Research Article

Prevalence of Fluoroquinolone Resistance Genes Among Carbapenem-Resistant Enterobacterales Isolates from a Tertiary Care Hospital in Egypt

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Abstract | Fluoroquinolones (FQs) are an optional treatment for serious infections caused by carbapenem-resistant Enterobacterales (CREs), which pose a considerable risk in the hospitalized patients. Quinolone resistance arises mainly from chromosomal mutations within the resistance-determining region (QRDR) of the *parC* or *gyrA* genes and from plasmid-mediated quinolone resistance (PMQR)- encoded efflux pumps. This study aimed to detect the *gyrA, parC, qepA, and aac(6')-Ib-cr genes* prevalence in *CREs* isolates from Ain Shams Hospital. A total of 100 *Enterobacterales* strains were obtained from diverse clinical samples and tested for their response to the various antimicrobial agents. To detect carbapenemase-producing *Enterobacteriaceae* (CPE), isolates that showed resistance to imipenem and meropenem by disc diffusion were tested by the modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM). Ciprofloxacin minimum inhibitory concentrations **(**MICs) were measured by the resazurin microtiter plate method for carbapenemase-producing isolates. Isolates that displayed ciprofloxacin resistance by MICs were examined by two multiplex polymerase chain reactions (PCRs), one for QRDRs *(gyrA and parC)* genes and the other for PMQR genes (*qepA and aac(6')-Ib-cr)* genes. Most of the clinical specimens were obtained from intensive care unit (ICU) patients (72%), and blood was the most collected specimen (28%), followed by sputum (24%). 78 isolates were multidrug-resistant (MDR). 50 isolates were examined for *gyrA* and *parC*,*qepA*, and *aac(6')-Ib-cr* genes. *Aac (6')-Ib-cr* gene was detected in every tested isolate (100%), *gyrA and parC* genes were present in 47 and 46 isolates, recording 94 and 92%, respectively. Only four isolates contained the *QepA* gene. Our findings showed high frequencies of PMQR genes among the CREs isolates.

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Introduction

Carbapenem-resistant Enterobacterales (CREs) are a growing global health issue. They are resistant to many antibiotics, including carbapenems, a class of last-resort β-lactam antibiotics, resulting in severe, often untreatable infections (Tamma *et al.*, 2024). Fluoroquinolones (FQs) are considered

alternative treatments for severe infections caused by CREs, including urinary tract infections (UTIs), bloodstream infections, and pyelonephritis (Ávila-Núñe, *et al*., 2023). The World Health Organization (WHO) regarded FQs as "Critically Important Antimicrobials" as they are broad-spectrum. Furthermore, they are crucial in treating diseases in humans and livestock (WHO, 2017). FQs influence DNA repair and replication *via* affecting DNA gyrase and DNA topoisomerase IV enzymes (Kabbani *et al*., 2018). Recent meta-analyses indicate that FQs currently demonstrate the highest resistance rates among the remarkable antibiotic classes for *Enterobacterales*, with global resistance rates surpassing 40%, which are escalating over time (Muteeb, 2023). Various pathways contribute to the FQs resistance. Chromosomally-mediated resistance arises from mutations within the quinolone resistance-determining region (QRDR), affecting DNA gyrase (*gyrA*, *gyrB*) topoisomerase IV (*parC*, *parE*), causing changes in regulatory genes of the chromosomally encoded efflux pumps, and reducing drug uptake elicited by the porin loss. This is the principal mechanism and is transmitted vertically (Kareem *et al*., 2021). Plasmid-mediated quinolone resistance (PMQR) is attributed to a different resistance mechanism, involving efflux pump genes such as *OqxAB* and *qepA*, the *aac(6)-Ib-cr* aminoglycoside-modifying gene, and *qnr* genes that confer quinolone resistance (Zeng *et al*., 2020). These resistance genes are transferred *via* horizontal and vertical mechanisms, and they may be present even in the absence of the FQs (Rodríguez *et al*., 2016). The emergence of FQs resistance in CREs underscores the critical need for a comprehensive surveillance, effective antimicrobial stewardship, and the development of novel treatment approaches to limit the dissemination of these potent microorganisms. The objective of this study was to determine the prevalence of FQs resistance among the CREs by detecting the presence of *gyrA*, *parC*, *QepA*, and *aac(6')-Ib-cr* genes using multiplex polymerase chain reaction (PCR).

Materials and Methods

Bacterial strains and their identification

During the period from April 2024 to October 2024, Enterobacterales isolates were isolated from sputum, urine, blood, cerebrospinal fluid (CSF), and central line samples collected from Ain Shams Hospital's Medical Microbiology laboratory using the streak plate method (Katz, 2008). The obtained isolates were cultivated on blood, MacConkey, and CLED agar. Colony identification was conducted using morphological traits and biochemical reactions, adhering to the methodologies outlined by Collee *et al*. (1996).

