrB1* and *qnrB4*, found in 6.4% (6 out of 64) and 1.6% (1 out of 64) of the isolates, respectively. Three quinolone-resistant isolates harbored two *qnr* encoding gene; *qnrA* with *qnrS1* in an *E. coli* strain, *qnrS1* with *qnrB1* in *Pseudomonas*, and *qnrB1* with *qnrA* in *K. pneumoniae*. However, *qepA* was not detected in any of the isolates. The distribution of quinolone resistance genes among the studied isolates, categorized by specimens and species, of the isolates is presented in Table 4. The *qnrA* gene was found in nine ESBL-producing isolates, while *qnrS1* and *qnrB1* were detected in five and three ESBL-producing isolates, respectively. Among the quinolone resistant Gram-Negative isolates, the gene *aac(6')-Ib-cr* was present in 26.6% (17 out of 64). A combination of *qnr* genes with *aac(6')-Ib-cr* was identified in 15.6% (10 out of 64) of the isolates. One ESBL-producing *K. pneumoniae* isolates harbored *aac(6')-Ib-cr, qnrB1*, and *qnrA*, while one *Pseudomonas* isolate carried *qnrS1, aac-6'-ib-cr*, and *qnrB1*. Among the tested PMQR genes, the majority of *qnr* genes were found in *Enterobacteriaceae* isolates except for one non-fermenter isolate that carried *qnrS* and *qnrA* genes. The *aac(6')-Ib-cr* gene was predominantly detected in *Pseudomonas* isolates (Table 4).

Identification of β-lactamase genes

In the quinolone-resistant isolates, various β-lactamase genes were identified. The $bla_{\text{CTX-M}}$ genes were detected in 50% (32/64) of quinolone resistant isolates. The $bla_{\text{CTX-M-15}}$ was the most frequently gene detected in 26 isolates, followed by $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-1}}$ were found in three and two isolates, respectively. While $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-27}}$ were found in one isolate. The $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-1}}$ genes were identified in 13 and 6 isolates, respectively. Class B beta-lactamase genes bla_{VIM} that were detected in carbapenem-resistant isolates, three variants of bla_{VIM} were identified ($bla_{\text{VIM-28}}$ in 2 *K. pneumoniae,* $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-2}}$ in *Pseudomonas* spp.). Three β-lactamase genes ($bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$) and ($bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-1}}$, $bla_{\text{VIM-28}}$) were present in association with PMQR genes in 6 *K. pneumoniae* isolates (Table 4).

Resistance mechanisms to Non- β-lactam antimicrobial agents

Resistance mechanisms to non-β-lactam antimicrobial agents were investigated in the quinolone-resistant Gram-negative isolates. Table 4 shows the antibiotic resistance genotypes of the quinolone-resistant Gram-Negative isolates. Thirty-two of the quinolone resistant isolates harbored *sul* genes (*sul1*: 30 isolates; *sul2*: one isolate; *sul1* + *sul2*: four isolates; and *sul1* + *sul3*: one isolate). The *aac(3)-II* gene was found in thirty aminoglycoside-resistant isolates and the *aac(3)-IV* gene in twelve isolates. Twenty-nine of the isolates harbored *tet* genes [*tetA*: nineteen isolates; *tetB*: four isolates; and *tetA* + *tetB*: six isolates].

Characterization of integrons

Class 1 integron has been identified in 31 of quinolone resistant isolates. They were in 13 *E. coli* isolates; 11 *Pseudomonas* spp., 5 *K. pneumoniae*, 1 *E. cloacae* and 1 *S. liquefaciens.* These integrons were detected in 17 ESBL producer isolates and in 14 non-ESBL producer isolates. The *QacEΔ1* and *sul1* genes were documented in integrons in 24 isolates while seven of those integrons lacked the *qacEΔ1* and *sul1* genes. The gene cassette in the variable region of the integron was demonstrated that encoded for resistance to trimethoprim (*dhfr*) and /or spectinomycin (*aadA*) with the following gene cassette arrangements: *dhfr17* + *aadA5* (6 isolates), *dfrA12* + *aadA2* (2 isolates), *dfrA1* + *aadA1* (1 isolate), *aadA6* (4 isolates), and *aadA1* (1 isolate) (Table 4). The genetic cassette content differs between bacterial species, in *E. coli* only *dhfr17* + *aadA5*, in *Pseudomonas* spp. only *aadA6*, and in *K. pneumoniae dfrA12* + *aadA2, dfrA1* + *aadA1*, and *aadA1*.

