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Plasmid-Mediated Quinolone Resistance Genes *qnrA, qnrB, qnrC, qnrD, qnrS* and *aac (6')-Ib* in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAMA designed the study, shared in the laboratory work, managed the literature searches, managed the analyses of the results and wrote the first draft of the manuscript. Authors RMMH and SMK wrote the protocol, participated in the designing of the study, collected the specimens, shared in the laboratory work and reviewed of the manuscript. Author AMAS shared in collection of specimens, shared in the laboratory work and shared in statistical work. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The emergence and spread of antimicrobial-resistant bacterial isolates is of great concern, especially to commonly used antimicrobials as Quinolones.
Aim of the Study: Estimation of the prevalence of quinolone-resistant *P. aeruginosa* and *A. baumannii* in various clinical specimens, detection and characterization of the pattern of plasmid-mediated quinolone resistance genes present.
Materials and Methods: Bacterial identification of *P. aeruginosa* (92) and *A. baumannii* (69) strains was performed using standard laboratory methods. Antimicrobial susceptibility to other antibiotic

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was determined by the standard Kirby- Bauer disk diffusion method. All collected quinolones resistant isolates (100) were identified and further subjected to conventional PCR for detection of quinolones resistant genes; namely *qnrA, qnrB, qnrC, qnrD, qnrS and aac (6')-Ib,* using specific primers. Sequencing were done for all PMQR positive strains using A BigDye Terminator v3.1 cycle sequencing kit.

Results: PCR revealed 46% of examined quinolone-resistant isolates have one or more of PMQR genes, *A. baumannii* 28/58 (48.3%) and *P. aeruginosa* 18/42 (42.9%). The predominant gene detected was *aac (6')-Ib* either alone or in combination with other genes. There was significant isolates harboring *qnrS* gene among *P. aeuroginsa* which was mainly isolated from outpatients. Sequencing of the PMQR positive strains has confirmed the results of PCR without detection of any new varients or disconcordant strains.

Conclusions: The prevalence rate of quinolone-resistant *P. aeruginosa* and *A. baumannii* harboring PMQR genes is high in our hospitals. The *acc(6)-1b* gene is the PMQR predominant gene coding for quinolone resistance.

Keywords: PMQR; aac (6')-Ib; sequencing; P. aeruginosa and A. baumannii.

1. INTRODUCTION

The emergence and spread of antibiotic-resistant bacterial strains are of great concern worldwide. Quinolones are antibacterial agents that target bacterial DNA gyrase and topoisomerase IV [1]. Widespread use of these agents, lead to the rise of bacterial quinolone resistance. Quinolone resistance is mainly due to chromosomal mutations which lead to alteration of the drug target enzymes DNA gyrase and DNA topoisomerase IV, or activation of the efflux systems [2].

Although transmissible resistance to quinolones has long been thought not to exist, plasmid-mediated quinolone resistance (PMQR) genes have been identified in various *Enterobacteriaceae* isolates, and their prevalence is increasing worldwide [3].

The mechanisms by which PMQR have been described to mediate fluoroquinolones resistance include; the protection of DNA gyrase from fluoroquinolone by proteins from a penta-peptide family encoded by, *qnrA, qnrB, qnrC, qnr D* and *qnrS* [4] and the production of a variant of aminoglycoside acetyltransferase capable of acetylating several fluoroquinolones, *aac (6')-Ib* [5]. Although the expression of PMQR genes per se only mediates low-level resistance, they facilitate the selection of additional chromosomal resistance mechanisms, leading to a higher level of quinolone resistance and enabling bacteria to become fully resistant [6]. Antibiotic treatment against infections caused by isolates harbouring PMQR genes is more complicated because of their high potential for transmitting antibiotic resistance among different bacterial species [7].

Pseudomonas aeruginosa and *Acinetobacter baumannii* are noted for their high intrinsic resistance to antibiotics and for their ability to acquire genes encoding resistance determinants [8]. The accumulation of multiple mechanisms of resistance leads to the development of multiple resistant or pan resistant strains [9]. As both genera are frequent pathogens in patients in intensive care units, so resistance to multiple classes of antibiotics seriously compromises the treatment outcome of the infected patients especially when they are immunocompromised [10].