Antibiotic susceptibility testing (AST)

All isolates underwent antibiotic susceptibility testing through the Kirby-Bauer disk diffusion technique. Briefly, colonies of each isolate were picked up and suspended in saline to make a suspension equivalent in density to the opacity standard (McFarland standard 0.5). Then, a sterile swab was immersed in the suspension and squeezed of excess fluid against the side of the tube and then rubbed over the plate of Mueller-Hinton (MH) agar (HI Media Laboratories, Mumbai, India). Commercially prepared antibiotic discs, 6 mm in diameter (Oxoid, England), were used (Kirby *et al*., 1957).The antibiotics comprised: β-lactams/β-lactamase inhibitors: Ampicillin, Ampicillin/sulbactam (SAM 10/10 µg), Amoxicillin/clavulanic acid (AMC 20/10 µg), Piperacillin/tazobactam (TZP 100/10 µg); Cephalosporins: Ceftazidime (CAZ 30 µg), Cefotaxime (CTX 30 µg), Cefoperazone (CEF 75 µg), Cefepime (FEP 30 µg); Carbapenems: Imipenem (IMP 10 µg), Meropenem (MEM 10 µg); Aminoglycosides: Gentamicin (10 µg), Amikacin (AK 30 µg); Fluoroquinolones: Ciprofloxacin (5 µg) and Levofloxacin (5 µg). *E. coli* ATCC 25922 strain served as the quality control. An isolate was designated multidrug-resistant (MDR) when it demonstrated resistance to at least three antimicrobials (Magiorakos et al., 2012).

Phenotypic identification of carbapenemase-producing Enterobacterales

Isolates exhibiting resistance to meropenem and imipenem *via* Kirby-Bauer disc diffusion were analyzed using the modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) to identify the carbapenemase-producing Enterobacterales (CPE) and specifically to recognize the metallo-β-lactamases (MBLs) from the other carbapenemases. Two separate tubes were filled with 2 ml of Tryptic Soy Broth (TSB) and a 1-µl aliquot of bacterial culture suspension (~1.5 × 10^8 cfu/l). One tube contained plain TSB for mCIM and the other for eCIM, which were supplemented with EDTA at a final concentration of 5 mM (obtained through mixing 2 ml TSB with 20 µl EDTA (0.5 M)). A meropenem disk was then added to each tube and they were kept for 4 h (±15 min.)

at 35˚C. The disks were released after incubation and placed aseptically onto the surface of MH agar plates, which were streaked with a 0.5 McFarland suspension of *E. coli* ATCC 25922. For 16 to 20 h, the plates were incubated at 35˚C. The mCIM test was considered positive when the pinpoint colonies were detected in inhibition zones (IZs) measuring 16-18 mm or 6-15 mm using a calibrated ruler, whereas was considered negative when the IZ was 19 mm or larger. Compared to mCIM, eCIM results were assessed as positive when IZ diameter increased by ≥ 5 mm, and negative when IZ: Zone diameter increased by < 4 mm (Sfeir *et al*., 2019).

Assessing minimum inhibitory concentration (MIC) using the resazurin microtiter plate assay

The ciprofloxacin MICs were ascertained by the resazurin microtiter plate assay for carbapenemase-producing isolates (Sarker *et al*., 2007). In summary, 100 µl of Mueller-Hinton broth (MHB) were allocated into each well of the 96-well microtiter plate. Subsequently, 100 µl of ciprofloxacin was introduced into the wells of the first row (1:2 dilution), followed by serial dilutions in the subsequent wells (maximum, 1:256). A bacterial suspension (10 µl, containing 10^6 cfu/ml) was subsequently added to each well. The plate was sealed and underwent incubation for 24 h at 37 ˚C. Afterward, 15 µl of resazurin solution was introduced into each well, followed by an additional incubation period of 3 h. The MIC was defined as the minimal ciprofloxacin concentration that prevented a color shift from blue to pink (Sarker *et al*., 2007). According to CLSI criteria, isolates with MIC values ≥ 4 µg/ml were designated as resistant (CLSI, 2025). *E. coli* ATCC 25922 served as the control strain.

Molecular identification of Quinolone resistance genes

Isolates exhibiting ciprofloxacin resistance as determined by MICs were analyzed using two multiplex PCR assays: one targeting QRDR genes (*gyrA and parC*) and the other targeting PMQR genes (*qepA and aac(6')-Ib-cr*). The primers utilized are stated in Table 1. The primers were supplied by Sigma Oligos, India. Multiplex PCR was conducted utilizing QIAGEN Multiplex PCR Kits (Qiagen, Germany). DNA extraction was performed utilizing the DNeasy Ultraclean Microbial DNA Isolation Kit (Qiagen, Germany), according to the manufacturer's protocols.

Amplifications of the gyrA, parC, qepA, and aac (6')-Ib-cr genes

Employing the thermal cycler PXE 0.2 X (Thermo USA), a final reaction volume of 50 µl was created, comprising 25 µl of 2x Qiagen multiplex PCR master mix with $MgCl_2$, 5 µl of 2 µM forward and reverse primers, 10 µl of template DNA, and 10 µl of PCR water. The PCR cycling condition settings for *gyrA and parC* were as follows: Initial activation step: 2 min. at 95˚C; Three-step cycling: Denaturation for 45 sec at 94˚C, annealing for 45 sec at 60˚C, extension for 30 sec at 72˚C, and final extension for 7 min. at 72˚C for 35 cycles (Gopal *et al*., 2016). The PCR cycling temperature conditions for *qepA* and *aac(6')-Ib-cr* were: Denaturation at 95 ˚C for 10 min., followed by 34 amplification cycles including 45 sec of denaturation at 94˚C, 45 sec of annealing at 55˚C, and 45 sec of extension at 72 ˚C (Kim *et al*., 2009). The amplified products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light before photography. The 1000 bp DNA ladder was utilized to determine the size of the PCR results on the gel.

