

Prevalence of Plasmid-Mediated Quinolone Resistance in Multidrug-Resistant Gram Negative Bacilli in Egypt

Mohamed Abd ElSalam¹, Doaa Gamal², Manal El Said², Dalia Salem², Aisha Abu Aitta² and Mamdouh S El Gamal¹

¹Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

²Department of Microbiology, Theodor Bilharz Research Institute, Giza, Egypt.

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Resistance to quinolone has increased significantly and one of the most reasons is plasmid-mediated quinolone resistance (PMQR). The aim of this study is to detect the prevalence of PMQR in multidrug-resistant (MDR) Gram negative bacilli and to characterize these resistance genes. A total of 420 Gram negative bacilli clinical isolates were collected from patients attending Misr children hospital. Isolates were identified by biochemical reactions, while antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method. Minimum inhibitory concentrations (MIC) of ciprofloxacin were detected by E-test, whereas combined test method was used to detect extended-spectrum β -lactamase (ESBL) production. *QnrA*, *qnrB*, and *qnrS* genes were determined by multiplex polymerase chain reaction (PCR). MDR Gram negative bacilli represented 68% (268/420); most of them were recovered from blood culture specimens (21%). Among these MDR isolates 21% (60/268) were ciprofloxacin resistant; with MICs $>32\mu\text{g/ml}$ in 95% of the isolates. ESBL production was detected in 11.7% of the studied isolates. The *qnr* genes were detected in 60%. *QnrS* and *qnrB* were the detected genes in 77.8% and 16.7% of the isolates respectively. Both *qnrB* and *qnrS* genes were determined simultaneously in 5.5%. *QnrB* gene was found alone in only one isolate (14.3%) that was ESBL-producer. The most MDR isolates were recovered from blood culture; this confirms the occurrence of these superbugs and their ability to cause life threatening infections. The prevalence of quinolone resistant Gram negative bacilli clinical isolates is high. The mostly prevalent PMQR gene is *qnrS* followed by *qnrB*.

Keywords: ESBLs; Gram Negative Bacilli; Multiplex PCR; Plasmid-Mediated Quinolone Resistance.

The incidence of serious infections due to multi drug resistant (MDR) Gram-negative bacteria has increased and these infections now constitute a serious threat worldwide.¹ These MDR bacteria are major causes of various types of infections; respiratory tract, urinary tract, bloodstream, and wound infections.² These pathogens have the ability to produce various β -lactamases such as extended-spectrum β -lactamases (ESBLs).³ MDR Gram-negative bacteria worldwide become a problem for clinicians and infection control staff due to few therapeutics possibilities⁴.

Quinolone are a group of broad-spectrum antibiotics that are widely used in routine clinical practice.⁵ Low side effects, large range of activities and adequate oral absorption, make quinolone the first-line of drug options to treat many infections.⁶ The widespread and inappropriate use of quinolone leads to a significant increase of resistant Gram-negative isolates.⁷⁻⁹

Resistance to quinolone is often due to several mechanisms such as point mutations in chromosomal genes such as DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*),¹⁰

decreased permeability of efflux pumps.^{9,11} Recently, plasmid-mediated quinolone resistance (PMQR) has also been demonstrated in several studies worldwide.⁹ Three mechanisms of PMQR have been described (i) target alteration by Qnr, (ii) drug modification by the aminoglycoside acetyltransferase AAC(6')-Ib-cr, which can reduce ciprofloxacin activity, and (iii) efflux pump activation by two quinolone efflux pumps.¹¹⁻¹⁴ Qnr proteins protect DNA gyrase and topoisomerase IV from the inhibitory activity of quinolone. Currently, there are six different qnr genes: qnrA, qnrB, qnrC, qnrD, qnrS, and the most recently reported; qnrVC.¹³

The aim of this study is to detect the prevalence of PMQR in multidrug-resistant Gram negative bacilli isolates and to determine their resistance genes.