This study was performed to estimate the prevalence of quinolone resistant *P. aeruginosa* and *A. baumannii* in various clinical specimens and to detect and characterize the pattern of plasmid-mediated quinolone resistance genes present.

2. MATERIALS AND METHODS

2.1 Study Design & Specimen Collection

A total of 161 clinical isolates of *P. aeruginosa* (92) *and A. baumannii* (69) strains were isolated over a period of 6 months, from different samples sent to the main microbiology laboratory of Cairo University Hospital, Egypt (sputum specimens, urine specimens, wound culture, blood cultures, ear discharge, drain culture, central venous line (CVL) and bed sore culture). This study was conducted on 100 clinical isolates of Quinolones resistant *P. aeruginosa* and *A. baumannii*, in the period from February, 2017-till July , 2017. The study was limited only to bacterial isolates obtained from routine patient's samples and patients were not directly involved in the study.

2.2 Identification and Antimicrobial Susceptibility Testing

Bacterial identification was initially performed using standard laboratory methods (Versalovic et al. 2011). Antimicrobial susceptibility was determined using the modified Kirby- Bauer disk diffusion method on Mueller Hinton agar (MHA). Antibiotic disks (Mast Group Ltd., UK) included were meropenem (10 µg), imipenem (10 µg), cefotaxime (30 µg), cefepime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin/tazobactam (110 µg) and co-trimoxazole (trimethoprim/ sulfamethoxazole) (1.25/23.75 µg), ciprofloxacin (5 ug) and levofloxacin (5 ug).

Pseudomonas aeruginosa ATCC 27853 were used as quality control reference strain for antimicrobial susceptibility testing. Susceptibility testing was performed and interpreted following the Clinical and Laboratory Standards Institute (CLSI) [11].

Isolates resistant to quinolones (ciprofloxacin &/or levofloxacin); *P. aeruginosa* (42) and *A. baumannii* (58) were stored at -20°C in 5% glycerol trypticase soy broth till further processing.

2.3 Molecular Detection of Plasmid Mediated Quinolone Resistance Genes by Conventional PCR

Genotypic identification of *A. baumannii* by detection of the *bla*OXA-51-like gene as previously described [12].

All quinolones resistant isolates (100) were subjected to conventional PCR for detection of PMQR genes, using specific primers as described previously; namely *qnrA, qnrB, qnrS,* [13] *qnrC* [14], *qnrD* [15] *and aac (6')-Ib* [16] (Table 1). Plasmid DNA was extracted using the plasmid extraction kit (Zyppy™ Plasmid Miniprep Kit) according to the manufacturer's instructions. PCR Amplification reactions were carried out in a thermocycler (Applied Biosystems, USA). The reaction mixtures were prepared in a total volume of 25µl (1µl of template DNA plus 24µl of PCR master mix) including 2.0U of Taq DNA polymerase in 2x PCR buffer containing Tris·Cl, KCl, (NH4)2SO4, 15 mM MgCl2; pH 8.7, 400 µM of each dNTP and distilled water. The cycle condition for each gene was adjusted as shown in the Table (2). A negative control to detect reagent contamination was included in each PCR, containing all components except the DNA extract, which was replaced by 2 µl of Sterile distilled H_2O. The PCR products were electrophoresed on 2% agarose gel (5-8µl of the PCR products was run in parallel with a PCR size marker ladder) and then were stained with ethidium bromide solution and visualized in a gel documentation system (UVtec, UK).

2.4 Sequencing of the PCR Products of Representative Positive Isolates

Amplified PCR products of representative positive isolates were purified using PureLink® PCR Purification Kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. A BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) was used to sequence the PCR amplified products from the positive cases according to manufacturer's instructions. The sequenced products were run on a 3500 Genetic Analyzer (Applied Biosystems). Sequences were compared with those available in the GenBank database using the basic local alignment search tool (BLAST, www.ncbi.nlm.nih.gov).