Statistical analysis

Statistical analysis was conducted utilizing SPSS version 27 (IBM Inc., Armonk, NY, USA). Data were given as frequency and percentage (%) and analyzed using the Chi-square test or Fisher's exact test. A two-tailed *P* value of < 0.05 was deemed statistically significant.

Table 1: *Primer sequences included in this study.*

Target gene	Primer sequence 5' - 3'	Annealing temperature	Amplicon size	Reference
parC	F: ATG AGC GAT ATG GCA GAG CGC CTT GCG CTA R: ACG CGC CGG TAA CAT TTT CGG TTC CTG CAT	60 ˚C	480	Gopal *et al*., 2016
gyrA	F: ATG AGC GAC CTT GCG AGA GAA ATT ACA CCG R: TTC CAT CAG CCC TTC AAT GCT GAT GAT GTC TTC		630	Renuka *et al*., 2004
qepA	F: GCAGGTCCAGCAGCGGGTAG R: CTTCCTGCCCGAGTATCGTG	55 ˚C	199	Yamane *et al*., 2008
aac (6') Ib-cr	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT		482	Park *et al*., 2006

Table 2: *Relation between a bacterial isolate and a sample (n= 100).*

Sample used for isolation	N (100)	Bacteria			x^2	P
		Klebsiella pneumonia (n= 55)	*Proteus* spp. (n= 19)	*E. coli* (n= 26)		
Ascitic fluid	2	2 (3.6%)	0 (0.0%)	0 (0.0%)	0.992	^{MC}p=1.000
Blood	28	21 (38.2%)	4 (21.1%)	3 (11.5%)	6.778*	0.03**
Bronchoalveolar lavage	3	0 (0.0%)	1 (5.3%)	2 (7.7%)	4.324	^{MC}p=0.089
Central line	7	5 (9.1%)	1 (5.3%)	1 (3.8%)	0.646	^{MC}p=0.869
Cerebrospinal fluid	6	2 (3.6%)	3 (15.8%)	1 (3.8%)	3.393	^{MC}p=0.162
Chest tube	1	1 (1.8%)	0 (0.0%)	0 (0.0%)	1.146	^{MC}p=1.000
Pus	10	4(7.3%)	4(21.1%)	2(7.7%)	2.928	^{MC}p=0.254
Sputum	24	12(21.8%)	5(26.3%)	7(26.9%)	0.321	0.852
Urine	11	4(7.3%)	1(5.3%)	6(23.1%)	4.473	^{MC}p=0.102
Wound	8	4(7.3%)	0(0.0%)	4(15.4%)	3.132	^{MC}p=0.207

*Where; Data are presented as n (%). χ^2: Chi-square test. ^MC^p: p-value calculated by Fisher's Exact test (Monte Carlo method), where expected cell counts were < 5. *Statistically significant at p < 0.05.*

Results

Study cohort and bacterial strains

Approximately 100 Enterobacteriales isolates were obtained from 100 clinical specimens of patients attended to the Ain Shams University Hospitals. The patients' ages varied from 11 days to 87 years old, averaging 49.88 ± 24.306 year. Female patients comprised 54% of the total patient population. 73% of the clinical samples were obtained from ICU patients, 25% were from inpatients in different departments, and 2% from outpatients. Blood constituted the most commonly collected clinical sample that represented 28%, followed by sputum (24%), urine (11%), and pus (10%). Additional sample percentages are presented in Table 2. After morphological and biochemical identification of these clinical isolates, the predominant bacterium was *Klebsiella pneumoniae (K. pneumoniae)* (55%), followed by *E. coli* (26%), and *Proteus* sp. (19%). The predominant source of *K. pneumoniae* isolates was blood (21/55, 38.2%), with a significant *p*-value of 0.034. Table 2 displays the prevalence of the isolated bacteria obtained from the various clinical samples.

Antibiotic susceptibility assessment

Most isolates (85-90%) resisted β-lactam/lactamase drugs. Ampicillin exhibited the greatest bacterial resistance among the isolated bacterial isolates recording 90%, followed by SAM (89%), AMC and CEF (88%), whereas gentamicin demonstrated the lowest resistance rate of 77%. 78 isolates exhibited multidrug resistance (Table 3).

