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ORIGINAL ARTICLE

Co-occurrence of Plasmid-mediated Quinolone Resistance and Carbapenemases in *Klebsiella pneumoniae* Isolates in Assiut, Egypt

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ABSTRACT

Background: *Co-occurrence of carbapenem and fluoroquinolone resistance amongst K. pneumoniae strains created a problem in treating infections caused by these MDR organisms.* ***Objectives:*** *This study was carried out to evaluate the co-existence of carbapenemases and plasmid mediated quinolone resistance (PMQR) determinants in K. pneumoniae isolates in Egypt.* ***Methodology:*** *Forty-three K. pneumoniae isolates were collected from patients admitted to various intensive care units at Assiut University Hospital. Genes encoding for carbapenemases and PMQR were detected by PCR and sequencing. To determine the horizontal transfer of the PMQR and /or carbapenemases positive plasmids, conjugation experiments were performed.* ***Results:*** *Carbapenemases were detected in 34/43 (79.1%) of K. pneumoniae isolates. The positive rates of bla_{KPC} and bla_{NDM1} were (48.8%) and (74.4%), respectively. PMQR determinants were detected in 100% of K. pneumoniae isolates. The positive rates of qnrB, qnrS and aac(6`)-Ib-cr were (83.7 %), (81.4 %) and (23.3%), respectively. bla $_{NDM1}$ positive K. pneumoniae positive isolates co-harbored qnr S, qnrB and aac(6`)-Ib-cr at rates of (87.5%), (81.3%) and (25%) respectively, while (90.5%), (85.7%) and (9.5%) of bla_{KPC} positive isolates co-harbored qnrS, qnrB and aac(6`)-Ib-cr, respectively. qnrB and qnrS exhibited statistically significant association with bla_{KPC} and bla_{NDM1} ($p<0.001$).* ***Conclusion:*** *Our study revealed high rate of co-existence of carbapenemases and PMQR determinants in K. pneumoniae isolates in Egypt. To our best of knowledge, this is the first study to report the presence of a statistically significant relation between carbapenemases and PMQR.*

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is an important pathogen causing a broad range of community and hospital acquired infections [1]. These infections are usually caused by multidrug resistant (MDR) strains [2,3]. Carbapenems or fluoroquinolones (FQs) are frequently used for the management of infections initiated by such organisms. Unfortunately, the emergence of carbapenem or FQ resistant *K. pneumoniae* strains has been increasing [4].

The most important mechanism that confers resistance to carbapenems is the spread of plasmid-mediated carbapenemases of class A (KPCs) and class B (VIMs, IMPs, and NDM-1) β-lactamases [5]. Quinolone resistance may be chromosomally mediated or plasmid mediated [6]. Plasmid-mediated quinolone resistance (PMQR) determinants include: *qnr* determinants (*qnrA, qnrB, qnrC, qnrD* and *qnrS*) that act by protection of target enzymes from inhibition by fluoroquinolones [7], efflux pumps (*qepA, oqxAB*) [8] and aminoglycoside acetyltranferase (6')-Ib-cr variant enzyme that confers reduced susceptibility to ciprofloxacin by N-acetylation of its piperazinylamine [9]**.** An emerging co-existence of carbapenems and fluoroquinolone resistance in *K. pneumoniae* is causing major difficulty in treating infections caused by such pathogen [10].

In Egypt several studies had evaluated the presence of either carbapenemases or PMQR determinants in *K. pneumoniae* isolates [11-13]. As far as we know this is the first report that investigated the co-existence of carbapenemases and PMQR determinants in *K. pneumoniae* isolates in Egypt.

METHODOLOGY

Isolation and identification of *K. pneumoniae:*

Two hundred clinical samples were collected from patients hospitalized at Intensive Care Units of Assiut University Hospitals between May 2015 and June 2016. Seventy (35%) isolates were identified as *Klebsiella species* by conventional bacteriological methods. The API 20E system confirmed 35/70 (50%) as *K. pneumoniae* (BioMerieux, Marcy L'Etolie, France). Additionally, 8 *K. pneumoniae* isolates recovered from Pediatric ICU during a previous study were included in this study [3]. A total of 43 *K. pneumoniae* isolates were tested in the study.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility profile was determined for all *K. pneumoniae* isolates by Kirby–Bauer disk diffusion method using commercial antibiotic discs (Oxoid, UK): amoxacillin (10µg), amoxacillin-clavulanic acid (20,10µg), piperacillin (100µg), cefazolin (30µg), cefpodoxime (30µg), cefoperazone (75µg), ceftriaxone (30µg), aztreonam (30µg), gentamycin (10µg), amikacin (30µg) and tetracycline (30µg). The minimum inhibitory concentrations (MICs) of imipenem and ciprofloxacin were determined using E-tests (BioMérieux, Solna, Sweden). The results were interpreted according to the Clinical and Laboratory Standard Institute guidelines [14].