Table 1. Oligonucleotide primers of the studied genes

Primer	Sequence	Amplicon size	Reference
qnrA-F	5´-ATTTCTCACGCCAGGATTTG-3´	516-bp	13
qnrA-R	5´-GATCGGCAAAGGTTAGGTCA-3´.		
qnrB-F	5´-GATCGTGAAAGCCAGAAAGG-3´	469-bp	13
qnrB-R	5´-ACGATGCCTGGTAGTTGTCC-3´		
qnrC-F	5'- GGGTTGTACATTTATTGAATC -3'	447 bp	14
qnrC-R	5'- TCCACTTTACGAGGTTCT - 3'		
qnrD –F	5' -CGAGATCAATTTACGGGGAATA-3'	600bp	15
qnrD –R	5' -AACAAGCTGAAGCGCCTG-3'		
qnrS-F	5´-ACGACATTCGTCAACTGCAA-3´	417-bp	13
qnrS-R	5´-TAAATTGGCACCCTGTAGGC-3´		
aac (6')-Ib F	5' -TGA CCT TGC GAT GCT CTA TG-3'	509 bp	16
aac (6')-Ib R	5'-TTA GGC ATC ACT GCG TGT TC-3'		

(F=forward, R=reverse)

Table 2. PCR cycle conditions for plasmid mediated quinolone resistance genes

PCR program		*QnrA*	*QnrB*	*QnrC*	*QnrD*	*QnrS*	*Aac (6')-Ib*
PCR conditions	**Initial denaturation**	**94°C 2 min**	**94°C 2 min**	**94°C 5 min**	**94°C 5 min**	**94°C 2 min**	**94°C 2 min**
	PCR Cycles	32	32	30	30	32	32
	Denaturation	94°C 45 sec	94°C 45 sec	94°C 30 sec	94°C 1 min	94°C 45 sec	94°C 30 sec
	Annealing	53°C 45 sec	53°C 45 sec	50°C 30 sec	50°C 1 min	53°C 45 sec	58°C 30 sec
	Elongation	72°C 45 sec	72°C 45 sec	72°C 30 sec	72°C 1 min	72°C 45 sec	72°C 30 sec
	final extension	72°C 5 min	72°C 5 min	72°C 7 min	72°C 10 min	72°C 5 min	72°C 5 min

2.5 The Statistical Method

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data were summarized using frequency (count) and relative frequency (percentage). For comparing categorical data, Chi-square ($\chi 2$) test was performed. P-values less than 0.05 were considered as statistically significant.

3. RESULTS

In this study, isolates were (100%) resistant to ciprofloxacin and 99 isolates (99%) were resistant to Levofloxacin with one isolate sensitive to it but included in our study as it was resistant to ciprofloxacin. The prevalence of quinolones resistance in *P. aeruginosa* was 45.6 % (42/92) and in *A. baumannii* was 84% (58/69).

Quinolone-resistant *P. aeruginosa* and *A. baumannii* were isolated from samples of patients in ICU (57%), patients admitted to different inpatient departments of Cairo University Hospitals (non ICU) (40%) and outpatients (OP) in 3% (Fig. 1).

Isolates were (100%) resistant to ciprofloxacin and 99 isolates (99%) were resistant to Levofloxacin with one isolate sensitive to it but included in our study as it was resistant to ciprofloxacin Data regarding the sensitivity pattern of the isolates to other antibiotics by the disc diffusion method was collected and illustrated in table (3).In our study, Most of the isolates show a high level of resistance to other classes of antibiotics as well as quinolones., and the highest sensitivity was to Imipenem (23%), Meropenem (22%) and Amikacin(13%) in order (Table 3).

Using PCR, 46% of total examined quinolone-resistant isolates harbored at least one of PMQR genes (*qnrA*, *qnrB qnrC*, *qnrD*, *qnrS* or *aac (6')-Ib*); *A. baumannii* 48.3% (28/58) and *P. aeruginosa* 42.9% (18/42), all PMQR positive strains harbored *aac (6')-Ib* gene. The *aac (6')-Ib* gene could be detected lonely in 31/ 46 (67.7%) and in qomination with other genes in 15/46 (32.3%) (Fig. 2, Fig. 3, Fig. 4).

Two or more genes of PMQR were present in 15 out of the 46 (32.6%) isolates, in *A. baumannii* 9 /28 (32.14%) while 6/ 18 (33.33%) in *P. aeruginosa*. The most common combination was *aac(6')-Ib* with *qnrA* (13.04%) (Table 4).