Table 3: *Antibiotic susceptibility of the tested isolates (n= 100).*

Antibiotics	Sensitive	Intermediate resistance	Resistance
Ampicillin (AM)	10 (10.0%)	0 (0.0%)	90 (90.0%)
Ampicillin/sulbactam (SAM)	11(11.0%)	0 (0.0%)	89 (89.0%)
Amoxicillin/clavulanic acid (AMC)	12 (12.0%)	0 (0.0%)	88 (88.0%)
Piperacillin/tazobactam (TPZ)	15 (15.0%)	0 (0.0%)	85 (85.0%)
Ceftriaxone (CRO)	13 (13.0%)	1(1.0%)	86 (86.0%)
Cefotaxime (CTX)	14 (14.0%)	0 (0.0%)	86 (86.0%)
Ceftazidime (CAZ)	14 (14.0%)	1 (1.0%)	85 (85.0%)
Cefoperazone (CEF)	12 (12.0%)	0 (0.0%)	88 (88.0%)
Gentamicin (CN)	23 (23.0%)	0(0.0%)	77 (77.0%)
Amikacin (AK)	18 (18.0%)	2 (2.0%)	80 (80.0%)
Ciprofloxacin (CIP)	22 (22.0%)	0 (0.0%)	78 (78.0%)
Levofloxacin (LEV)	13 (13.0%)	1(1.0%)	86 (86.0%)
Imipenam (IMP)	20 (20.0%)	0 (0.0%)	80 (80.0%)
Meropenem (MEM)	20 (20.0%)	0 (0.0%)	80 (80.0%)
Sulfamethoxazole-trimethoprim (SXT)	15 (15.0%)	0 (0.0%)	85 (85.0%)

Phenotypic identification of carbapenemase-producing Enterobacterales

Out of the 100 Enterobacterales isolates, 80 exhibited resistances to IMP and MEM based on the disc diffusion assay. Of the 80 isolates, 75 (93.8%) displayed serine carbapenemases, as indicated by positive mCIM and negative eCIM results. Five isolates (6.2%) expressed metallo-β-lactamase activity as shown by positive mCIM and eCIM.

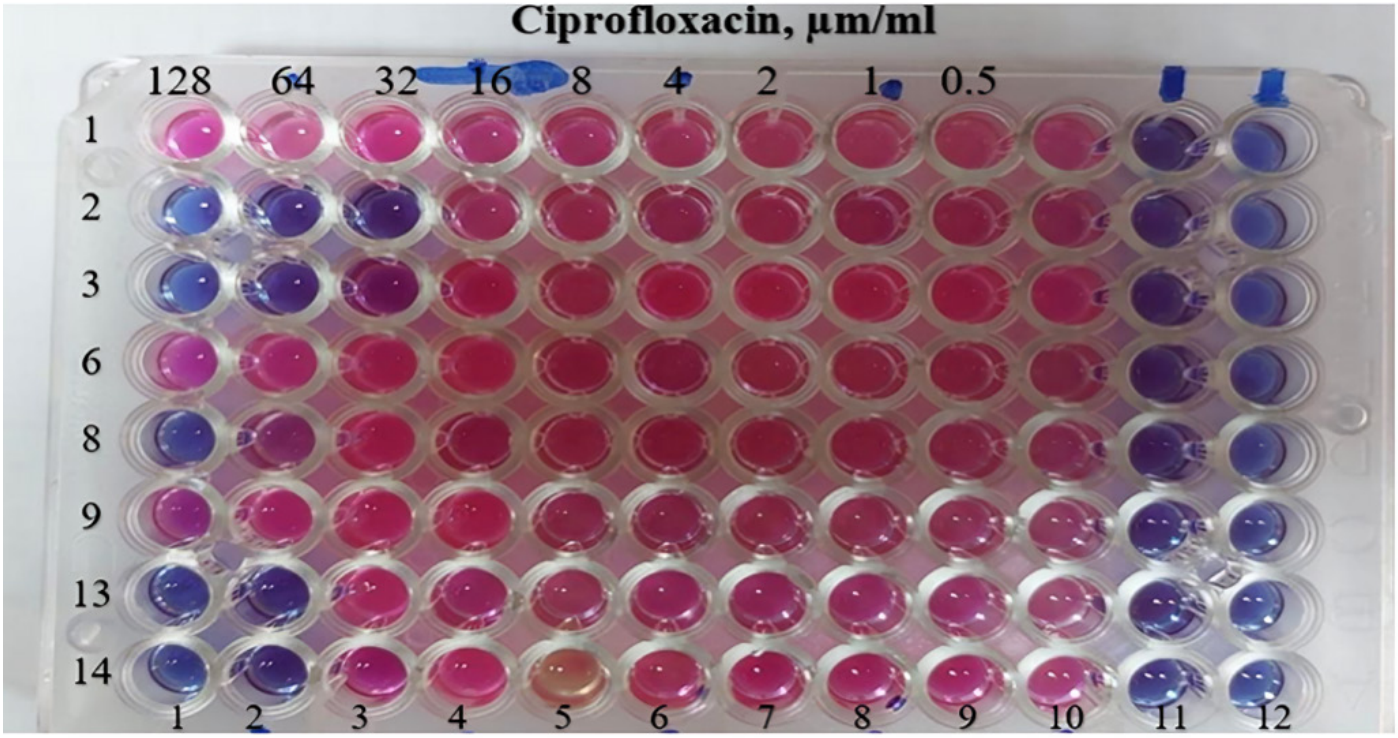

Figure 1: *Determination of ciprofloxacin MICs by the resazurin microtiter plate assay. Samples 1, 6, and 9 (red color) indicate bacterial growth) and indicate MIC ≥128 µg/ml. Sample 2: show a MIC of 32 µg/ml. Column 10: a positive bacterial control (bacteria + broth, no antibiotic), shows a change of resazurin's natural color to red. Columns 11: sterile control (broth only)– blue. Column 12: Antibiotic control (broth + antibiotic)– blue color of resazurin.*

Assessing MIC with the resazurin microtiter plate assay

The MICs of ciprofloxacin varied from 0.5 to 128 µg/ml, as illustrated in Figure 1. 25 isolates exhibited sensitivity to ciprofloxacin, recording MICs of 0.5 to 2 µg/ml. Most isolates (21%) displayed a MIC of 32 mg/ml. Substantial *p*-values were observed at MICs of 4, 8, and 16 µg/ml. Most *Klebsiella* isolates (29/55, 52.7%) expressed MICs of 4 and 8 µg/ml. Table 4 illustrates the MICs incidence of the isolated bacterial species.