MATERIALS AND METHODS

Clinical Bacterial Isolates

A total number of 420 Gram negative bacilli clinical isolates were collected from patients attending Misr children hospital during the period from June 2017 to February 2018. Two hundred and eighty six isolates (68%; 286/420) were multidrug resistant isolates; MDR bacteria were defined as resistant to one or more antimicrobials on three or more antimicrobial classes.¹⁵ Sixty isolates (21%; 60/268) were ciprofloxacin resistance by Kirby-Bauer disk diffusion method; they were isolated from different clinical infections as urinary tract, surgical wound, chest, blood stream, central line, and umbilical catheter infections. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a prior approval by TBRI institutional review board (FWA 00010609).

Identification of clinical isolates

Identification of the Gram negative isolates was done by; cultural characters and biochemical reactions using biochemical test media (Triple sugar iron (TSI), Lysine iron agar (LIA), Motility indole ornithine (MIO), citrate and urease) (Oxoid, England). Storage of bacterial isolates was done by adding 0.85 ml of an overnight incubated bacterial culture of the identified Gram negative isolates in nutrient broth (Oxoid, England) to 0.15 ml sterile glycerol in sterile cryotubes, which were vortexed and stored at -20°C.¹⁶

Antimicrobial Susceptibility Testing (AST)

Gram negative clinical isolates were screened by Kirby-Bauer (KB) disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁷ Resistance to quinolones was assessed using; nalidixic acid (NA; 30 µg), ciprofloxacin (CIP; 5 µg), and levofloxacin (LEV; 5 µg). Sensitivity to other antibiotics was tested using; amikacin (AK; 30 µg), gentamicin (CN; 10 µg), ceftriaxone (CRO; 30 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg) cefoperazone (CEP; 75 µg), amoxicillin/clavulanic (AMC; 30 µg), ampicillin/sulbactam (SAM; 20 µg), and imipenem (IPM; 10 µg) (Mast Diagnostics, U.K.). The interpretation of antimicrobial susceptibility results was according to current CLSI guidelines.¹⁷

Minimum Inhibitory Concentration (MIC) of Ciprofloxacin by E-test

E-test (AB Bio Disk Solna, Sweden) was used for detection of MIC of Gram negative clinical isolates to ciprofloxacin and was performed according to the instructions of manufacturer. MIC interpretive criteria for ciprofloxacin CLSI¹⁷ were used to interpret results; susceptible ≤1 mg/ml, intermediate = 2 mg/ml and resistant ≥ 4 µg/ml.

Detection of Extended Spectrum β-Lactamases (ESBLs)

Screening for ESBL by disk diffusion method

The multi drug resistant Gram negative clinical isolates were screened by the KB disk diffusion method where isolates with decreased susceptibilities to cefotaxime (zone diameter of <27 mm) and/or ceftazidime (zone diameter of <22 mm) were considered probable ESBL-producing pathogens according to CLSI guidelines.¹⁷

Confirmatory Test by Combination Disk Method

Phenotypic confirmation of potential ESBL-producing isolates was performed using cefotaxime (CTX 30 µg) and cefotaxime/clavulanic acid disks (CTC 30/10 µg) (Bio-Rad, France) according to CLSI recommendations.¹⁷ Interpretation of antimicrobial susceptibility results was according to CLSI guidelines; if the zone size increased in the presence of the inhibitor compared with the cephalosporin alone by 5 mm or more this is indicative of an ESBL-producing strain.¹⁷ *E. coli* ATCC 25922 was used as negative control in this method.

Multiplex PCR for detection of qnrA, qnrB, and qnrS genes

Primers Screening and Selection by Using BLAST Algorithm¹⁸

Several sets of PCR primer pairs sequences corresponding to different qnrA, qnrB, and qnrS gene clusters were taken from literature¹⁹⁻²⁴ and searched for homology to all qnrA, qnrB, and qnrS sequences available in Gen bank database using BLAST algorithm (www.ncbi.nlm.nih.gov). Primers with 100% homology to all retrieved qnrA, qnrB, and qnrS with no match to human genes have been selected to be synthesized (Table 1). Extraction of bacterial DNA was performed by boiling method.²⁰ Briefly, a bacterial colony was suspended in 200 µL of sterile distilled water and heated to 95°C for 10 min in water bath. Then, the suspensions were centrifuged at 6000 rpm for 20 minute. Supernatants were separated in sterile eppendorf tube and stored at -20°C for PCR assays. PCR reactions for all primer pairs have been carried out in multiplex using negative controls with no DNA sample. DNA extract of each isolate was added to each PCR tube to reach 50µl. PCR master mix that contained; dream Taq DNA polymerase, 2 x Dream Taq green buffer, Nucleotides mix (0.4