Other PMQR genes; *qnrA* gene was found in 6 out of 58 (10.3%) quinolone resistant *A. baumannii* , while 3 out of 42 (7.1%) quinolone resistant, were positive in *P. aeruginosa*, *qnrD* gene was found in 5 out of 58 (8.6%) quinolone resistant *A. baumannii* , while no positive isolates were detected among *P.* aeruginosa. Also, *qnrS* was detected in 4 out of 42 (9.5%) quinolone resistant *P. aeruginosa* but not detected in quinolone resistant *A. baumannii*. In the total 100 quinolone resistant isolates, neither *qnrB* nor *qnrC* genes could be detected (Table 5). As regards the relation between the type of organism and the PMQR genes; there was a statistically significant difference regarding the *qnrS* gene and *P. aeruginosa* (p-value 0.029) (Table 5).

The results of the sequencing of the 46 strains positive for PMQR genes have confirmed that obtained from the PCR without detection of any new variants or disconcordant strains (Fig. 5).

There was a statistically significant difference regarding the *qnrS* gene and the patient locations; as it was more prevalent among

resistant isolates in the outpatient samples than samples from patients admitted to the department than ICU patients' samples respectively (p-value 0.011) (Table 6).

There was no statistically significant difference between the sample type and the presence of different resistant genes (P value > 0.05) (Table 7).

4. DISCUSSION

Plasmid-mediated quinolone resistance had received considerable attention due to the emergence of many quinolone resistant strains in *Enterobactericae* [13]. Many studies focused on PMQR among *Enterobacteriaceae* while, few have investigated the occurrence of PMQR in *P. aeruginosa* and *A. baumannii* [2,6,12,13,15,16].

In our study, most of our quinolones resistant organisms (57%) were from patients admitted to the ICU followed by patients admitted to the different departments of Cairo University Hospitals (40%) then OP (3%) and most of our isolates obtained from wound culture samples (44%). This goes in line with the known fact that in intensive care patients, the administration of three or more antibiotics and the need for invasive medical devices as nasogastric or

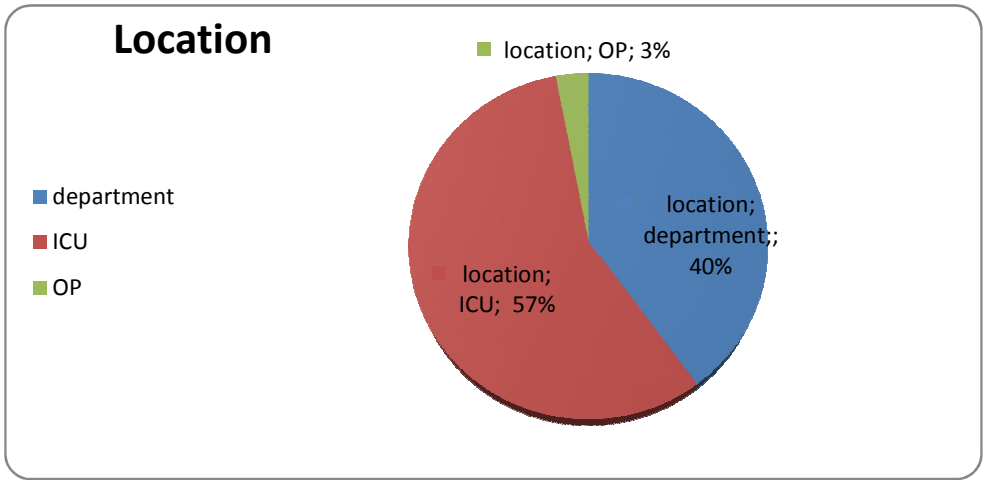

Fig. 1. Distribution of Quinolone resistant isolates detected from various specimens according to the departments.