Molecular identification of Quinolone resistance genes

Fifty isolates were selected from the 75 ciprofloxacin-resistant isolates and analyzed for the presence of QRDRs (*gyrA* and *parC*) and PMQR genes (*qepA and aac(6')-Ib-cr)*. The selected isolates comprised 36 *K. pneumoniae,* 11 *E. coli,* and 3 *Proteus* spp. based on their MICs. The *aac (6')-Ib-cr* gene was identified in all the tested isolates (100%). Meanwhile, the *gyrA and parC* genes were detected in most of the selected isolates, 47 and 46 out of 50 isolates (94% and 92%, respectively), revealing no significant differences (p = 0.65). On the other hand, the *qepA* gene was identified in only 4 isolates, 2 of which were *K. pneumoniae*, with no significant difference (p = 0.253), as illustrated in Table 5 and Figures 2 and 3. 42 out of 50 isolates (84%) possessed three genes, mainly *aac(6')-Ib-cr, parC,* and *gryA*. 4 isolates (8%) carried all the four tested genes. No significant relationships were noticed among ciprofloxacin MICs and *gyr A, par C,* and *qep A* genes, which demonstrated P-values of 0.928, 0.846, and 0.429, respectively.

Table 4: *Frequencies of ciprofloxacin MICs and their relation to the isolated bacteria (n= 100).*

Ciprofloxacin MIC (µg/ml)	N	*Klebsiella pneumonia* (n= 55)	*Proteus* spp. (n= 19)	*E. coli* (n= 26)	χ^2	^{MC}p
0.5	12	1(1.8%)	5(26.3%)	6(23.1%)	10.66*	0.0048*
1	10	3 (5.5%)	2(10.5%)	5(19.2%)	5.488*	0.047*
2	3	1 (1.8%)	2(10.5%)	0(0.0%)	4.217	0.095
4	16	13 (23.6%)	3(15.8%)	0(0.0%)	6.755*	0.032*
8	19	16 (29.1%)	0(0.0%)	3(11.5%)	6.560*	0.035*
16	6	1 (1.8%)	1(5.3%)	4(15.4%)	6.684*	0.016*
32	21	13 (23.6%)	4(21.0%)	4(15.4%)	0.427	0.880
64	7	5(9.1%)	1(5.3%)	1(3.8%)	0.346	1.000
128	6	2(3.7%)	1(7.1%)	3(11.5%)	3.024	0.126

*Where; MIC: Minimum Inhibitory Concentration. Resistance is defined as MIC ≥ 4 µg/ml per the CLSI. guidelines. χ^2: Chi-square test. ^MC^p: p-value calculated by Fisher's Exact test (Monte Carlo method), where expected cell counts were < 5. *Statistical values are significant at p < 0.05.*

Table 5: *Prevalence of resistance genes among the isolated pathogenic bacteria (n=50).*

Resistance genes			Bacteria						p value
			E. coli (n=11)		*Proteus* spp. (n=3)		*Klebsiella pneumonia* (n=36)		
			Number	%	Number	%	Number	%	
QRDR genes	*gyrA*	Positive (47) 94%	11	100%	3	100%	33	91.7%	0.646
		Negative (3) 6%	0	0%	0	0%	3	8.3%	
	parC	Positive (46) 92 %	11	100%	3	100%	32	88.9%	0.659
		Negative (4) 8%	0	0%	0	0%	4	11.1%	
PMQR genes	*qepA*	Positive (4) 8%	1	9.1%	1	33.33%	2	5.6%	0.253
		Negative (46) 92%	10	90.9%	2	66.67%	34	94.4%	
	aac(6´)-Ib -cr	Positive (50)100 %	11	100%	3	100%	36	100%	

*Where; The *aac(6´)-Ib-cr* gene was detected in all 50 isolates (100%). p-values were calculated by the Chi-square test through comparing gene prevalence across the bacterial species. No statistically significant differences were observed (p > 0.05).*

Figure 2: *Multiplex PCR for detection of PMQR genes (qepA and aac(6´)-Ib -cr). Lanes 3 and 6 show both the qep A gene at 199 bp, and the aac(6´)-Ib -cr gene at 482bp. All the other lanes show only the aac(6´)-Ib-cr gene (482 bp). Lane M: 1000 bp DNA ladder.*