mM each), and 4 mM MgCl₂. Amplification was carried out with the following thermal cycling (Biometra, UK) conditions; initial denaturation at 95°C for 5 min, followed by 25 cycles at 95°C for 105 sec, annealing at 56°C for 15 sec, and extension at 72°C for 15 sec. 25 DNA fragments were analysed by electrophoresis in a 2% agarose gel containing 0.05 mg ethidium bromide. The gel was photographed using the gel documentation system (Cleaver, UK).

Statistical Methods

The data were statistically articulated inexpressions of frequencies (number of cases) and relative frequencies (percentages). P value(probability value) less than 0.05 was considered statistically significant. All statistical estimations were performed using Microsoft Excel 2013 program (Microsoft Corporation, NY., USA) and SPSS (Statistical Package for the Social Science; IBM SPSS statistic) version 20 for Microsoft Windows.

RESULTS

The prevalence of multidrug resistant Gram negative bacilli were 68% (286/420), among

Table 1. Primers for detection PMQR genes

Primer name	Sequence	M.W	Ref.
<i>qnrA</i>	F: 5'-AGAGGATTTCTCACGCCAGG-3' R: 5'-TGCCAGGCACAGATCTTGAC-3'	580bp	[20]
<i>qnrB</i>	F: 5'-GGMATHGAAATTCGCCACTG-3' R: 5'-TTYGCBGYCYGCCAGTCGAA-3'	264bp	
<i>qnrS</i>	F: 5'-GCAAGTTCATTGAACAGGGT-3' R: 5'-TCTAAACCGTCGAGTTCGGCG-3'	428bp	

M.W = molecular weight, f= forward, r= reverse

Table 2. Distribution of bacterial isolates according to species and specimen type

Species (no. of Isolates)	Blood culture	No. (%) of isolates according to specimens				
		Urine	Endotracheal tube	Central line	Umbilical catheter	Wound swab
<i>K. pneumonia</i> (40)	17 (42.5)	2 (5)	15 (37.5)	3 (7.5)	1 (2.5)	2 (5)
<i>E. coli</i> (13)	6 (46.1)	5 (38.5)	0(0)	0(0)	0(0)	2 (15.4)
<i>E. cloacae</i> (7)	5 (71.4)	1 (14.3)	1 (14.3)	0(0)	0(0)	0(0)

Data are expressed as numbers (N) and percent (%).

them 21% isolates (60/286) were ciprofloxacin resistant. All the 60 clinical isolates were members of Enterobacteriaceae family; they were recovered from 39 males (65%) and 21 females (35%).

Most of clinical isolates were isolated from blood culture specimens 46.7% (28/60). The other isolates were taken from endotracheal tube (26.7%; 16/60), urine (13.3%; 8/60), wound swabs (6.7%; 4/60), central lines (5%; 3/60), and an umbilical catheter (1.6%; 1/60).

Identification of Clinical Isolates

Klebsiella pneumoniae (*K. pneumoniae*) was the most frequently isolated species (66.7%; 40/60), followed by Escherichia coli (*E. coli*) with

isolation rate of 21.7% (13/60), then Enterobacter cloacae (*E. cloacae*) in 11.6% (7/60). The distribution of studied isolates according to the species and specimen type is shown in table 2.

Antimicrobial Susceptibility Testing (AST)

Antibiotic susceptibility pattern of 60 multidrug resistant Enterobacteriaceae isolates to different antibiotics is shown in table 3.

Detection of ESBLs

Among the 60 clinical isolates 11.7% (7/60) isolates were confirmed phenotypically as ESBL-producers. They were *E. coli* isolates in 71.4% (5/60) and 2 isolates; one *K. pneumoniae* and another *E. cloacae* (14.3%, each).