Table 3. Antibiotic susceptibility testing of isolates

Antibiotic disc	Resistant		Sensitive	
	Count	%	Count	%
CTX	96	96.0%	4	4.0%
CFP	95	95.0%	5	5.0%
CRO	97	97.0%	3	3.0%
CAZ	97	97.0%	3	3.0%
MEM	78	78.0%	22	22.0%
IPM	77	77.0%	23	23.0%
AK	87	87.0%	13	13.0%
GM	90	90.0%	10	10.0%
PIP	91	91.0%	9	9.0%
TZP	94	94.0%	6	6.0%
SXT	95	95.0%	5	5.0%
LEV	99	99.0%	1	1.0%
CIP	100	100.0%	0	0%

cefotaxime (CTX), cefepime (CFP), ceftriaxone (CRO), ceftazidime (CAZ), meropenem (MEM), imipenem (IPM), amikacin (AK), gentamicin (GN), piperacillin (PIP), piperacillin/tazobactam (TZP), co-trimoxazole (SXT), levofloxacin (LEV) and ciprofloxacin (CIP)

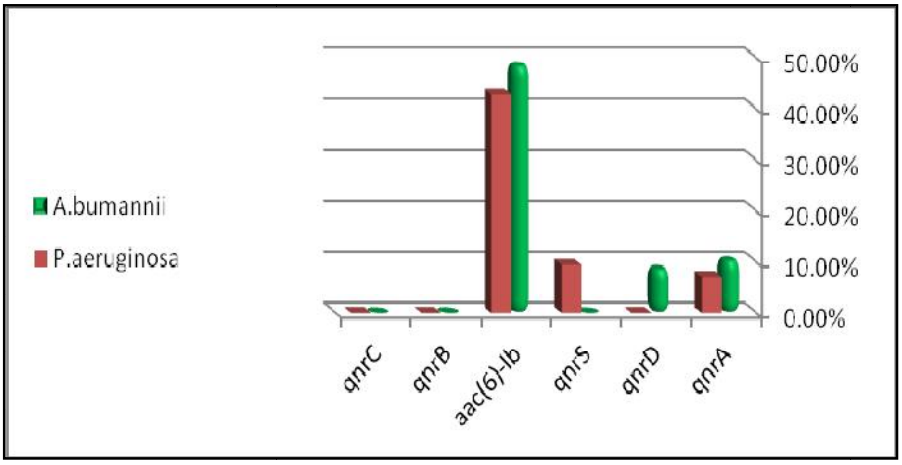

Fig. 2. Prevalence of different PMQR genes in *A. baumannii* and *P. aeruginosa* among qinolone resistant isolates

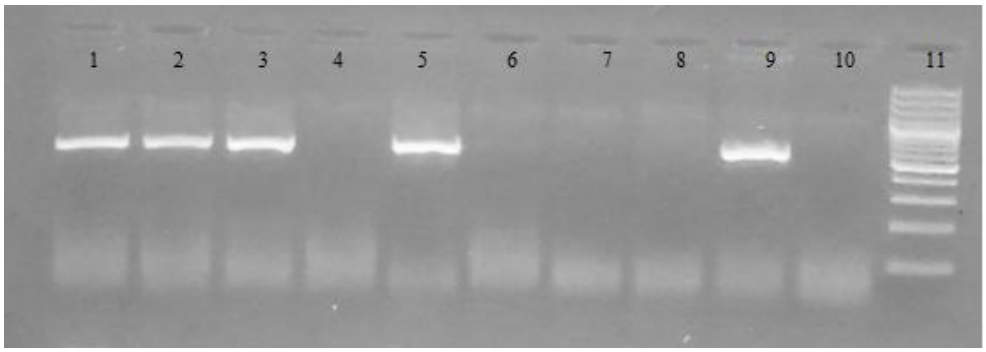

Fig. 3. Agarose gel electrophoresis and ethidium bromide staining for detection of *aac (6')-Ib* gene (amplicon at 509 bp); lanes 1,2,3,5 and 9 show positive isolates for *aac (6')-Ib* gene, lanes 4, 6, 7 and 8 show negative isolates, lane 10 show negative control and lane 11 show PCR size marker ladder.

