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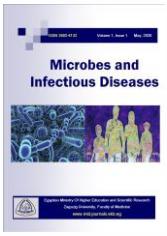

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Original article

Detection of plasmid-mediated quinolone resistance genes in *Acinetobacter baumannii* clinical isolates in Suez Canal University Hospitals in Ismailia

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ABSTRACT

Background: The Gram-negative bacterium, *Acinetobacter baumannii (A. baumannii)* has emerged as a significant threat in healthcare settings, causing severe opportunistic infections. This research aimed to identify plasmid-mediated quinolone resistance (PMQR) genes in *A. baumannii* clinical isolates in Suez Canal University Hospitals (SCUHs). **Methods:** *A. baumannii* isolates were collected and identified by conventional methods. The minimum inhibitory concentration index of ciprofloxacin was determined using the agar dilution method, and efflux pump activity was detected after the addition of carbonyl cyanide 3-chlorphenhydrazone (CCCP) efflux inhibitor. The PMQR genes were detected using conventional PCR. **Results:** The study included 44 *A. baumannii* isolates collected from 650 patients at a rate of 6.7%. The highest isolation was from the intensive care unit (56.8%) and respiratory specimens (52.3%). The highest antibiotic resistance was to ceftazidime and cefepime (95.5% and 86.4% respectively), and fluoroquinolones (FQs) (82%), and the lowest resistance was to doxycycline and tetracycline (45.5% and 54.5% respectively). After using efflux inhibitor, thirteen strains (36%) of quinolone-resistant isolates showed efflux pump activity. Using conventional PCR, the *aac (6')-Ib-cr* gene showed the highest frequency (89 %), *oqx*B gene (53 %), *qep*A gene (47 %) and *oqxA* gene (30.5%). **Conclusions:** Most PMQR genes were detected at unforeseen high levels. Strict adherence to infection control measures is important to restrain the horizontal spread of these genes. The addition of the newest arsenal of antibiotics to the Egyptian market becomes a national demand, that may partially solve this critical situation.

Introduction

Acinetobacter baumannii (*A. baumannii*) is a Gram-negative, aerobic, non-motile, glucose-non-fermenting coccobacilli and is the primary *Acinetobacter species* of clinical significance. Among opportunistic pathogens, *A. baumannii* stands out as one of the most dangerous threats in health-care settings, resulting in a wide range of life-threatening infections including ventilator-associated pneumonia (VAP), bloodstream infections and less frequently catheter-associated urinary tract infections and infections in the skin and soft tissues [1].

In addition to having inherent resistance determinants, it can get acquired resistance mechanisms resulting in multidrug, extensively

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drug and even pan drug resistance phenotypes with limited treatment options. Fluoroquinolones are synthetic antibiotics with wide-ranging antibacterial effects that disrupt bacterial DNA replication by targeting the enzymes DNA gyrase (topoisomerase II) and topoisomerase IV [2].

Mutations in the chromosomal quinolone resistance determining regions (QRDRs) of DNA gyrase and topoisomerase genes were described as main cause of resistance [3]. However, Martinez-Martinez and their team first identified plasmid-mediated quinolone resistance (PMQR) in 1998, revealing a new mechanism of quinolone resistance that has since become a growing concern [4].

To date, researchers have identified three distinct PMQR mechanisms: (i) Protection of target sites by qnr proteins; (ii) Modification of quinolones by a variant aminoglycoside acetyltransferase, aac (6')-Ib-cr (iii) and Efflux of quinolones via plasmid-encoded pumps, namely, quinolone efflux pump (qepA) and oqxAB [5].

The aac (6')-Ib-cr protein is a specialized form of aac (6')-Ib, distinguished by two amino acid substitutions, Trp102Arg and Asp179Tyr. Being a bifunctional variant, it can acetylate aminoglycosides and the amino group on the C7 piperazine ring of quinolones [6]. The OqxAB multidrug efflux pump is part of the Resistance-Nodulation Division (RND) family of efflux systems that provides resistance to nalidixic acid, norfloxacin, ciprofloxacin, chloramphenicol, and trimethoprim. It was identified on the conjugative plasmid pOLA52, which contains two open reading frames (ORFs), *oqxA and oqxB*, encoding the OqxA and OqxB proteins, respectively [7]. The QepA efflux pump is a member of the major facilitator (MFS) family and reduces susceptibility to hydrophilic fluoroquinolones, particularly ciprofloxacin and norfloxacin [8].

Attention should be paid to plasmid-mediated genes due to their ability to facilitate the horizontal spread of quinolone resistance, the selection of mutants with a higher-level of quinolone resistance, and the promotion of treatment failure [9]. Unfortunately, the majority of the studies on fluoroquinolone resistance in *A. baumannii* especially in Egypt are focused on chromosomal mutations. This study aimed to determine the prevalence and distribution of PMQR genes among clinical isolates of *Acinetobacter baumannii*. This would provide essential data to improve the local treatment guidelines and help prevent the inappropriate use of quinolones in treating this life-threatening pathogen.