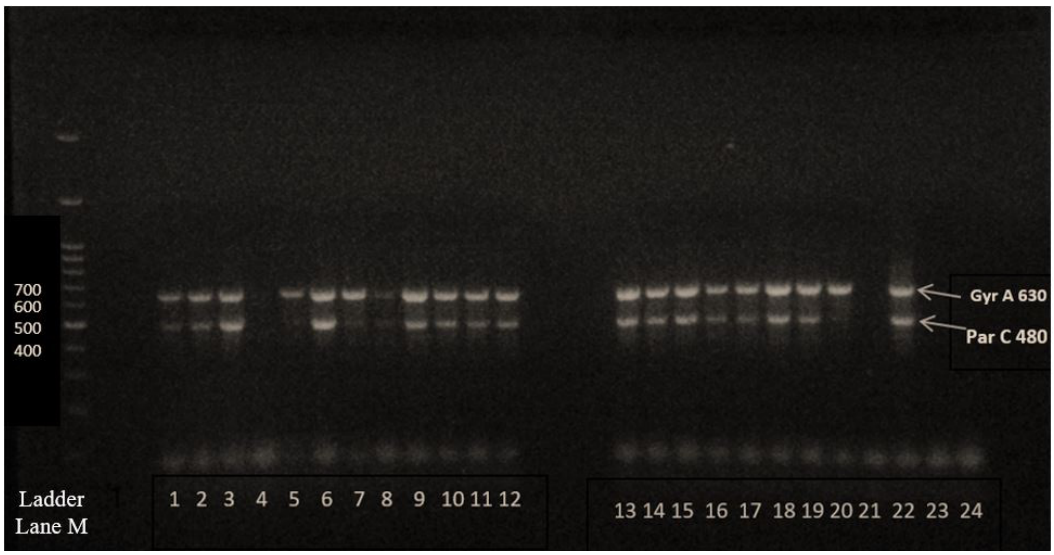

Figure 3: *Multiplex PCR detection of QRDR genes (gyrA and parC genes). Most isolates harbor both the gyrA (630 bp) and parC (480 bp) genes. Lanes 4 and 21 show the absence of both genes. Lane M: 1000 bp DNA ladder.*

Discussion

Carbapenem-resistant Enterobacterales (CREs) infections are consistently linked to increased mortality owing to limited antibiotic options and frequent co-resistance to other drug classes (Logan and Weinstein, 2017). CRE infections are also associated with substantially increased healthcare costs, with the median cost of a single CRE infection ranging from $22 484 to $66 031 in US hospitals (Bartsch *et al*., 2017). Fluoroquinolones, such as ciprofloxacin and levofloxacin, are preferred treatment for CREs infections such as cystitis (Tamma *et al*., 2021). This study sought to evaluate the FQ resistance in the carbapenem-resistant Enterobacterales and detect the *parC*, *gyrA*, *qepA*, and acc*(6) ib-cr* genes in these isolates.

In the current study, 100 *Enterobacterales* isolates were obtained, most of them (72%) were isolated from patients in the ICU, underscoring the considerable prevalence of infections in this critical care environment. This aligns with a previous study conducted by Negm *et al*. (2021) in Egypt, which assessed the various ICUs at an Egyptian tertiary care hospital.

In our investigation, blood samples were the predominant clinical specimen (28%), followed by sputum (24%), urine (11%), and pus (10%). This result agrees with Negm *et al*. (2021) study, which found the predominant infection site was blood (32.37%), followed by sputum and urine. However, urine samples were the most collected ones in two studies reported by El-Masry *et al*.(2023) and Tekele *et al*. (2020) in Saudi Arabia and Ethiopia, respectively. El-Masry *et al*. (2023) revealed that 25.4% of the samples were urine, 23.2% wounds, and 15.2% were blood, while the majority of gram-negative bacteria (GNB) were recovered from urine (72.2%) and then pus (18.6%) in the previous study conducted by Tekele *et al*. (2020). Additionally, Urooj *et al*. (2024) discovered that 58.59% (n=70) of the clinical isolates were derived from urine specimens and 34.97% (n=50) from blood specimens.

In this study, *K. pneumoniae* was the predominant isolated bacterium (55%) and the main contributor to the bloodstream infections (BSI). This bacterium was isolated from 38.2% (21/55) of the blood samples, with a p value of 0.034. A higher percentage was reported in an investigation conducted at Zagazig University Hospitals ICUs, Egypt, which detected *K. pneumoniae* BSI in 52.1% of the cases (Yahia and Wadan, 2026). According to Palmeiro *et al*. (2019); Parrott *et al*. (2021), *K. pneumoniae* isolates were recovered mainly from the blood cultures. In contrast to our result, a lower percentage of 6.25% was detected in a study conducted at Tanta University Hospitals (Taha *et al*., 2023). These obtained results emphasized the remarkable role of *K. pneumoniae* as a key contributor to bacteremia in the studied cohort. This is consistent with its known virulence potential and ability to invade the bloodstream from various primary infection sites (e.g., urinary tract, lungs, and wounds), leading to bacteremia and sepsis (Holmes *et al*., 2021).