Discussion

Limited research has been conducted in Palestine on the prevalence and molecular mechanisms of quinolone-resistant Gram-negative strains. This study aimed to fill this knowledge gap by investigating the prevalence of quinolone-resistant isolates, plasmid-mediated mechanisms of quinolone resistance and ESBL-, non-β-lactam antibiotic resistance-associated genes in these strains.

In our study, we observed that 64 out of 146 (43.8%) were resistant to quinolones and/or fluoroquinolones. This indicates a significant rate of quinolone resistance among clinical gram-negative isolates. However, our findings showed a lower quinolone resistance rate compared to previous reports from Egypt (57.2%), China (59.4%), and Iran (68%) [6, 20, 21]. Interestingly, our results contrast with a study

Table 4 Characteristics of the 64 of quinolone-resistant isolates recovered from clinical samples

No.	Bacteria	Hospital	Samples	Resistance phenotype	*ESBL Ph	*B-lact. genes	*Quin. genes	Class 1 integron	
								*Int/qac	*IntI st
1	*E. coli*	Al-Shifa	Urine	AMP, NAL, CHL, CIP			*qnrS1*		
8	*K. pneumoniae*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{\text{CTX-M-14}}$, *aac (3) II, Sul1*	*qnrS1*	+/+	*aadA1*
13	*K. pneumoniae*	Al-Shifa	Urine	CTX, GM, AMP, IMP, NAL, SXT, TOB, CIP	+	$bla_{\text{CTX-M-15}}$, bla_{TEM-1}	*aac(6')-Ib-cr, qnrS1, aac (3) II, sul2*		
14	*E. coli*	Al-Shifa	Urine	CTX, AMP, SXT, CIP	+	$bla_{\text{CTX-M-15}}$	*sul1*	+/+	*dhfr17 + aadA5*
15	*E. coli*	Al-Shifa	Wound	CTX, AMP, NAL, SXT	+	$bla_{\text{CTX-M-15}}$, bla_{TEM-1}	*sul1,sul2*		
16	*E. coli*	Al-Shifa	Urine	GM, AMP, KAN, NAL, SXT, TOB, CIP			*aac (3) II, sul1*	+/+	
22	*Pseudomonas* spp.	Al-Shifa	Wound	CTX, IMP, KAN, AMK, NAL, FOX,			*aac-6'-ib-cr*		
28	*E. coli*	Al-Remal	Urine	AMP, NAL, SXT, CIP		bla_{TEM-1}	*sul1, sul2, qnrA, qnrS1*	+	
38	*Pseudomonas spp.*	Balsam	Blood	CTX, GM, IMP, KAN, NAL, FOX			*aac-6'-ib-cr, aac (3) II*		
39	*Pseudomonas spp.*	Balsam	Wound	CTX, GM, AMP, IMP, KAN, NAL, AMK, FOX, TOB, CIP			*aac (3) II*		
40	*E. coli*	Balsam	Urine	CAZ, CTX, GM, AMP, KAN, NAL, SXT, TOB, CIP, TET	+	$bla_{\text{CTX-M-15}}$	*aac (3) II, aac(6')-Ib-cr, sul1, tetB*		
41	*Pasteurella pneumotropica*	Balsam	Urine	NAL			*qnrA*		
43	*Pseudomonas spp.*	Balsam	Urine	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*aac-6'-ib-cr*		
48	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET			*aac-6'-ib-cr, aac (3) II, sul1, tetA*	+/+	*aadA6*
51	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET			*aac (3) II, sul1*	+/+	
52	*Pseudomonas spp.*	Al-Shifa	Pleural fluid	CTX, GM, KAN, NAL, FOX, TOB, CHL, CIP			*qnrS1, aac-6'-ib-cr, aac (3) II, aac (3) IV, qnrB1*		
53	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*sul1, tetA, qnrS*		
55	*E. coli*	Al-Shifa	Urine	CTX, AMP, NAL, SXT, CIP	+	$bla_{\text{CTX-M-15}}$	*qnrA, Sul1*		
56	*E. coli*	Al-Shifa	Wound	CAZ, CTX, GM, AMP, KAN, NAL, SXT, TOB, CHL, CIP, TET	+	$bla_{\text{CTX-M-15}}$, bla_{TEM-1}	*aac (3) II, aac (3) IV, aac(6')-Ib-cr, qnrA, sul1, tetA, tetB*		
57	*K. pneumoniae*	Al-Shifa	Urine	CAZ, CTX, AMP, KAN, NAL, AMK, SXT, TOB, CIP	+	$bla_{\text{CTX-M-15}}$, bla_{TEM-1}, bla_{SHV1}	*aac(6')-Ib-cr, sul1, qnrA*	+/+	*dfrA12 + aadA2*
58	*E. coli*	Al-Shifa	Urine	CAZ, CTX, AMP, IMP, NAL, SXT, CIP, TET	+	$bla_{\text{CTX-M-15}}$	*sul1, tetA*		
60	*Enterobacter cloacae*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CIP, TET	+	$bla_{\text{CTX-M-55}}$	*aac (3) II, qnrB1, tetA*	+	