Phenotypic detection of carbapenemases:

Carbapenemase production by *K. pneumoniae* isolates was screened by Modified Hodge test (MHT), as previously described [15]. MBLs activity was determined by combined disk (CD) test, using EDTA as inhibitor [16] and by E-test MßLs (Liofilchem, Italy).

Detection of genes encoding carbapenemases and PMQR determinants by PCR:

DNA was extracted from *K. pneumoniae* isolates by boiling method [17]. Screening of genes encoding carbapenemases (bla_{KPC}, bla_{VIM1}, bla_{IMP} and bla_{NDM1}) and PMQR determinants (*qnrA*, *qnrB*, *qnrS*, *qepA* and *aac(6`)-Ib-cr*) was performed by PCR. Amplification was done in a thermal cycler (BioRadT100, USA) using hot start taq DNA polymerase (Biolabs, New England) in a 50-µl volume. Annealing temperatures and sequences of oligonucleotide primers used for PCR amplification are listed in table (1). The sequences obtained for each gene were compared with published sequences on the National Center for the Biotechnology website (http://www.ncbi.nlm.nih.gov).

After PCR amplification, the *aac(6')-Ib* positive products were further subjected to digestion with the restriction enzyme BtsCI (Jena Bioscience, Germany) for detection of the *cr* variant. The detection of 272-bp and 210-bp DNA fragments was suggestive of *aac(6')-Ib*, while an undigested fragment was suggestive of *aac(6')-Ib-cr* variant.

Table 1: Oligonucleotide primers and annealing temperatures used for PCR amplification of different genes.

Gene	Sequence (5'-3')	Size	Tm	Reference
bla_{NDM1}	F: GGTTTGGCGATCTGGTTTTC	621 bp	55 °C	[31]
	R: CGGAATGGCTCATCACGATC			
bla_{VIM1}	F: TCTACATGACCGCGTCTGTC	747 bp	58 °C	[32]
	R: TGTGCTTTGACAACGTTCGC			
bla_{IMP}	F: CTTGATGAAGGCGTTTATGTT	610 bp	54 °C	[33]
	R: TAACCGCCTGCTCTAATGTAAG			
bla_{KPC}	F: ATGTCACTGTATCGCCGTCT	893 bp	57 °C	[34]
	R: TTTTCAGAGCCTTACTGCCC			
qnrA	F: ATTTCTCACGCCAGGATTTG	518 bp	54 °C	[35]
	R: GATCGGCAAAGGTTAGGTCA			
qnrB	F: GATCGTGAAAGCCAGAAAGG	469 bp	54 °C	[36]
	R: ACGATGCCTGGTAGTTGTCC			
qnrS	F: CAATCATACATATCGGCACC	641 bp	56 °C	[37]
	R: TCAGGATAAACAACAATACCC			
aac(6`)-Ib	F: TTGCGATGCTCTATGAGTGGCTA	482 bp	58 °C	[38]
	R: CTCGAATGCCTGGCGTGTTT			
qepA	F: GCAGGTCCAGCAGCGGGTAG	218 bp	58 °C	[39]
	R: CTTCCTGCCCGAGTATCGTG			

Conjugation experiment:

To determine the transmissibility of the resistance determinants, conjugation experiment was performed on all *K. pneumoniae* isolates. Horizontal transfer of antimicrobial resistance genes was investigated with the conjugation method modified from Miller (1972) [18]. All isolates were grown on Luria–Bertani (LB) broth with *E. coli* J53 as the recipient. Selection of transconjugants was done on LB agar plates supplemented with sodium azide (200 µg/ml) for counter selection, and imipenem (0.5 µg/ml) or ciprofloxacin (2 µg/ml). The presence of PMQR and carbapenemases in transconjugants was determined by PCR.

RESULTS

Antimicrobial susceptibility pattern:

Forty *K. pneumoniae* isolates were found to be MDR while 3 isolates expressed Pan Drug resistant (PDR) phenotype (resistant to all drug classes). Twenty-three isolates were resistant to imipenem when tested by E-test®. Carbapenemase production was detected in 31/43 (72.1%) of *K. pneumoniae* isolates by MHT, while MßLs production was determined in 25/43 (58.1%) of *K. pneumoniae* isolates by both CD test and E-test MßLs.