The second most common isolated bacterium was *E. coli* (26%), which was isolated from the urine samples (6/26, 23.1%). This result aligns with a previous study conducted by El-Kholy *et al*. (2020), which reported that *E. coli* accounted for 28.4% of Gram-negative isolates in three tertiary hospitals in Cairo, Egypt, with the majority recovered from urine specimens. Similarly, Sahle *et al*. (2022) found that *E. coli* was the most frequently isolated pathogen (32.5%) among the hospitalized patients in Ethiopia, with urine samples accounting for the highest proportion of isolates. Likewise, Sisay and Mulugeta (2025) reported that *E. coli* represented 29.8% of Enterobacterales isolates obtained from hospitalized patients in Northeast Ethiopia, and urine was the most common clinical specimen that yielded this pathogen. These consistent findings across different geographic regions confirm the established role of E. coli as the leading cause of urinary tract infections in healthcare settings). These results confirm that *E. coli* was the main cause of UTIs, as it was frequently isolated from the catheterized patients, complicating the UTI treatment and increasing the likelihood of hospital-acquired complications and death (Duque-Sánchez *et al*., 2024).

In this investigation, 78% of the isolates were MDR, with ampicillin displaying the highest resistance rate among the β-lactam antibiotics. This result align with many studies conducted in different regions, indicating high rates of MDR (Tekele *et al*., 2020; El-Kholy *et al*.,2020; Sisay and Mulugeta,2025). In this study, the prevalence rate of carbapenem resistance was 80%. This rate was higher than the rates of CRE detected

in previous surveys conducted by Amer *et al.* (2016) in Kotb *et al.* (2020), who reported the carbapenem resistance rates of 62.7% and 54.1%, respectively. Also, our result was supported by the WHO Global antimicrobial resistance and use surveillance system (GLASS) report, which listed Egypt among the countries it monitors, with prominently elevated resistance levels to IMP, MEM, ertapenem, and doripenem (WHO, 2022). However, it contradicts the previous results obtained by Defrawy *et al.* (2023); Alkhawagah *et al.* (2025), who reported CRE rates of 6.5% and 19.1%, respectively. These discrepancies observed in the carbapenem resistance rates across the different studies may stem from the differences in study populations, hospital settings, and detection methods. These factors affect the reported prevalence and explain the variations observed in the different research findings.

Carbapenem-resistant *K. pneumoniae* (CRKP) is the prevalent and clinically substantial CRE reported worldwide. In this study, it was the most prevalent CRE. Comparable results have been observed in Egypt. Ali and Mohamed (2019) documented the predominance of CRKP among CRE isolates in Sohag University Hospitals. Similarly, El-Kholy *et al.* (2020) reported that *K. pneumoniae* accounted for 52.7% of MDR Gram-negative isolates from three tertiary hospitals in Cairo. In addition, El-Defrawy *et al.* (2023) found that *K. pneumoniae* constituted 68% of CRE isolates at a tertiary care hospital in Egypt. To mitigate the overuse of carbapenems, FQs are frequently advocated as the first-line of therapy for infections caused by Gram-negative bacteria. Furthermore, they may be employed in conjunction with carbapenems to manage the carbapenem-resistant bacteria.

Out of 100 isolates, 75% were resistant to ciprofloxacin based on their MICs, which ranged from 4 to 128 µg/ml. Similar to our results, a study conducted at Al-Azhar University reported a similar percentage of ciprofloxacin resistance, 73.5% (Solyman *et al.*, 2017). However, Jomehzadeh *et al.* (2022); Rezaei *et al.* (2024) documented reduced ciprofloxacin resistance levels of 18.5% and 18.6%, respectively. 50 out of 75 ciprofloxacin-resistant isolates were inspected for QRDRs (*gyrA and parC*) and PMQR genes (*qepA and aac(6')-Ib-cr*). Most of the current isolates possessed the *gyrA* and *parC* genes (94% and 92%, respectively), but specific nucleotide mutations within the QRDR regions were not identified. DNA sequencing would be required to precisely characterize the mutations (e.g., Ser83→Phe in *GyrA* or Ser80→Ile in *ParC*). Consistent with our findings, another PCR-based study conducted by Abdallah *et al.* (2025) detected the *gyrA* and *parC* genes in 53% and 37% of the ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates, respectively. Moreover, they reported a strong, statistically significant link between the presence of the *gyrA* gene and the ciprofloxacin resistance [Odds Ratio (OR)= 33.5, 95% Confidence Interval (CI): 5.97–188.2, False Discovery Rate (FDR)-adjusted $p < 0.001$] after a multivariable logistic regression. Mutations in *gyrA* and *parC* were reported in many studies. Zhan *et al.* (2021) studied the CRKP in China and reported that *gyrA* was detected in 75.2% of the isolates and *parC* in 74.5%. In addition, Onishi *et al.* (2022) reported that mutations in the *gyrA* and the *parC* genes occurred in 49.5% and 61% of *K. pneumoniae*, respectively. Moreover, Rezaei *et al.* (2024) identified mutations in the *gyrA* and *parC* genes in 87.5% and 85% of isolates, respectively. These results indicated that chromosomal changes were the main cause of elevated fluoroquinolone resistance in these Enterobacterales (Hooper and Jacoby, 2015). The complete presence of these genes in *E. coli and Proteus* spp. isolates, in contrast to 91.7% of *gyrA* and 88.9% of *ParC* in *K. pneumoniae*, along with the lack of a significant difference in the distribution of these mutations across the three species (p=0.646 for *gyrA*; p=0.659 for *parC*), suggested potential genus-specific variations in the gene acquisition. In 2006, *aac(6')-Ib-cr* gene was first reported and now it is widely disseminated, belongs to the PMQR group, and can also modify the aminoglycosides, giving it a broader selective advantage over the fluoroquinolone-specific efflux pump encoded by *qepA*. In the present study, the *aac(6')-Ib-cr* gene was identified in all the tested isolates (100%), and *qepA* was detected in four isolates, two of which were *K. pneumoniae*. Compared to a study conducted in Iraq by Kareem *et al.* (2021), *aac(6')-Ib-cr* occurred in 92.5% of *K. pneumoniae* isolates. In addition, *aac(6')-Ib-cr* was the most identified PMQR gene in a study conducted on *E. coli* isolates, which was detected in 61.1% of the isolates, followed by *qnrS* (43.3%), *qnrB* (22.2%), and *qepA* (10%) (Hooper and Jacoby, 2015). Vieira *et al.* (2020) conducted a systematic evaluation of PMQR in Enterobacterales across Latin America. They concluded that most of the analyses indicated the presence of PMQR genes in Enterobacterales derived from the human samples