Methods

Study design

This cross-sectional descriptive study was conducted during the period from March 2022 to August 2023.

Sample size:

The estimated sample size was **44 *A. baumannii* clinical isolates. According to the following equation.**

$$n = [(Z_{\alpha}/_2 / E)^2] \times P(1 - P)$$ **[10]**

Where:

n= sample size = 44 *A. baumannii* clinical isolates

$Z\alpha/2$ = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail)

P = the prevalence of *A. baumannii* infections in Suez Canal area = (2.9%) **[11]**

E = the margin of error = 0.05

Subjects

The study included patients of all age groups and both sexes admitted to various wards at Suez Canal University Hospitals in Ismailia, including the Intensive Care Unit (ICU), Neonatal Intensive Care Unit (NICU), Pediatric Intensive Care Unit (PICU), Internal Medicine, Surgery, Urology, Orthopedics, and Burn Unit. Participants also had at least two signs of systemic inflammatory response, such as fever or hypothermia, tachycardia, tachypnea, leukopenia, or leukocytosis.

Consent was taken from all patients to use their data in the research work. Patients who received antibiotic treatment in the last 48 hours were not included in the study.

Specimens

Specimens included urine, sputum, endotracheal aspirate (ETA), pus, wound exudate, blood, and pleural fluid and were properly collected under complete aseptic conditions.

Ethical approval

The study protocol was approved by the Research Ethics Committee, Faculty of Medicine Suez Canal University (Approval No. 4824 /2022). Informed consents were obtained from all participants in the study for the use of the samples.

Processing of specimens:

Specimens were streaked onto blood and MacConkey agar plates, incubated aerobically at 35-37°C for 24 - 48 hours. All isolates were identified based on standard biochemical reactions such as oxidase, catalase, indole, citrate utilization test, triple sugar iron agar, motility in the Motility Indole Ornithine (MIO) medium, and oxidation-fermentation (OF) medium [12].

Confirmation of *A. baumannii* by amplification of the *blaOXA-51*-like gene

A pair of primers: F:5 -TAA TGC TTT GAT CGG CCT TG and R: 5 -TGG ATT GCA CTT CAT CTT GG was used to amplify a **353** bp fragment of gene encoding the intrinsic **OXA-51-**like enzymes of *A. baumannii.* The amplification conditions were initial denaturation at 94 ◦C for 5 min, followed by 30 cycles of 94 ºC for 25 s, 52 ºC for 40 s, and 72 ºC for 50 s, and a final elongation at 72 ºC for 6 min. PCR mixture was done in a total volume of 25 µl including 1 µl MgCl2 (1.5 mM), 0.3 µl Taq DNA polymerase (500 U), 2.5 µl 10x PCR buffer, 0.5 µl dNTP (200 µM), 1 µl of each primer) 10 pmol/ml) and 2 µl of DNA template (5 ng genomic DNA) [13,14]. *A. baumannii* ATCC 19606 was used as a positive control.

Antibiotic susceptibility testing was done using the **Kirby-Bauer disk diffusion method** (CLSI, 2023) [15]. The following antibiotic disks were used: Cefepime (30µg), Ceftazidime (30µg), Meropenem (10µg), Gentamycin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Doxycycline (30µg). Fluoroquinolone resistance was detected if the zone diameter was ≤ 15mm around ciprofloxacin and/ or ≤ 13mm around levofloxacin disks**.**

Agar dilution method for detection of minimum inhibitory concentration (MIC) of ciprofloxacin in *A. baumannii* isolates

Muller Hinton-agar plates were prepared containing ciprofloxacin (**Sigma Aldrich, Germany)**. According to **CLSI (2023),** serial dilutions starting from 0.125 $\mu g/ml$ to 1024 $\mu g/ml$ and antibiotic-free Muller-Hinton plates (as growth control) were prepared [15]. Brain heart infusion broth suspension of different isolates was made from blood agar after 18–24hour incubation and turbidity was adjusted to be equivalent to 0.5 McFarland standard (1-2 x10^8 CFU /mL). Then, 1:10 dilution of each bacterial suspension was done to reach 1 x10^7 CFU /mL bacteria density and a 32-well replicating apparatus was used to transfer 1 μl of each bacterial suspension to a series of Muller-Hinton agar plates. After incubation of inoculated Muller-Hinton plates for 24 hours, ciprofloxacin-resistant *A. baumannii* isolate was defined if the lowest concentration of the antibiotic that showed no turbidity by naked eye was ≥ 4µg/ml [16].

Detection of fluoroquinolone active efflux pump

Efflux pump mechanism was detected by adding the efflux pump inhibitor Carbonyl Cyanide 3-Chlorophenylhydrazone (CCCP) **(Sigma-Aldrich, Germany)** to each plate of the Muller Hinton agar containing 0.125 to 1024 μg/ml ciprofloxacin. The final concentration of CCCP in the Muller Hinton agar was 10 mg/liter [17]. A muller Hinton agar plate without CCCP was used as a growth control. Ciprofloxacin MIC was measured before and after adding CCCP. In presence of active efflux pump, adding CCCP reduced MIC ≥ 4 folds.