Prevalence of carbapenemases among *K. pneumoniae* isolates:

Carbapenemase encoding genes were detected by PCR in 34/43 (79.1%) of *K. pneumoniae* isolates. bla_{KPC} was detected in 21 (48.8%), while bla_{NDM1} was detected in 32 (74.4%) of the isolates (Table 2). bla_{KPC} and bla_{NDM1} co-existed in 19/34 (55.9%) of carbapenemase positive isolates. None of the isolates harbored bla_{VIM1} or bla_{IMP}. Both bla_{KPC} and bla_{NDM1} were statistically significant positively correlated to imipenem resistance pattern (r=0.44, p=0.004), (r=0.39, p=0.010) respectively (Table 3). There was statistically significant positive weak correlation between bla_{NDM1} and bla_{KPC} (r=0.36, p=0.018).

Table 2: Carbapenemases, PMQR determinants and associated MICs for imipenem and ciprofloxacin in *Klebsiella pneumoniae* isolates (n=43)

ID	MIC IMP	Carbapenemases	MIC CIP	PMQR genes	Phenotype
Carbapenem resistant isolates					
K1	**>32 (R)**	*KPC, NDM1*	**>32(R)**	*qnrB, qnrS*	MDR[1,2,3,4,7,8]
K2	**>32 (R)**	*KPC, NDM1*	**>32(R)**	*qnrB, qnrS*	PDR
K3	**>32 (R)**	*KPC, NDM1*	**>32(R)**	*qnrB, qnrS*	MDR[1,2,3,4,5,7,8,9]
K4P	**>32 (R)**	*KPC, NDM1*	**>32 (R)**	*qnrB, qnrS*	MDR[1,2,3,4,7,8,9]
K5	**>32 (R)**	*KPC, NDM1*	**>32 (R)**	*qnrB, qnrS*	MDR[1,2,3,4,5,7,8,9]
K6	**>32 (R)**	*KPC, NDM1*	**>32(R)**	*qn B, qnrS, aac(6`)-Ib-cr*	PDR
K7	**16 (R)**	*NDM1*	**>32(R)**	*qnrB, aac(6`)-Ib-cr*	MDR[1,2,3,4,6,7,8]
K8	**16 (R)**	*KPC, NDM1*	**>32 (R)**	*qnrS*	MDR[1,2,3,4,7,8,9]
K9	**12 (R)**	*NDM1*	**>32(R)**	*qnrB, qnrS, aac(6`)-Ib-cr*	MDR[1,2,3,4,6,7,8]
K10P	**12 (R)**	*KPC, NDM1*	**>32 (R)**	*qnrB, qnrS*	MDR[1,2,3,4,7,8,9]
K11	**12 (R)**	*NDM1*	**1(S)**	*qnrB*	MDR[1,2,3,4,5,6,8,9]
K12	**8 (R)**	*KPC, NDM1*	**0.125 (S)**	*qnrB, qnrS*	MDR[1,2,3,4,6,8,9]
K13	**8 (R)**	*KPC, NDM1*	**>32 (R)**	*qnrB, qnrS*	MDR[1,2,3,4,7,8,9]
K14	**6 (R)**	*KPC, NDM1*	**>32(R)**	*qnrS, aac(6`)-Ib-cr*	MDR[1,2,3,4,7]
K15	**6 (R)**	*NDM1*	**>32 (R)**	*qnrS*	MDR[1,2,3,4,7,8,9]
K16	**6 (R)**	*NDM1*	**>32 (R)**	*qnrB, qnrS, aac(6`)-Ib-cr*	MDR[1,2,3,4,5,7,9]
K17	**4 (R)**	*NDM1*	**>32(R)**	*qnrB, aac(6`)-Ib-cr*	MDR[1,2,3,4,6,7,8,9]
K18	**4 (R)**	*NDM1*	**>32(R)**	*qnrS, aac(6`)-Ib-cr*	PDR
K19	**4 (R)**	*NDM1*	**>32 (R)**	*qnrS*	MDR[1,2,3,4,7,8,9]
K20	**4 (R)**	-	**>32(R)**	*qnrB, qnrS, aac(6`)-Ib-cr*	MDR[1,2,3,4,5,7,8,9]
K21	**4 (R)**	-	**6(R)**	*qnr B*	MDR[1,2,3,4,7,8,9]
K22	**4 (R)**	*NDM1*	**1(S)**	*qnr B, qnrS*	MDR[1,2,3,4,5,6,8]
K23	**4 (R)**	*KPC, NDM1*	**0.75(S)**	*qnr B, qnrS*	MDR[1,2,3,4,5,6,8]
K24	**4 (R)**	*KPC, NDM1*	**0.75(S)**	*qnrS*	MDR[1,2,3,4,8,9]
K25P	**4 (R)**	*KPC, NDM1*	**0.38 (S)**	*qnrB, qnrS*	MDR[1,2,3,4,8,9]
K26P	**4 (R)**	*KPC, NDM1*	**0.06 (S)**	*qnrB, qnrS*	MDR[1,2,3,4,8,9]
K27P	**4 (R)**	*KPC, NDM1*	**0.06 (S)**	*qnr B, qnrS*	MDR[1,2,3,4,8,9]
K28P	**4 (R)**	*KPC, NDM1*	**0.04(S)**	*qnrB*	MDR[1,2,3,4,8,9]
Carbapenem intermediate isolates					
K29	**3 (I)**	-	**>32(R)**	*qnrS,, aac(6`)-Ib-cr*	MDR[1,2,3,5,7,8]
K30	**3 (I)**	*NDM1*	**>32 (R)**	*qnr B, qnrS, aac(6`)-Ib-cr*	MDR[1,2,3,5,7,8]
K31P	**3 (I)**	*KPC, NDM1*	**0.75(S)**	*qnr B, qnrS*	MDR[1,2,3,8,9]
K32	**2 (I)**	-	**4(R)**	*qnr B*	MDR[1,2,3,5,6,7,8,9]
K33	**1.5 (I)**	-	**6(R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K34P	**1.5 (I)**	*KPC, NDM1*	**0.12 (S)**	*qnr B, qnrS*	MDR[1,2,3,6,8,9]
Carbapenem sensitive isolates					
K35	**1 (S)**	*NDM1*	**>32(R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K36	**0.75 (S)**	*KPC*	**>32(R)**	*qnr B*	MDR[1,2,3,5,6,7,8,9]
K37	**0.75 (S)**	*KPC*	**>32(R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K38	**0.75 (S)**	*NDM1*	**>32(R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K39	**0.5 (S)**	-	**8(R)**	*qnr B*	MDR[1,2,3,5,6,7,8,9]
K40	**0.25 (S)**	-	**4 (R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,9]
K41	**0.25 (S)**	-	**6 (R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K42	**0.125 (S)**	*NDM1*	**4 (R)**	*qnr B, qnrS*	MDR[1,2,3,5,6,7,8,9]
K43	**0.125 (S)**	-	**2 (I)**	*qnr B, qnrS*	MDR[1,2,3,5,6,9]