Table 4 (continued)

No.	Bacteria	Hospital	Samples	Resistance phenotype	*ESPh	*B-lact. genes	*Quin. genes	Class 1 integron	
								*Int/qac	*IntI st
64	*E. coli*	Al-Shifa	Urine	GM, AMP, KAN, NAL, TOB, CIP, TET		Bla_{OXA1}	*aac (3) II, aac(6')-Ib-cr, tetA, tetB, qnrA*		
66	*K. pneumoniae*	Al-Shifa	Urine	AMC, CAZ, CTX, GM, AMP, KAN, NAL, AMK, SXT, TOB, CIP, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV1}	*aac (3) II, aac(6')-Ib-cr, qnrB1, sul1, Sul2, tetA*	+/+	*dfrA12 + aadA2*
68	*E. coli*	Al-Shifa	Urine	CAZ, CTX, AMP, NAL, CHL, CIP, TET	+	$bla_{CTX-M-15}$	*tetA, tetB, qnrA*		
70	*K. pneumoniae*	Al-Shifa	Wound	CAZ, CTX, GM, AMP, KAN, NAL, FOX, TOB, CIP, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV1}	*aac (3) II, aac(6')-Ib-cr, qnrB1, tetA, qnrA*		
71	*E. coli*	Al-Shifa	Urine	AMC, CAZ, CTX, GM, AMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-15}$	*aac (3) II, aac(6')-Ib-cr, sul1, sul3, tetA, tetB*	+	*dhfr17 + aadA5*
73	*S. liquefacients*	Al-Shifa	Urine	AMC, CAZ, CTX, GM, AMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-15}$	*aac (3) II, aac-6'-ib-cr, sul1, tetA*	+/+	
77	*Pseudomonas spp.*	Al-Shifa	Sputum	AMC, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET			*qnrB1, aac-6'-ib-cr, aac (3) II, aac (3) IV, sul1, tetA*	+/+	
79	*E. coli*	Al-Shifa	Wound	AMC, CAZ, CTX, AMP, NAL, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}	*tetB, qnrA, aac-6'-ib-cr*		
80	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL, CIP, TET			*qnrB1, aac-6'-ib-cr, aac (3) IV, sul1, tTetA, tetC*	+/+	*aadA6*
81	*E. coli*	Al-Shifa	Wound	GM, AMP, NAL, SXT, TOB, CHL, CIP			*aac (3) II, sul1*	+/+	
83	*E. coli*	Al-Shifa	Wound	CTX, AMP, NAL, SXT, CIP	+	$bla_{CTX-M-15}$	*sul1*	+/+	
87	*E. coli*	Al-Shifa	Urine	AMP, NAL, CHL, CIP					
88	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET		$bla_{CTX-M-15}$	*aac (3) IV, tetA*		
92	*E. coli*	Al-Shifa	Urine	AMC, CAZ, CTX, AMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP	+	$bla_{CTX-M-3}$	*aac (3) II, aac(6')-Ib-cr, sul1, sul2*	+/+	*dhfr17 + aadA5*
93	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, CHL, CIP, TET			*aac-6'-ib-cr, aac (3) IV, sul1, tetA*	+/+	
94	*K. pneumoniae*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-14}$, bla_{VIM-28}	*aac (3) II, qnrS1, sul1, tetA, tetB*	+/+	*dfrA1 + aadA1*
95	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL			*aac-6'-ib-cr, aac (3) IV*	+/+	*aadA6*
98	*E. coli*	Al-Shifa	Urine	AMC, CAZ, CTX, GM, AMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-15}$	*aac (3) II, aac(6')-Ib-cr, sul1,tetB, qnrA*	+	*dhfr17 + aadA5*
100	*Pseudomonas spp.*	Al-Remal	Ear	NAL, SXT, CIP					