PCR-detection of Plasmid-mediated Quinolone Resistance Genes in quinolone-resistant *A. baumannii* isolates*:*

Plasmid DNA was extracted from the test isolates by using the QIAprep Miniprep Kit **(QIAGEN, Germany).** Conventional PCR was done to screen the quinolone-resistant *A. baumannii* strains for PMQR genes namely: **1. Quinolone efflux genes;** *qepA*, *oqx*A and *oqx*B. **2. Quinolone modifying enzyme gene**; *aac(6')-1b-cr*. **Gene-specific primers are shown in Table 1.**

PCR reaction mix (25 µl) was prepared as 2µl of template plasmid DNA, 12.5 ul of 2X ABT Red master mix **(Applied Biotechnology Co. Ltd, Egypt),** and 20 pmol of both forward and reverse primers; all in distilled water. Reaction mixtures without a DNA template served as negative controls.

PCR-Amplification was carried out in a thermal cycler **(Peltier Thermal cycler, MJ Research, USA)** with the following thermal cycling conditions: initial denaturation for 5 min. at 94°C and 35 cycles consisting of: 30 sec denaturation at 94°C; 30 sec annealing at 60° C for ***aac (6') Ib-cr* genes**, at 67° C for ***oqx*A,** at 68° C for ***oqx*B** and at 55° C for ***qepA*** gene; 30 sec of extension at 72°C; and 5 minutes at 72°C for the final extension [2].

PCR Amplicons were analyzed by gel electrophoresis **(Major Science, Taiwan)** in 1% agarose gel in 1 X Tris-Borate-EDTA (TBE) buffer containing 5 µl/mL ethidium bromide at 100 volts for 90 min. and visualized with UV light. The amplicon size (bp) of the tested genes was

identified, compared to a 100 bp molecular size standard DNA ladder **(Applied Biotechnology Co. Ltd, Egypt).**

Results:

From 650 clinical specimens**, 44 *A. baumannii*** isolates (6.7%) were identified co; from ICU (56.8%), internal medicine wards (13.6%), surgery wards (11.4%), NICU (6.8%), burn unit (4.5%) and from the PICU, urology and orthopedics departments, 2.3 % for each. The highest isolation 52.3% was from respiratory specimens; (34.1% from ET aspirate,18.2% from sputum) while 18.2% of isolates were obtained from urine and 13.6% from blood. The isolation rates of *A. baumannii* strains from pus, wound swabs, and pleural effusion were 9%, 4.5%, and 2.3% respectively. All isolates were confirmed to be *A. baumannii* by the amplification of the *blaOXA-51*-like gene, **figure (1)**.

Antibiogram of *A. baumannii* isolates:

The highest antibiotic resistance rates were to ceftazidime and cefepime at rates of 95.5% and 86.4% respectively. Resistance to ciprofloxacin and levofloxacin was 82%. The lowest resistance rate was for doxycycline and tetracycline at rates of 45.5% and 54.5% respectively, **figure (2)**.

Phenotypic detection of efflux pump activity

The MIC of ciprofloxacin was measured again after the addition of 10 µg /ml Carbonyl-cyanide-3 chlorophenylhydrazone (CCCP) efflux pump inhibitor (EPI) to the Muller-Hinton agar (MHA). Out of the 36 quinolone-resistant isolates, thirteen strains (36%) showed efflux pump activity with ≥ 4-fold-reduction in MIC of ciprofloxacin, while six strains (16.7%) showed a 2-fold-reduction, and 17 strains (47.3%) didn't show any change in MIC. Comparing the MIC values before and after the addition of EPI showed a statistically significant result (***p* < 0.05, Wilcoxon Signed-Rank test), figures 3,4.**

GC: Growth Control; MIC: Minimum Inhibitory Concentration**; CCCP:** Carbonyl Cyanide 3-Chlorphenhydrazone. **Figure (3)** shows the values of MIC of ciprofloxacin of 22 isolates before the addition of CCCP using the agar dilution method. The 1st well contains crystal violet, and the other wells contain isolated strains.

Significant ***p* value = 0.014** (significant test at $p < 0.05$, Spearman correlation test; **correlation coefficient 0.4**). There was **a statistically significant direct correlation** between the number of the PMQR mechanisms detected and the MIC of ciprofloxacin in the quinolone-resistant strains.