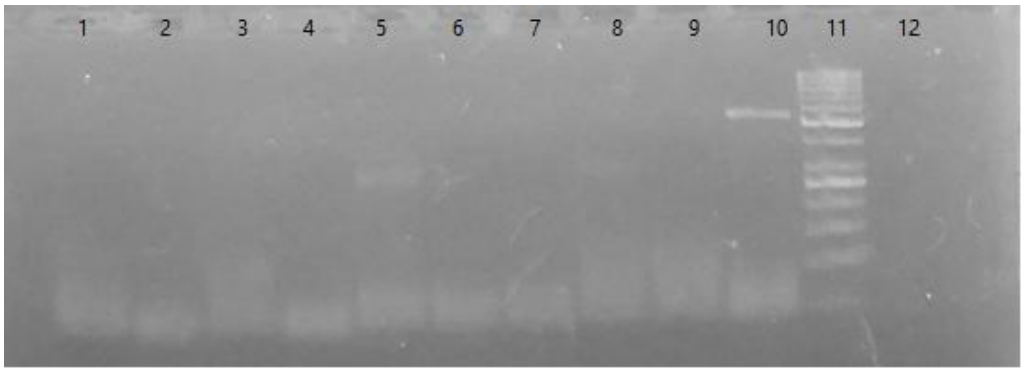

Fig. 4. Agarose gel electrophoresis and ethidium bromide staining for detection of *qnrS* gene (amplicon at 417 bp); lane 10 shows positive isolate for *qnrS*, lanes 2 to 9 show negative isolates, Lane 1 shows negative control and lane 11 show PCR size marker ladder.

endotracheal tubes, drains, and urinary or intravenous catheterization, are all risk factors associated with increased rates of infection by resistant strains of *P. aeruginosa* and *A.*

baumannii [17,18,19]. *P. aeruginosa* and *A. baumannii* are of the most common resistant organisms identified in wound infections [20,21].

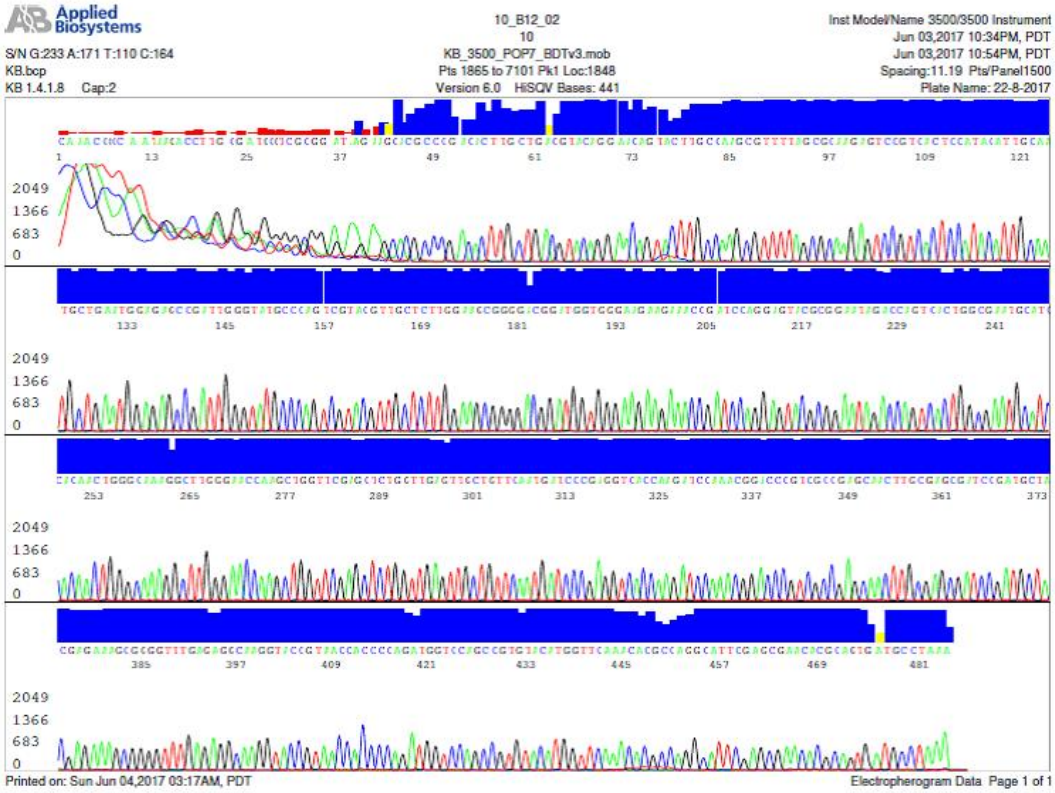

Fig. 5. Sequence analysis of *aac (6')-Ib* gene of a representative isolate

Table 4. The distribution of PMQR genes detected by PCR among the different isolates of *A. baumannii* and *P. aeruginosa*