Table 4 (continued)

No.	Bacteria	Hospital	Samples	Resistance phenotype	*ESBL Ph	*B-lact. genes	*Quin. genes	Class 1 integron	
								*Int/qac	*IntI st
106	*Klebsiella*	Balsam	Urine	CAZ, CTX, AMP, IMP, KAN, SXT, CHL, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV1}	*aac(6')-Ib-cr, qnrS1, tetB*	+	
108	*E. coli*	Al-Remal	Urine	AMC, CAZ, CTX, AMP, NAL, SXT, FOX, TET	+	$bla_{CTX-M-15}$	*tetA*		
115	*E. coli*	Al-Remal	Urine	AMP, NAL, SXT, TET	+	bla_{TEM-1}	*qnrA*	+	
117	*E. coli*	Balsam	Urine	AMC, CTX, AMP, NAL, SXT, FOX, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}	*qnrA*	+	*dhfr17 + aadA5*
119	*Pseudomonas spp.*	Balsam	Blood	CTX, NAL, SXT, FOX			*aac-6'-ib-cr, sul1*		
121	*E. coli*	Balsam	Urine	CAZ, CTX, AMP, KAN, NAL, AMK, SXT, TOB, CIP, TET	+	$bla_{CTX-M-15}$	*aac (3) II, aac(6')-Ib-cr, sul1, tetA*	+/+	*dhfr17 + aadA5*
122	*Pseudomonas spp.*	Balsam	Urine	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*aac-6'-ib-cr*		
126	*E. coli*	Balsam	Urine	CTX, AMP, KAN, NAL, CHL, CIP, TET	+	$bla_{CTX-M-1}$	*tetA*		
128	*Pseudomonas spp.*	Balsam	Urine	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*aac (3) IV, aac-6'-ib-cr*		
133	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET		bla_{VIM-1}	*aac-6'-ib-cr, aac (3) II, aac (3) IV, Sul1, tetA*	+/+	*aadA6*
134	*E. coli*	Balsam	Urine	CTX, GM, AMP, KAN, NAL, AMK, SXT, TOB, CHL, CIP, TET	+	$bla_{CTX-M-14a}$	*aac (3) II, aac(6')-Ib-cr, sul1*	+/+	
138	*E. coli*	Balsam	Urine	CTX, AMP, KAN, NAL, SXT, CIP	+	$bla_{CTX-M-15}$, bla_{TEM-1}	*sul1*	+/+	
139	*K. pneumoniae*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, TET	+	$bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV1}	*aac(6')-Ib-cr, tetA*		
140	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET		bla_{VIM-2}	*aac-6'-ib-cr, aac (3) II, aac (3) IV, sul1*	+/+	
142	*E. coli*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-15}$	*aac (3) II, aac(6')-Ib-cr, sul1,tetB*		
143	*K. pneumoniae*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	+	$bla_{CTX-M-15}$, bla_{SHV1}, bla_{VIM-28}	*aac (3) II, aac(6')-Ib-cr, qnrS1, sul1, tetB*		
144	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CAZ, CTX, GM, IMP, KAN, NAL, SXT, FOX, CHL, CIP, TET			*aac-6'-ib-cr, aac (3) II, aac (3) IV, sul1*	+/+	
145	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*tetA*		
147	*E. coli*	Balsam	Urine	CTX, AMP, KAN, NAL, AMK, CIP, TET	+	$bla_{CTX-M-27}$	*tetA*		
150	*Pseudomonas spp.*	Al-Shifa	Wound	AMC, CTX, KAN, NAL, SXT, FOX, CHL, TET			*sul1, tetA*	+	
151	*E. coli*	Balsam	Urine	CTX, AMP, KAN, NAL, CHL, CIP, TET	+	$bla_{CTX-M-1}$	*tetA*		