Despite possessing the same set of PMQR genes, high variability in fluoroquinolone (FQ) level of resistance (i.e., ciprofloxacin MIC) among isolates was noted. Two isolates harbored only aac *(6')-Ib-cr* gene, however, showed high variation in MIC: 4 µg and 1024 µg. Another two isolates had *aac (6')-Ib-cr & qepA* genes but showed MIC of 512 and 1024 µg /ml. The *aac (6')-Ib-cr & oqxB* genes were detected in three different isolates with MIC 4, 256 and 1024 µg /ml. MICs of 32, 128, 256, 512, and 1024 µg /ml were found in different isolates with the same set of genes, aac *(6')-Ib-cr*, *oqxA*, and *oqxB.*

Twelve out of the 13 isolates (92.3 %) with efflux pump activity were proved to have at least one PMQR efflux pump gene (*oqx*A, *oqx*B, *or qep*A). Only one strain showed positive phenotypic efflux pump activity with no PMQR efflux pump genes detected.

The association between phenotypic efflux pump activity and the presence of PMQR efflux pump genes among quinolone-resistant *A. baumannii* strains is shown in **Table 3.** There was no statistically significant association between phenotypic efflux pump activity and PMQR efflux pump genes, **except for *the qepA* gene**. * Statistically significant result ($p < 0.05$), Chi square test was used to test significance.

The association between aminoglycoside resistance and the presence of the bifunctional aminoglycoside acetyl transferase variant **(*aac (6')-Ib-cr*)** gene among 36 *A. baumannii* strains is illustrated in **Table 4.** Thirty-one strains 88.6 % out of 35 aminoglycoside-resistant isolates harbored the *aac (6')-Ib-cr* gene. The only aminoglycoside sensitive isolate was found to have the *aac (6')-Ib-cr* gene also and this difference was statistically significant ($p < 0.05$).

Table 1. Gene-specific primers of the PMQR genes.

Target gene	Primer Sequence (5` to 3`)		Amplicon size (bp)	Annealing temperature ℃	Ref.
aac (6') Ib-cr	**F:**	CTTGCGATGCTCTATGAGTGG			**[2]**
	R:	GAATGCCTGGCGTGTTTGAA	**480**	**60**	
***qep*A**	**F:**	TCTACGGGCTCAAGCAGTTG			
	R:	ACAGCGAACCGATGACGAAG	**312**	**55**	
***oqx*A**	**F:**	CTCTCCTTTCTGCTCGTCGG			
	R:	AATAGGGGCGGTCACTTTGG	**489**	**67**	
***oqx*B**	**F:**	TAGTGCTGGTGGTGCTGGTA			
	R:	GGGTAGGGAGGTCTTTCTTCG	**480**	**68**	

Table 2. Correlation between Ciprofloxacin MIC and number of PMQR genes detected in quinolone-resistant strains (N=36).

Ciprofloxacin MIC (µg /ml)	**Nº. of strains (%)**	**Nº. of PMQR genes**
4	1 (2.8%)	2
32	1 (2.8%)	3
64	1 (2.8%)	1
	1 (2.8%)	2
128	1 (2.8%)	2
	1 (2.8%)	3
	1 (2.8%)	3
256	2(5.6%)	2
	1(2.8%)	3
512	4(11.1%)	3
	1(2.8%)	2
	1(2.8%)	4
≥1024	7	3
	6	2
	3	4
	3	1
	1	5
Total	**36**	

Table 3. The association between phenotypic efflux pump activity and the presence of plasmid-mediated quinolone efflux pump genes**.**

Efflux pump gene Isolate no.	**Efflux pump activity (n= 13)**		**No efflux pump activity (n= 23)**		***p*- value**
	No.	**%**	**No.**	**%**	
***oqx*A (n= 11)**	2	15.3%	9	39.1%	**0.46**
***oqx*B (n= 19)**	3	23 %	16	69.6 %	**0.12**
***qep*A (n = 17)**	12	92.3 %	5	21.7 %	**0.00***

Table 4. The association between Aminoglycoside resistance and the presence of the aac (6')-Ib-cr gene (N=36).

Aac (6')-Ib-cr gene	Aminoglycoside Resistance (n= 35)		Aminoglycoside Sensitive (n= 1)		*p*- value
	No.	%	No.	%	
Positive (n= 32)	31	88.6%	1	100%	**0.000***
Negative (n= 4)	4	11.4%	0	0%	

* Statistically significant result ($p < 0.05$), Chi square test was used to test significance

Figure 1. Agarose gel electrophoresis of *OXA-51* like gene amplicons (353 bp). Lane M: 100 bp DNA ladder; lane 1 positive control for the gene; lanes 2-16: amplicons of *OXA-51* gene from different isolates.

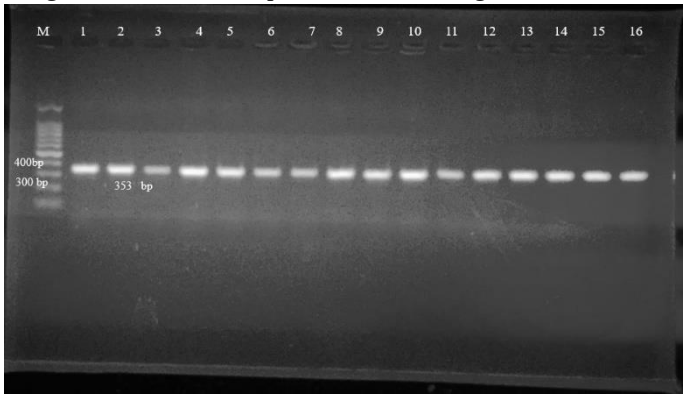


Figure 2. Antibiotic susceptibility testing of the isolated *A. baumannii* strains (N=44).