Table 3. Antibiotic susceptibility pattern of 60 multidrug resistant *Enterobacteriaceae* isolates to different antibiotics

Antibiotics	<i>K. pneumoniae</i> (No.40)			<i>E. coli</i> (No.13)			<i>E. cloacae</i> (No.7)		
	SN(%)	IN(%)	RN(%)	SN(%)	IN(%)	RN(%)	SN(%)	IN(%)	RN(%)
Nalidixic acid	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Ciprofloxacin	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Levofloxacin	0(0)	0(0)	40(100)	1(7.7)	0(0)	12(92.3)	1(14.3)	0(0)	6(85.7)
Amikacin	3(7.5)	0(0)	37(92.5)	13(100)	0(0)	0(0)	2(28.6)	0(0)	5(71.4)
Gentamicin	7(17.5)	0(0)	33(82.5)	9(69.2)	0(0)	4(30.8)	0(0)	0(0)	7(100)
Imipenem	5(12.5)	2(5)	33(82.5)	13(100)	0(0)	0(0)	2(28.6)	2(28.6)	3(42.8)
Ceftriaxone	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Ceftazidime	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Cefoperazone	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Cefotaxime	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Amoxicillin/ clavulanic	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)
Ampicillin/ sulbactam	0(0)	0(0)	40(100)	0(0)	0(0)	13(100)	0(0)	0(0)	7(100)

*S= Susceptible, I= Intermediate and R= Resistant; Data are expressed as numbers (N) and percent (%)

Table 4. Quinolone resistance genes and their distribution among studied isolates according to specimens and species of the isolate

gene Organism	<i>qnrS</i>			<i>qnrB</i>		<i>qnrB</i> and <i>qnrS</i>			
	<i>E. cloacae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Specimen	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
Blood	2(7.1)	1(3.6)	11(39.3)	0(0)	0(0)	1(16.7)	0(0)	0(0)	2(100)
Urine	0(0)	0(0)	0(0)	0(0)	0(0)	2(33.3)	0(0)	0(0)	0(0)
Endotracheal tube	0(0)	0(0)	10(35.7)	0(0)	0(0)	2(33.3)	0(0)	0(0)	0(0)
Central line	0(0)	0(0)	2(7.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Wound swab	0(0)	0(0)	1(3.6)	0(0)	0(0)	1(16.7)	0(0)	0(0)	0(0)
Umbilical catheter	0(0)	0(0)	1(3.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total (36)		28 (77.8)			6(16.7)		2(5.5)		

Data are expressed as numbers (N) and percent (%)

E-Test

The overall prevalence of the 60 Gram negative isolates which demonstrated resistance to ciprofloxacin (MICs >32µg/ml) was 95%. They were 38 *K. pneumoniae* isolates (66.7%), 12 *E. coli* isolates (21%), and 7 *E. cloacae* isolates (12.3%).

The remaining isolates demonstrate different levels of resistant to ciprofloxacin; where one *K. pneumoniae* isolate (1.7%) was resistant to 12µg/ml and 8µg/ml respectively. One *E. coli* isolate (1.7%) showed intermediate susceptibility with MIC 3µg/ml.

Molecular detection of PMQR Genes

Blast alignment of selected primers: the sequences of selected primers which described previously by Cattoir *et al*²⁰ were subjected to Blast alignment against the Gene Bank no redundant genes database. 18 Data showed complete sequence homology and conservation of these primers with *qnrA*, *qnrB* and *qnrS* genes in all of the affiliated targeted species but not with non-targeted species.

Multiplex PCR detection of *qnrA*, *qnrB*, and *qnrS* genes in isolated bacteria

A multiplex PCR reaction was used for simultaneous detection of *qnrA*, *qnrB* and *qnrS* genes in each isolated bacteria (Figure 1).

The *qnr* genes were detected in 60% (36/60) of quinolone resistant Gram negative bacilli isolates. The *qnrS* was the main gene detected in 77.8% (28/36) while *qnrB* was detected in 16.7%

(6/36). Both *qnrB* and *qnrS* genes were determined simultaneously in two *K. pneumoniae* isolates (5.5%; 2/36). However, *qnrA* was not detected in any of the studied isolates. Quinolone resistance genes and their distribution among studied isolates according to specimens and species of the isolates are illustrated in table 4. The *qnrB* gene was detected in one ESBL-producer *K. pneumoniae* isolates (1/7; 14.3%), while *qnrS* gene was not detected in ESBL-producer isolate.