Table 4 (continued)

No.	Bacteria	Hospital	Samples	Resistance phenotype	*ESBL Ph	*B-lact. genes	*Quin. genes	Class 1 integron	
								*Int/qac	*IntI st
153	*Pseudomonas spp.*	Al-Shifa	Wound	CTX, KAN, NAL, FOX			*aac (3) IV*		
155	*Pseudomonas spp.*	Balsam	Urine	CTX, AMP, NAL, SXT, FOX, CHL		$bla_{CTX-M-15}$	*aac(6')-Ib-cr, qnrB4, sul1*	+/+	

* *ESBL Ph, ESBL Phenotyp;* *B-lact genes, B-lactmase genes; Quin. Genes, Quinolone resistance genes and others; *IntI/*qac*., IntI-1/*qacEΔ1* + *sul1*; *IntI st, Integron structure

conducted in the United States, which reported a quinolone resistance rate of 21% among uropathogenic *Enterobacteriaceae* [22]. This discrepancy suggests regional variations in resistance patterns and highlights the importance of local surveillance studies. The high rate of quinolone resistance observed in our country may be attributed to several factors. Overuse or misuse of these antibiotics in Palestine, both in human medicine and veterinary practices, could contribute to the development and spread of resistance. Additionally, the easy availability of quinolones and the lack of an antimicrobial treatment policy in Palestine may further exacerbate the problem. Addressing the issue of quinolone resistance in Palestine requires an accurate approach, including promoting judicious use of antibiotics, implementing effective infection control measures, and developing and enforcing antimicrobial stewardship programs. These interventions are essential to preserve the effectiveness of quinolones and combat the growing threat of antimicrobial resistance in our region. In this study, we found that quinolone-resistance isolates exhibited high levels of resistance to β-lactams, aminoglycosides and trimethoprim-sulfamethoxazole. Remarkably, 97% of the isolates were classified as multidrug-resistant (MDR), displaying resistance to at least one agent in three or more classes of antibiotics. The presence of transferable plasmid-mediated quinolone resistance determinants in quinolone-resistant Gram-negative isolates is a significant contributing factor to their resistance profiles. These resistance genes are often co-located on plasmids alongside resistance genes for other antibiotic classes, such as β-lactams and aminoglycosides [5]. This genomic organization helps elucidate the high rates of resistance to these other antibiotic classes, as well as the high prevalence of MDR strains observed in this study.

Interestingly, the prevalence of carbapenem resistance (32.8%) was significantly higher in our quinolone-resistance isolates compared to other studies conducted in Iran (3.2) and Egypt (14%) [5, 6]. However, it should be noted that another study reported a much higher rate of 60% of quinolone-resistant isolates being resistant to carbapenems [2]. In recent years, the emergence of carbapenem-resistant bacteria has become a clinical problem worldwide. These drugs are considered the most potent antibiotics for treating infections caused by MDR bacteria.