	Acineto (58)		Pseudo (42)		Significance	
	NO	%	NO	%	X2	P value
Presence of two or more PMQR genes						
aac(6')-Ib + qnrA+ qnrD	2	3.44	0	0	1.459	0.227
aac(6')-Ib + qnrA+ qnrS	0	0	1	1.380	0.480	0.240
aac(6')-Ib + qnrA	4	6.89	2	4.76	0.194	0.659
aac(6')-Ib + qnrD	3	5.17	0	0	2.216	0.136
aac(6')-Ib + qnrD	0	0	0	0	-	-
aac(6')-Ib + qnrS	0	0	3	7.14	0.422	0.0398*
Presence of one of PMQR genes alone						
aac(6')-Ib	19	32.75	12	28.57	0.496	0.485
qnrA	0	0	0	0	-	-
qnrB	0	0	0	0	-	-
qnrC	0	0	0	0	-	-
qnrD	0	0	0	0	-	-
qnrS	0	0	0	0	-	-

Table 5. Relation between organisms and the resistant genes

PMQR genes		Organism				P value
		Acineto (58)		Pseudomonas (42)		
		Count	%	Count	%	
qnrA	Positive	6	10.3%	3	7.1%	0.730
	Negative	52	89.7%	39	92.9%	
qnrB	Positive	0	0 %	0	0%	---
	Negative	58	100.0%	42	100.0%	
qnrC	Positive	0	0%	0	.0%	---
	Negative	58	100.0%	42	100.0%	
qnrD	Positive	5	8.6%	0	0%	0.072
	Negative	53	91.4%	42	100.0%	
qnrS	Positive	0	0%	4	9.5%	0.029*
	Negative	58	100.0%	38	90.5%	
aac(6)-Ib	positive	28	48.3%	18	42.9%	0.592
	negative	30	51.7%	24		

**P value < 0.05 is considered significant*

Table 6. Relation between different patient locations and the PMQR genes

PMQR genes		Location						
		Department		ICU		OP		P value
		Count	%	Count	%	Count	%	
qnrA	positive	3	7.5%	6	10.5%	0	0%	0.799
	negative	37	92.5%	51	89.5%	3	100%	
qnrB	positive	0	0%	0	0%	0	0%	----
	negative	40	100%	57	100%	3	100%	
qnrC	positive	0	0%	0	0%	0	0%	----
	negative	40	100%	57	100%	3	100%	
qnrD	positive	1	2.5%	4	7.0%	0	0%	0.697
	negative	39	97.5%	53	93.0%	3	100 %	
qnrS	positive	3	7.5%	0	0%	1	33.3%	0.011*
	negative	37	92.5%	57	100%	2	66.7%	
aac(6)-Ib	positive	23	57.5%	22	38.6%	1	33.3%	0.158
	negative	17	42.5%	35	61.4%	2	66.7%	

**P value < 0.05 is considered significant*

The prevalence of PMQR genes among the collected isolates of phenotypically resistant *P. aeruginosa* and *A. baumannii* in our study were 42.9% and 48.3% respectively, with an overall prevalence of 46% and that is relatively higher prevalence than the prevalence reported by most of the previous studies conducted on the same organisms [22,23]

In a study conducted by Jiang et al. two (1.8%) isolates carried *qnrB* gene and 15.4% of isolates were positive for *aac (6')-Ib* from a total of 110 isolates of A. baumannii [22]. In another study three (7.7%) isolates harbored qnrB gene and 1 (2.6%) isolate harbored *qnrS* among 39 isolates of *A. baumannii* [24]. A similar study found *qnrA1* gene in 1 strain while *qnrB, qnr C, qnrD ,qnrS* and *aac(6')-Ib-cr* were not detected Among 256 *P. aeruginosa* clinical isolates [25]. While another study demonstrated 28 % isolates harboured *aac(6')-Ib* gene and 20 % harboured *qnrB* among 25 quinolone resistant *P. aeruginosa* [23].