Lanes : 26,28,34,35,36,37,38,55,57,58, and 60;isolates with positive results to *qnrS* (427bp), lane 56;isolate with positive result to *qnrB* (263bp). Lanes: 33, 39, and 59 isolates show negative PCR to PMQR tested genes. Lane NC (negative control) and lanes: M; molecular size marker (Gene ruler TM 100 bp DNA ladder).

The *qnr* genes were detected in 60% (36/60) of quinolone resistant Gram negative bacilli isolates. The *qnrS* was the main gene detected in 77.8% (28/36) while *qnrB* was detected in 16.7% (6/36). Both *qnrB* and *qnrS* genes were determined simultaneously in two *K. pneumoniae* isolates (5.5%; 2/36). However, *qnrA* was not detected in any of the studied isolates. Quinolone resistance genes and their distribution among studied isolates according to specimens and species of the isolates are illustrated in table 4. The *qnrB* gene was detected in one ESBL-producer *K. pneumoniae* isolates (1/7; 14.3%), while *qnrS* gene was not detected in ESBL-producer isolate.

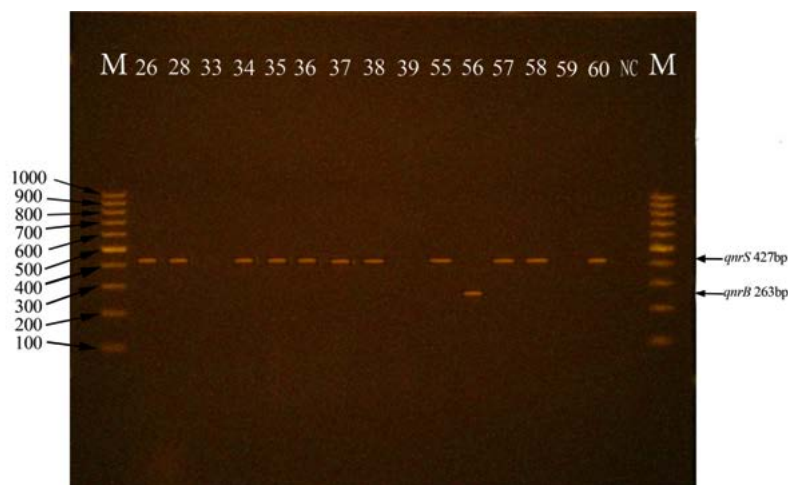


Fig. 1. Agarose gel electrophoresis (2%) of the multiplex PCR reactions for detection of PMQR genes in (15) *K. pneumoniae* isolates

DISCUSSION

The increased prevalence of infections caused by MDR Gram-negative bacteria constitutes a serious threat to global public health due to the limited current available treatment options and the lack in development of new antimicrobial agents. Infections caused by these pathogens are allied with elevated morbidity and mortality rates and prolonged hospital stay.²

Quinolones is a widely used antibiotic with a broad spectrum. Inhibiting the normal functions of bacterial type II topoisomerase is the target of quinolones.¹⁰ Quinolone resistance is mainly caused by mutations in chromosomal genes encoding the quinolone targets, such as DNA gyrase and topoisomerase IV; however recently PMQR genes have been increasingly reported in most area of the world.¹¹

The aim of this study was to determine the prevalence of PMQR among the multidrug Gram negative bacilli isolates through detection of qnr genes.

The prevalence of MDR Gram negative bacilli isolates among other similar isolates was 68% (286/420). This finding is in agreement with another study conducted in Egypt by Tohamy *et al*²⁶ who identified MDR in 68.6% of isolated bacteria. This could be attributed to the irrational use of antibiotics against pathogenic bacteria.¹⁵

In this study, 60 (21%) of multidrug resistant Gram negative isolates were ciprofloxacin resistant. Most of isolates were isolated from blood culture specimens (47%). Whereas in the National Cancer Institute (NCI) in Egypt Helmy and Kashef²⁷ reported that majority of his MDR isolates were collected from urine (36%). Our finding confirms the ability of these superbugs MDR bacteria to cause life threatening infections.