Regarding the sources of quinolone-resistance isolates, a substantial proportion (47%) was isolated from urine samples, which aligns with the findings reported by Helmy and Kashef, where the majority of resistant isolates (36%) were obtained from urine [23]. These findings underscore the alarming levels of multidrug resistance among quinolone-resistant isolates and the need for effective infection control measures and antimicrobial stewardship programs to combat the spread of resistance. Furthermore, the high prevalence of carbapenem resistance highlights the urgency of implementing strategies to prevent the emergence and dissemination of carbapenem-resistant bacteria in our healthcare settings.

In this study, we observed that among the 64 quinolones-resistant Gram-negative isolates, *Pseudomonas* spp. was the most frequently isolated species (68.6%), followed by *E. coli* (40.6%) and *K. pneumoniae* (33.3%). This distribution differs slightly from a similar study conducted in Turkey, where *P. aeruginosa* was the most resistant to quinolones (60.0%), followed by *E. coli* (38.6%) [24]. Another clinical study reported that *K. pneumoniae* was the most frequently resistant species (66.7%), followed by *E. coli* (21.7%) [2]. These variations in species distribution highlight the regional differences in resistance patterns and the importance of local surveillance studies.

Among the quinolones resistance isolates in our study, 51.5% (33/64) were identified as ESBL-producers using the double-disk synergy method. These findings are consistent with the studies conducted by Taha, Omar [5] and Rao et al. [25], which reported that more than half of the *Enterobacteriaceae* isolates were ESBL-producers. However, in contrast to our findings, Azargun et al. [6] reported that 34.2% of the isolates were ESBL-producers. Furthermore, a study conducted in Morocco reported that 20% (39/188) of Gram-negative isolates were ESBL producers [26]. These variations in ESBL production rates among quinolone-resistant isolates highlight the dynamic nature of resistance patterns and the importance of continuous surveillance and monitoring of resistance mechanisms. Understanding the prevalence and distribution of ESBL-producing strains is crucial

for the development of effective treatment strategies and infection control measures.

In the current study, we found that among the ESBL-producing isolates, *E. coli* accounted for 66.6% (22/33), while *K. pneumoniae* represented 27.3% (9/33) of the isolates. These findings differ from an Iranian study where 53.5% of ESBL-producing isolates were *K. pneumoniae* and 33.8% were *E. coli* [6]. Similarly, in India, 61.4% of *E. coli* were ESBL producers, while 46.2% of *K. pneumoniae* isolates exhibited ESBL production [25]. These variations in the distribution of ESBL producing strains can be attributed to several factors such as different regions, the hospitalization period, antibiotic usage patterns, and local antimicrobial resistance policies.

In our study, the most predominant plasmid-mediated quinolone resistance (PMQR) gene was *aac(6')- Ib-cr*, which is consistent with the findings of other studies [6, 27]. The high prevalence of the *aac(6')- Ib-cr* gene can be attributed to its broad spectrum of activity against both quinolones and aminoglycosides. Similar studies conducted in different regions have also identified *aac(6)-Ib-cr* as the most prevalent PMQR gene in quinolone-resistant Gram-Negative isolates. For example, Badamchi et al. [28] reported a 24% prevalence of the *aac(6)-Ib-cr* gene in uropathogenic *E. coli*, while Ma et al. [29] found an 18.8% prevalence of this gene in *E. coli* isolates.

The *aac(6')-Ib* gene encodes aminoglycoside-modifying enzymes responsible for resistance to tobramycin, kanamycin, and amikacin. The *aac(6')-Ib-cr* variant gene can confer resistance to both aminoglycosides and fluoroquinolones [28]. The existence of the *qnr* with *aac(6')-Ib-cr* genes further promotes the development of multidrug resistance isolates, as demonstrated in previous studies conducted in China [30]. Considering the widespread use of aminoglycosides and fluoroquinolones for patient treatment in Palestine, it is plausible that the high prevalence of *aac(6')-Ib-cr* and its association with multidrug resistance contribute to the emergence of MDR isolates in our study population. These findings underscore the need for judicious use of antibiotics, effective infection control measures, and continuous surveillance of resistance patterns to mitigate the development and spread of MDR bacteria.