(77%), predominantly from Brazil (37.7%), Mexico (18%), and Uruguay (11.5%). Nine distinct PMQR genes were identified in Latin America, with *aac-(6')-Ib-cr* (60.6%) and *qnrB* (42.6%) being the most prevalent, while *qepA* was observed in 6.5%. *E. coli* (65.6%) was the predominant species identified as harboring PMQR genes, followed by *K. pneumoniae* (39.3%). Moreover, each isolate that showed resistance to ciprofloxacin carried at least single PMQR gene, with *aac(6')-Ib-cr* present in 62.5% of them. Jomehzadeh *et al.* (2022) documented that 88% of the resistant isolates possessed the PMQR determinants and *aac(6')-Ib-cr* was detected in 50% of the isolates. However, Onishi *et al.* (2022) reported an elevated *aac(6')-Ib-cr* prevalence of 85% among the human clinical isolates. The *qepA* gene was not detected in several studies, including Onishi *et al.* (2022); Rezaei *et al.* (2024). The *qepA* gene is often linked to specific mobile genetic elements (e.g., IS26, ISCR3C) and may require co-selection with other resistance genes (*i.e.*, *rmtB or ESBLs*) for successful spread, limiting its independent dissemination (Vieira *et al.*,2020). In the current study, 84% of the isolates possessed the three genes: *aac(6´)-Ib-cr, parC,* and *gyrA*. Four isolates (8%) had all the four tested genes. Zhan *et al.* (2021) observed a lower prevalence, reporting that 68.5% of the isolates carried both the PMQR genes and the mutations in the QRDRs of *gyrA and parC*. Similarly, Rezaei *et al.* (2024) reported a lower prevalence, with the coexistence of two and three PMQR genes in 57.5% and 30% of the ciprofloxacin-resistant isolates, respectively. The diverse findings observed among the different studies concerning the prevalence rates of ciprofloxacin resistance and the presence of PMQR genes were attributed to several factors, such as the genetic background of the bacterial populations, the way of antibiotic usage, and the implementation of a stewardship program. This underscores the role of tailored interventions to regulate the spread of drug-resistance effectively.

Conclusions and Recommendations

This study demonstrated that 80% of the Enterobacterales isolates obtained from Ain Shams University Hospital were carbapenem-resistant, with 75% of them displayed resistance to ciprofloxacin, and *Klebsiella pneumoniae* was the predominant pathogenic bacterium. The *gyrA* and *parC* genes were present in more than 90% of these isolates and the plasmid-mediated *aac(6')-Ib-cr* gene was present in all the tested isolates. Further studies are recommended to conduct DNA sequencing to identify the specific point mutations within the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes, along with expansion of the PCR panels to include the *qnr* variants and the carbapenemase genes.

Novelty Statement

This study is the first Egyptian study to investigate both the chromosomal and the plasmid-mediated quinolone resistance genes and carbapenem resistance in several pathogenic clinical isolates obtained from Ain Shams University Hospital, Egypt, whereas the previous Egyptian studies have examined either carbapenem or quinolone resistance. Additionally, this study is among the few studies that document the co-carriage of the three resistance genes aac(6´)-Ib-cr, gyrA, and parC in 84% of the tested bacterial isolates, highlighting a threat to the use of fluoroquinolones as an alternative therapy for CRE infections.

Authors' Contribution

Raghda Hager: Conceptualization, Methodology, investigation, writing-original draft, Writing-review and editing.
Shimaa A. Abdel Salam: Methodology, investigation, writing-original draft, writing-review, and editing.
Nagwa M Abo El-Magd: Methodology, investigation, and writing-original draft.
All authors read and approved the final version of the manuscript.

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Ethical approval
The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (Approval No. FMASU R62/2024, dated 20 March 2024). All work was performed according to the guidelines of the International Council on Harmonization (ICH) and the Islamic Organization for Medical Sciences (IOMS), the United States Office for Human Research Protections, and the United States Code of Federal Regulations, and operated under Federal Wide Assurance No. FWA 000017585, as well as the 1964 Helsinki Declaration and its subsequent

amendments.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI-assisted technology were used in the creation of this manuscript.

Conflict of interests

The authors have declared that there are no conflicts of interest regarding the publication of this article.

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