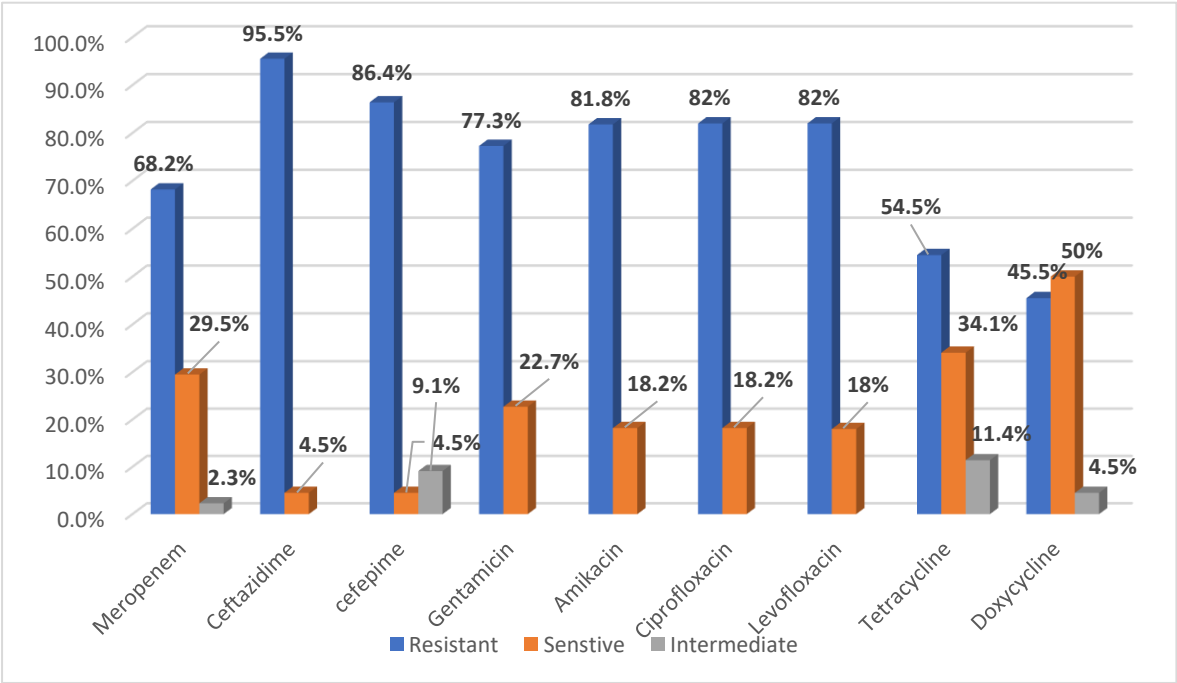


Figure 3. MIC of ciprofloxacin of 22 strains before the addition of CCCP using the agar dilution.

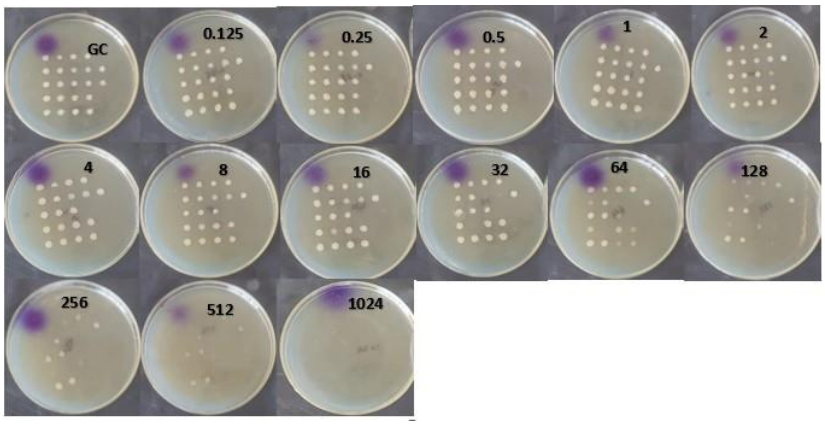
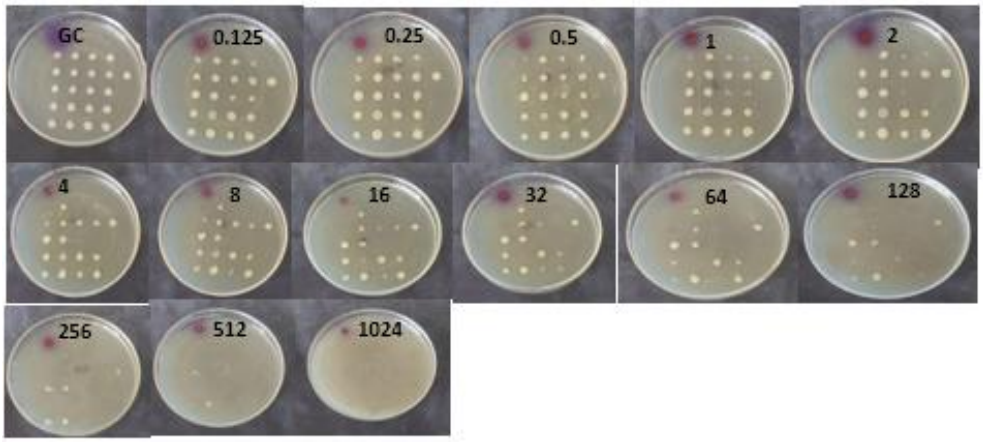