In our study, we found that 39% (25/64) of quinolone-resistant isolates carried *qnr* genes, which is consistent with a previous study [31]. However, this prevalence was lower than reported in two studies from Egypt (60%) and Iran (89.1%) [2, 6], and higher than in an Italian study that reported a prevalence rate of 17% [32]. Among the *qnr* genes, *qnrA* was the most dominant, which aligns with the findings of previous studies [5]. The presence of three *qnr* genes (*qnrA*, *qnrS1* and *qnrB*) among our isolates has been reported in previous studies [4, 6, 33]. Additionally, three quinolone-resistant isolates in our study harbored two qnr encoding genes, including *qnrA* with *qnrS1* in *E. coli* strain, *qnrS1* with *qnrB1* in *Pseudomonas*, and *qnrB1* with *qnrA* in *K. pneumoniae*. These findings are consistent with studies from Poland, China and Algeria [34–36].

Interestingly, our results revealed that 60% of PMQR genes (*aac(6')-Ib-cr*, *qnrA*, *qnrS1* and *qnrB1*) were found in a significant portion of ESBL-producing isolates (72.7%, 24/33). The presence of these genes in ESBL producers may be due to the coexistence of plasmids carrying ESBL and PMQR genes, which can explain the co-resistance to beta-lactams and fluoroquinolones [36]. Furthermore, we found that most of the qnr-positive *Enterobacterales* isolates in our study were ESBL producers. Among the qnr-carrier isolates, 33 produced CTX-M, 13 produced TEM-1 and 6 produced SHV-1. Several studies have also reported the association between qnr-positive isolates and ESBL production [4, 7, 8, 26].

CTX-M-15 was the most predominant ESBL type, which is consistent with findings from other studies [6]. In a Palestinian study, CTX-M-15, CTX-M-56, OXA-1, SHV-1, and TEM-1 genes were associated with PMQR genes, including *aac(6')-Ib-cr* and *qnrB2* in ESBL-positive strains [14]. One significant finding of our study is the significant association between *aac(6')-Ib-cr* and *qnr* with β-lactamase genes ($bla_{\text{CTX-M}}$, bla_{TEM-1}, bla_{SHV1}) in *Enterobacterales* isolates, which has also been detected in clinical Enterobacterial isolates from Iran, Uruguay, and Togo [4, 6, 37]. *Qnr* genes are commonly found in plasmids that carry different resistance determinants, including β-lactamase genes [38]. The important association of ESBL-producing isolates with PMQR genes is clinically significant due to the limited therapeutic options for these isolates and may lead to treatment failure and increased mortality rates in patients.

One of the significant results of this study is the detection of class B beta-lactamase genes bla_{VIM} among carbapenem-resistant isolates, specifically in three variants $bla_{\text{VIM-1}}$,$bla_{\text{VIM-2}}$ and $bla_{\text{VIM-28}}$. This is noteworthy because the production of VIM enzymes is associated with a significant decrease in susceptibility to various β-lactam antibiotics, particularly carbapenems, which are crucial for treating multidrug-resistant Gram-negative bacteria. We also observed a significant association between bla_{VIM} and PMQR genes (*aac(6')-Ib-cr, qnrS1*), which aligns with the findings of an Italian study where *qnrS1* was found in 21 out of 24 VIM-positive isolates [39]. A study conducted in Taiwan demonstrated a high prevalence of *qnr* (78.6%) among producers of bla_{IMP}, another type of metallo-beta-lactamase [40]. The production of VIM enzymes is indeed associated with significant reductions in susceptibility to different β-lactam antibiotics, especially carbapenems. Carbapenems are crucial drugs for treating multidrug-resistant

Gram-negative bacteria, and when these bacteria produce VIM enzymes, it poses a challenge in terms of limited treatment options.

CTX-M-type enzymes belong to a group of class A ESBLs that are plasmid-mediated [4]. In our isolates, the simultaneous production of CTX-M-type with other plasmid-mediated β lactamase types such as TEM, SHV, VIM is noteworthy. The presence of both ESBLs and VIM-MBL, in the same isolates has been reported in *Enterobacteriaceae* in Italy [39]. This association suggests a concerning integration and co-localization of diverse resistance genes on individual plasmids or integrons. The concurrent presence of both MBLs and ESBLs in PMQR-positive bacteria highlights the accumulation of critical resistance determinants. This observation raises alarming concerns about the further development and dissemination of MDR strains. The emergence of such extensively resistant strains can result in significant mortality and morbidity, as therapeutic options become severely limited.