In the present study, among the 60 ciprofloxacin resistant Gram negative isolates, *K. pneumoniae* was the most frequently isolated species (66.7%), followed by *E. coli* (21.7%), then *E. cloacae* (12%). These results are in accordance with Jlili *et al*²⁸ who reported that *K. pneumoniae* is the main species (59%) followed by *E. coli* (23.4%), then *E. cloacae* (7.9%). However, another study in tertiary care cancer hospital in Cairo, Egypt stated that the most frequent isolates in immunocompromised cancer patients were

E. coli (39.7%) followed by *K. pneumoniae* (34.3%), then *E. cloacae* (3.3%).²⁹ Many studies^{30, 31}, showed *K. pneumoniae* the most frequently Gram negative species isolated from blood cultures and this explained the majority of the *K. pneumoniae* isolates in this study.

Among the 60 studied clinical isolates 7 (11.7%) were ESBL-producer by combined disk method. These results are in accordance with Charfi *et al*³² who reported that, out of the 283 Gram-negative isolates, 46 (16.25%) isolates were ESBL producers, but was slightly lower than the findings of Bouchakour *et al*³³ who reported that, out of 188 gram-negative isolates included in their study, 39 (20%) were ESBL producers. However, this figure may be underestimated, as most of our isolates (60%) were imipenem resistant and phenotypic detection of ESBL is not reliable in presence of other β -lactamases as production of carbapenemases, although not investigated in the current study, is the main mechanism of carbapenem resistance³⁴.

In this study, qnr genes were detected in 60% (36/60) of ciprofloxacin resistant Gram negative bacilli isolates. That was consistent with another Egyptian study by Tohamy *et al*²⁶ who found qnr genes in 42.9% (30/70) of MDR isolates, while that was higher than in two studies from Iran and Italy that reported a detection rate of 19% and 17% respectively.^{11,35}

In the current study, qnr genes were found more frequently in *K. pneumoniae* isolates. As they were detected in 82.5% (33/40) of *K. pneumoniae* isolates. Nearly similar findings were reported from Italy (68.1%), Japan (66.7%), China (65.5%) and Korea (63.1%).³⁵⁻³⁸ Lower rate (52.2%) was reported in Iran.¹¹ However, in a study conducted in both Norway and Sweden very low rate (8.3%) was reported.³⁹ In the present study, the prevalence of qnr genes was much lower in *E. coli* isolates (7.7%) than in other species of Gram negative bacilli isolates. These results are in accordance with the findings of many studies that reported a low detection rate of qnr genes in *E. coli* isolates.^{11,24,35}

In this study, qnrS was the main qnr gene (77.8%) found. This was in accordance to Poirel *et al*⁴⁰ who identified qnrS in 61.5% of the Enterobacteriaceae isolates. The qnrS was detected in the current study in 62.5% of *K. pneumoniae* and in 7.7% of *E. coli* isolates.

Lower prevalence rate was determined in a study conducted in Egypt in which *qnrS* was found in 9.5% of *K. pneumoniae* isolates, and in 2.7% of *E. coli* isolates.²⁷ While a higher rate of *qnrS* (16.6%) was detected in ESBL producing *E. coli* isolates in another Egyptian study.⁴¹

In our study, the *qnrS* was detected in 28.6% of *E. cloacae*. This was in accordance with Yang *et al*³⁷ who reported its presence in 17.1% of *E. cloacae* isolates. However, other studies in Korea and Morocco reported absence of *qnrS* in *E. cloacae* isolates.^{33,42}

In the current study, *qnrB* was the second predominant *qnr* detected gene (16.7%); *qnrB* gene alone was only detected in 10% of *K. pneumoniae*. Similarly, a study from Spain identified *qnrB* gene in 14.3 % of the Gram negative bacilli isolates.²¹ An Egyptian study reported a detection of *qnrB* gene in 9.5% of *K. pneumoniae* isolates.²⁷ Higher values were reported from Morocco in which *qnrB* gene was found in 28.5% of *K. pneumoniae* isolates.³³ It worth to mention that both *qnrB* and *qnrS* genes were detected simultaneously in 5% of our *K. pneumoniae* isolates. That was in accordance with other studies that reported simultaneous detection of both genes in 2.5% and 1.8% of *K. pneumoniae* isolates.^{28,37}