Figure 4. The values of MIC of ciprofloxacin of 22 strains after the addition of CCCP using the agar dilution method.



Detection of PMQR genes

Figure 5. Frequency distribution of the different PMQR genes.

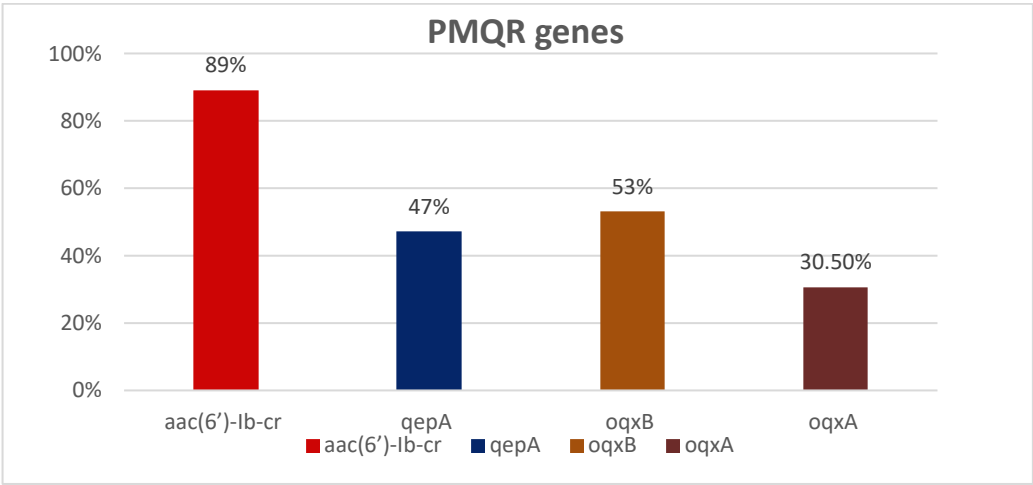


Figure 6. Agarose gel electrophoresis of *aac (6')-Ib-cr* gene amplicons (480 bp). **Lane M**: 100 bp DNA ladder; **lane 1: negative control, lane 2: positive control, lanes: 3-9**; amplicons of *aac (6')-Ib-cr* gene from different isolates.

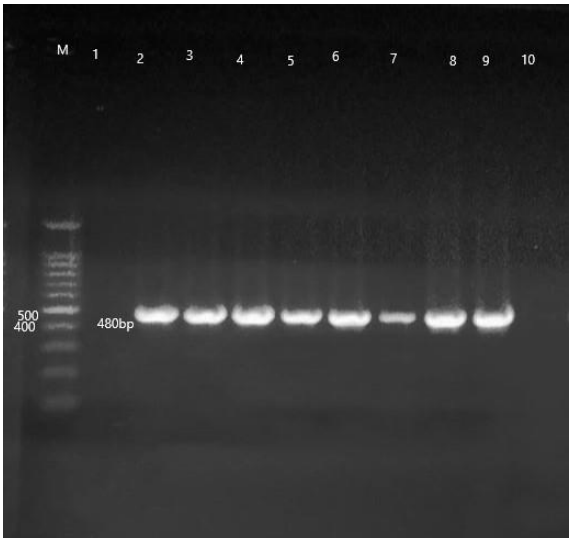


Figure 7. Agarose gel electrophoresis of *qepA gene* amplicons (312 bp). Lane M shows 100 bp DNA ladder. **Lane 1** was negative control and **lane 2** was positive control for the gene; **lanes 3-10** show amplicons of *qepA* gene from different isolates.

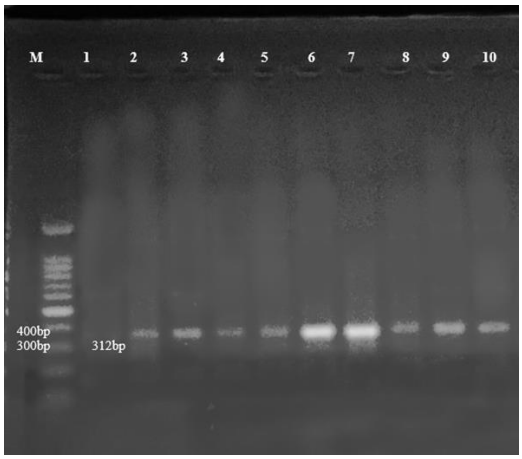


Figure 8. Agarose gel electrophoresis of *OqxB gene* amplicons (480 bp). Lane M: 100 bp DNA ladder. **Lane 1**: negative control; **lane 2**: positive control for the gene; lanes 3,5-8,10,11: amplicons of *OqxB* gene from different isolates.