In this study, we found class I integrons were found in 31 out of 64 (48%) of the isolates. Similarly, in an Egyptian study, class I integrons were detected in 59 (44%) out of 134 clinical isolates of *E. coli* [9]. Abbasi, Ghaznavi-Rad [41] detected class 1 integron in 71.4% of quinolone-resistant *Salmonella* species isolated from diarrheic children in Iran. Integrons are carried by mobile genetics elements within bacterial cells, allowing them to spread to other bacteria. Integrons play a crucial role in carrying and disseminating antimicrobial resistance genes among bacteria through horizontal transfer, which is one of the most significant routes for the distribution of these genes [9].

Five different gene cassettes were found among the 14 intI-1-positive isolates by the sequencing of the amplified variable regions. Three genes of dihydrofolate reductase (dfr family) that encoded for resistance to trimethoprim (*dhfr17, dfrA12, dfrA1*) and four aminoglycosides resistance genes (*aadA1*, *aadA2*, *aadA5*, and *aadA6*). The occurrence of cassettes encoded for resistance to trimethoprim and aminoglycosides (*dfrA, aadA*) in quinolone-resistant isolates has been reported [41–43]. Gene cassettes *dfrA17-aadA5* and *aadA6* were detected in six and four isolates, respectively, which may be due to the transfer of the integrons between bacterial species. The dissemination of integrons is carried out by the cross-transmission of integron-carrying clones from one patient to the other in hospital settings [44]. The two gene cassettes (*dfrA17-aadA5*, and *aadA6*) were documented in Gram-negative strains in several regions worldwide [9, 45–47]. The spread of these genes in other regions may involve self-transferable plasmids within the host (humans and animals) [48].

Interestingly, we did not detect any PMQR genes in the gene cassettes. Although the presence of integrons did not influence the susceptibility to the tested quinolones in the present study, it is important to note that the prevalence of integrons has played a significant role in the development of MDR bacteria. The quinolones and other antibiotic-resistance genes except *dfrA* and *aadA* genes are generally located outside the integrons. The association between quinolone resistance and the presence of an integron is not yet fully understood and has been the subject of ongoing research [48, 49].

Conclusion

The study confirmed a high prevalence of quinolone resistance, ESBL and integrons among the clinical Gram-negative isolates. PMQR determinants were widely reported, with particularly high prevalence of both *aac(6')*-Ib and *qnrA* genes. The important association between ESBL-producing with PMQR genes is clinically significant, as it potentiates the development of MDR phenotypes and severely limits therapeutic options for these isolates. This study also revealed a high prevalence of integrons, often harboring the most dominant gene cassettes conferring resistance to trimethoprim and aminoglycosides. While the integrons did not directly impact quinolone susceptibility (as the resistance genes were not cassette-borne), they played a crucial role in the development of MDR bacteria. To prevent outbreaks of PMQR, integrons and ESBL-producing isolates, it is crucial to implement robust infection control measures and establish continuous antimicrobial resistance surveillance. This can be achieved through the application of an antimicrobial resistance monitoring system to control their dissemination and the linked health hazard. Further studies are needed to study whole genome sequencing and determine plasmids and insertion elements, providing a better understanding of the genetic factors contributing to antimicrobial resistance in these isolates.

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Author contributions Gh.T. designed the study, performed the experimental work (the microbiological and molecular tests), collected the data, analyzed and interpreted the data and drafted the manuscript. I.F. and M.B.S. contributed to final writing and editing the manuscript. A.B. participated in the project design and contributed to final writing and editing the manuscript. K.B.S. designed and supervised the study. All authors read and approved the final version of the manuscript.

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Data availability The datasets generated during and analyzed during the current study are available in this manuscript.

Declarations

Ethics approval and consent to participate Not applicable.

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