Our results showed the dominance of *aac (6')-Ib* gene among quinolone resistant isolates. It was detected in 28 out of 58 *A. baumannii* (48.3%) and in 18 out of 42 quinolone resistant *P. aeruginosa* isolates (42.9%), either alone or in combination with other genes.

The higher detection frequency of PMQR genes in our study may indicate that the rate of PMQR is increasing and reflects that there is inappropriate and extensive use of broad-spectrum antibiotics which has resulted in the emergence of resistant isolates in our hospital settings. Moreover, the high resistance rate found among the isolates in this study

Table 7. Relation between different samples and the PMQR genes

PMQR genes		Sample type																P value
		Sputum (28)		Urine (7)		Wound (44)		Blood (11)		Ear discharge (2)		Drain (6)		central venous line (1)		bed sore (1)		
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
qnrA	P	3	10.7	0	0	4	9.1	2	18.2	0	0	0	.0%	0	0	0	0	0.87
	N	25	89.3	7	100	40	90.9	9	81.8	2	100	6	100	1	100	1	100	
qnrB	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	---
	N	28	100	7	100	44	100	11	100	2	100	6	100	1	100	1	100	
qnrC	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	---
	N	28	100	7	100	44	100	11	100	2	100	6	100	1	100	1	100	
qnrD	P	1	3.6	2	28.6	2	4.5	0	0	0	0	0	0	0	0	0	0	0.28
	N	27	96.4	5	71.4	42	95.5	11	100	2	100	6	100	1	100	1	100	
qnrS	P	1	3.6	1	14.3	1	2.3	0	0	0	0	1	16.7	0	0	0	0	0.27
	N	27	96.4	6	85.7	43	97.7	11	100	2	100	5	83.3	1	100	1	100	
aac(6)-Ib	P	13	46.4	1	14.3	23	52.3	6	54.5	0	0	2	33.3	1	100	0	0	0.35
	N	15	53.6	6	85.7	21	47.7	5	45.5	2	100	4	66.7	0	0	1	100	

*P: positive; N: Negative, *P value < 0.05 is considered significant*

emphasizes the need for an implementation of strict antibiotic policy in our hospitals for monitoring the administration of antibiotics and emergence of antibiotic resistance in bacterial isolates present in our hospital settings.

Our study demonstrated a predominance of *aac(6)-Ib* genes among the studied isolates. The prevalence of *aac(6)-Ib* gene was lower in previously published reports 7% and 35% [26,27]. Some Recent reports showed the similar and even higher prevalence of *aac(6)-1b* gene than our study with rates of 64.5% and 78.9% respectively [28,29]. In the present study, *acc(6)-1b* gene was detected in combination with other genes and as single gene in MDR strains, this is worrisome as this gene can produce phenotypic resistance to both quinolones and aminoglycosides, adding to the presence of resistance to cephalosporines with or without resistance to carbapenems in this study collection.

In our study, Most (87%) of the samples were MDR and the highest sensitivity was to imipenem, meropenem and amikacin in order. It was nearly consistent with another study as regarding susceptibility pattern of *P. aeruginosa* that reported the highest susceptibility to imipenem, meropenem and amikacin among all antibiotics included in that study [30]. This was discordant to another study that reported a higher effectiveness of amikacin (80.5% sensitive) over imipenem (66.7% sensitive) against *P. aeruginosa* [31]. This finding represents an alarm for implementation of infection control strategies and strict antibiotic setting trying to limit emergence and spread of MDR organisms.

Moreover, our study revealed that PMQR genes were positive in samples collected from ICU patients more than those from other hospital locations except *aac(6')-Ib* and *qnrS* which were more identified in samples collected from different departments. The prevalence of *qnrS* gene, among *P. aeruginosa* was statistically significant and it was more detected in samples collected from different departments. This can be explained by the fact that *qnrS* gene in our study, was detected only in *P. aeruginosa* that were isolated in large numbers from samples collected from different departments compared to *A. baumannii* (20 samples versus 17 samples).

5. CONCLUSIONS

From our results, it can be concluded that MDR *P. aeruginosa* and *A. baumannii* become of major concern in our hospitals. The *acc (6)-1b* is a PMQR predominant gene coding for quinolone resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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