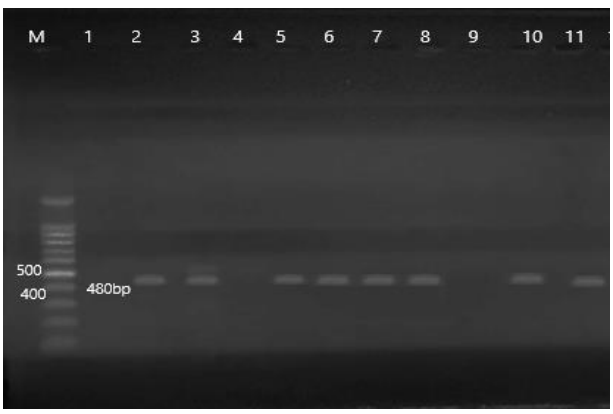
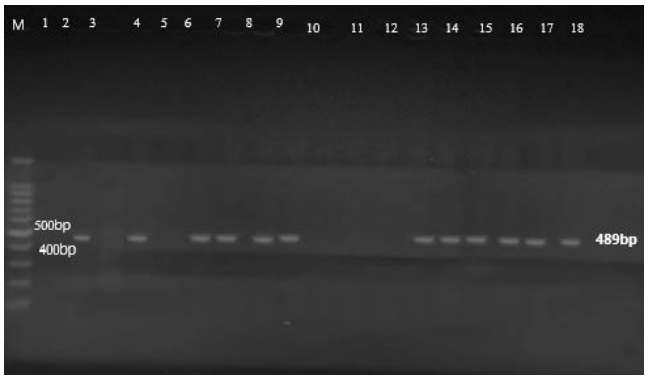


Figure 9. Agarose gel electrophoresis of *OqxA gene* amplicons (489 bp). Lane M: 100 bp DNA ladder. **Lane 1** negative control; **Lane 2** positive control for the gene; lanes 4,6-9, and 13-18: *Oqx*A gene from different isolates.



Discussion

Acinetobacter baumannii has emerged in recent years as an important infectious agent causing severe healthcare-associated infections [18]. Difficulty in treating this organism is attributed to its intrinsic resistance and ability to acquire multiple resistance determinants [19]. Quinolone antibiotics have broad-spectrum antibacterial activity [20] and the rapid increase in quinolone-resistant *A. baumannii* has become a major threat in clinical settings [21]. Mutations in the chromosomal quinolone resistance-determining region (QRDR), have the main impact on quinolone resistance. However, the acquisition of PMQR genes has contributed to quinolone resistance in this organism [2].

The majority of patients in the ICU suffer from various comorbidities and the use of urinary, I.V catheters, mechanical ventilation or intubation, puts them at risk of obtaining opportunistic pathogens such as *A. baumannii*. This could explain the fact that the highest rate of *A. baumannii* isolation in our study was from the ICU (56.8%) and from respiratory secretions (52.3%). The continuous administration of a wide array of empirical broad-spectrum antibiotics is responsible for losing efficacy of those drugs which is reflected in the antibiogram of our study results. Most isolates showed high resistance to most used antibiotics with special concern to meropenem (68%), cephalosporins (86.4-95.5%), and quinolones (82%)

Quinolones are broad spectrum antibiotics the inhibit DNA gyrase and topoisomerase IV enzymes [21]. About (72.7%) of our isolates were highly quinolone-resistant with MIC ≥128 µg /ml and these results agreed with other works in Egypt [22, 23] who reported MIC with nearly two-third of strains had MIC ≥128 µg /ml. This high level of quinolone resistance could be due to the combined actions of chromosomal and plasmid-mediated resistance mechanisms including activated multidrug efflux pumps, increased outer membrane permeability, enzymatic modification of drugs, and target gene mutation [21].

In disparity to our results, the study by **Jahromy and that by Moazzen** reported that only 5% and 25% of strains showed efflux pump activity respectively. The reported variations may be explained by differences in patterns of antibiotic usage, geographic characteristics, and environmental factors in various countries [24,25]

Although PMQR genes by themselves confer a low level of quinolone resistance, they can have an additive effect, with the chromosomal QRDR mutations contributing to higher-level quinolone resistance that can spread between *A. baumannii* strains [2]. Moreover, they provide a favorable environment for increasing rates of multidrug resistance phenotypes by carrying transferable resistance genes to other groups of antimicrobials such as the bifunctional *aac (6)-Ib-cr* gene that confers resistance to both quinolones and aminoglycosides [26].