In this study, the *qnrA* gene was not detected in any of the studied isolates. This was consistent with two studies in Egypt and other studies from Iran, Korea and France.^{5,11,13,23,27,43} In contrast other Egyptian studies by Tohamy *et al* found that of the 70 MDR Gram negative bacilli isolates 2 (2/70, 2.8%) isolates carried *qnrA* gene.²⁶ Esmat and Mohamed⁴⁴ who detected *qnrA* in 11 % *K. pneumoniae* isolates. In Kuwait Vali *et al*⁴⁵ reported that only one isolate out of 173 *K. pneumoniae* isolates had *qnrA* and another Japanese study reported that the prevalence of the *qnrA* gene was 0.8% in *E. coli*³⁶. This worldwide relatively low prevalence may explain its absence among our isolates.

Qnr genes were detected mainly in blood culture samples (47.2%). Whereas, Peymani *et al*⁵ reported that *qnr*-positive isolates were mostly recovered from urine 42.9% followed by trachea secretion 36.7%, wound swabs 12.2%, and were least in blood culture specimens (2%) This may be explained by the difference in the number of each type of specimens in the two studies; as blood was

the major specimen in the current study whereas urine was the predominant one in the later. Again this figure confirms serious consequences of these resistant strains.

In the present study, all the tested isolates (100%) were resistant to at least one quinolone, while 96.7 % were resistant to all of them. All of the 40 (100%) isolates of *K. pneumoniae*, the most frequently isolated species, were resistant to nalidixic acid, ciprofloxacin and levofloxacin. These results are in accordance with the findings of study in Portugal that reported 100% resistance of *K. pneumoniae* isolates to nalidixic acid and ciprofloxacin.²¹

Regarding quinolones susceptibility within *qnrS* positive isolates; all isolates showed resistance to all quinolones except one *E. cloacae* isolate that was sensitive only to levofloxacin. Similar findings by Jlili *et al*²⁸ who reported that of the 23 *qnr*-positive *K. pneumoniae* isolates, 95.6%, 100%, and 69.5% were non-susceptible to nalidixic acid, ciprofloxacin, levofloxacin, respectively. These minor fluctuations in sensitivity patterns to various quinolones could be explained by Jacoby,⁴⁶ who reported that quinolone susceptibility is decreased by PMQR however, this resistance is not detected in the clinical level, but it enhances other resistance mechanisms and aid further mutations to higher levels of resistance. Also, other mechanisms of resistance rather than PMQR may be involved. In our study; 40% (24/60) of our isolates were negative to *qnr* genes. These isolates probably had other mechanisms of resistance that were not investigated in this study. *Qnr* positive isolates infections may consequently augment the selection of these resistant mutants and increase the spread of this type of resistance.

The alliance of quinolone resistance with resistance to other antibiotics especially aminoglycosides was analyzed in this study. Among *qnr* positive isolates, 97% were resistant to amikacin. The *qnr* genes presence were significantly correlated with amikacin ($p < 0.001$). That was similar to a Korean study found that all *qnr* positive clinical isolates were resistant to amikacin.³⁸ Another Iranian study by Majlesi *et al*.¹¹ stated that the prevalence of plasmid-mediated quinolone resistance due to the *qnr* and *aac(6)-Ib-cr* genes was high among quinolone-resistant clinical isolates of Enterobacteriaceae where only 41% of

quinolone-resistant clinical isolates were positive for PMQR genes. This could be attributed to collocate of PMQR genes with other resistance determinants within transposons and/or integrons in multidrug resistance plasmids. Quinolones and aminoglycosides can act as potential coselectors for antimicrobial resistance. Also the presence of widely prevalent *aac* (6')-Ib-cr47 which confers resistance to kanamycin, tobramycin, netilmicin, amikacin, and ciprofloxacin, although it was not attempted in the current study, may explain the associated high aminoglycosides resistance.⁴⁸

CONCLUSION

The prevalence of quinolone resistant Gram negative bacilli clinical isolates in the current study was high. The mostly detected PMQR genes were *qnrS* followed by *qnrB*. Gram-negative bacteria expressed high antibiotic resistance common antibiotics lead to loss of convenience for treatment of many infections. More knowledge about trends of antibiotic resistance and mechanisms of resistance is required to reduce the risk of antibiotic treatment failure. The overuse of quinolone has led to increased resistance, making them less effective.

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