As a bifunctional variant, the presence of the *aac (6)-1b* gene at a high rate (89%) in our study is worrisome as this gene can produce phenotypic resistance to both quinolones and aminoglycosides, adding to the multidrug resistance phenotype in our isolate. **Roy et al.** also reported a similarly high rate **(**74%) among *A. baumannii* strains [27].

An earlier Egyptian study in (2021) at Assiut University Egypt, reported different rates of

the plasmid-mediated quinolone efflux pump genes; the *oqx*A gene (73.25%), *oqx*B (39.53%), while *qep*A (0%) [2]. **Venkataramana** and his co-workers in (2022) reported lower rates of efflux pump genes: *oqxAB* gene (2.2%) and *qep*A (0%) of the study isolates [28]. At a higher rate than our study, the *qep*A gene was detected in 56% of *A. baumannii* strains in the study conducted by **Abdel-Rahim et al.** at Assiut University Hospital, Egypt [29].

High variation in the prevalence of plasmid-mediated quinolone resistance genes may be explained by the increased spread of such genes via horizontal spread over time and variation in genetic composition between different isolates in different geographic areas. Also, the excessive consumption of fluoroquinolones has created a transferable resistance among bacteria. In addition, differences in surveillance strategies, and variability in following up antibiotic stewardship among organizations can result in variations in the distribution of PMQR determinants in different health-care settings.

A remarkable result of this study is the high variability of FQ level of resistance among *A. baumannii* isolates despite possessing the same set of PMQR genes. This variability could be attributed to differences in the expression of PMQR genes among isolates, caused by variations in regulatory regions, and promoter strength that control gene expression. The plasmid carrying the PMQR genes may differ in its copy number, stability, or conjugative ability, which can affect the level of resistance [30].In addition, other resistance mechanisms such as chromosomal *gyr*A/*par*C mutations, the reduction in outer membrane porin diffusion channels or other PMQR genes that were not analyzed in this study could be cause of these variations [31].

Among 13 isolates that had phenotypic efflux pump activity, only one strain didn't harbor any PMQR efflux pump genes. Such efflux pump activity could be attributed to chromosomally encoded efflux pumps that were not evaluated in our study.

Our study gives a red alert that not only most PMQR genes were detected at unforeseen high levels in *A. baumannii* but also their co-existence is a worrisome finding. This storm of association is due to genome plasticity of *A. baumannii* and its great ability for continuous changes such as the introduction of mobile genetic elements like plasmids which mediate new genes, integrative conjugative elements, and transposons. These properties give rise to pan drug resistant (PDR), extensive drug resistant (XDR), and multidrug resistant MDR phenotypes [32].

In the study carried out by **Roy et al.**, higher MIC of FQ (>8-folds) was detected among quinolone-resistant isolates which harbored both chromosomal and PMQR gene mutations in comparison to the strains where only chromosomal mutations were detected. The existence of PMQR genes facilitates the generation of *gyr*A/*par*C mutations [27]. This may explain the result that most of our isolates (72.7%) were highly resistant with MIC ≥128 μg /ml

This study also showed that 31 strains (88.6%) out of 35 aminoglycoside-resistant isolates harbored the plasmid *aac (6')-Ib-cr* gene. The aac (6')-Ib-cr enzyme is a variant of aac (6')-Ib enzyme and is not only able to acetylate aminoglycosides: tobramycin, amikacin, and kanamycin but also can confer resistance to quinolones (mainly ciprofloxacin) **[**33]. The absence of the plasmid *aac (6')-Ib-cr* gene in 4 of aminoglycoside-resistant isolates can be explained by the presence of other aminoglycoside modifying genes that were not assessed in our study.

The insufficient adherence to infection prevention and control measures, along with noncompliance with antimicrobial stewardship practices, may account for the observed findings of high antibiotic resistance across most antibiotic classes and the coexistence of multiple PMQR genes.

Conclusions and recommendations

This study highlights the alarming resistance of *A. baumannii* in SCUHs, particularly its high resistance to fluoroquinolones, which reduces treatment options and increases mortality. The co-existence of PMQR genes at unexpected levels is concerning, emphasizing the need to understand the local resistance patterns in developing countries for better treatment strategies.

To mitigate this resistance, we recommend strict antimicrobial stewardship, continuous surveillance, and revised antibiotic use protocols in hospitals. Improved infection control measures, cautious quinolone use, and the adoption of more effective antibiotics like Ticarcillin, Cefiderocol, and Doripenem are crucial. Further research into

additional PMQR genes and chromosomal mutations is necessary to enhance treatment outcomes and combat the spread of resistance.

Conflict of interests

All authors declare no conflict of interest with respect to research, authorship, and publication of this article.

Contributions

All authors contributed greatly to the design, writing and practical work of this study. In addition, all authors contributed to preparation and editing the final study manuscript.

Limitations

The study did not evaluate all PMQR genes or the mutations in the quinolone resistance determining region, which could provide additional insights for further analysis of the findings.

Conflicts of Interest:

Non declared.

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