Abbreviations: IMP, imipenem; CIP, ciprofloxacin; MDR, multi drug resistance; PDR, pan drug resistance.
Key: 1=penicillins; 2=cephalosporins; 3=monobactams; 4= carbapenems; 5=tetracyclines; 6=chloramphenicol; 7=fluoroquinolone; 8= trimethoprim sulfonadmide; 9 = aminoglycosides.

Table 3: Distribution of carbapenemases and its relation to carbapenem resistance pattern in *K. pneumoniae* isolates.

Carbapenemases	Total (43)	R (28)	I (6)	S (9)	r	p value
	No. (%)	No. (%)	No. (%)	No. (%)		
bla$_{KPC}$	21 (48.8%)	17 (60%)	2 (33.3%)	2 (22.2%)	0.44	0.004**
blaNDM1	32 (74.4%)	26 (92.9%)	3 (50%)	3 (33.3%)	0.39	0.010

R=Resistant I=Intermediate S=Sensitive
* Statistically significant correlation (p<0.05)
** Statistically significant correlation (p<0.01)

Prevalence of PMQR among *K. pneumoniae* isolates:

One or more of PMQR determinants were detected in each of *K. pneumoniae* isolates. Thirty six (83.7%) isolates harbored *qnrB*, 35 (81.4%) isolates harbored *qnrS*, while 10 (23.3%) isolates harbored *aac(6`)-Ib-cr* (Table 2). No *qnrA* or *qepA* was detected in any isolate. *qnrB* and *qnrS* were present in (90.9%), (81.8%) of ciprofloxacin susceptible isolates, respectively. Statistically significant weak positive correlation between *aac(6`)-Ib-cr* and ciprofloxacin resistance (r=0.49, p=0.001) was detected, while the presence of *qnrB* and *qnrS* had no relation to ciprofloxacin resistance pattern (Table 4).

Table 4: Distribution of PMQR determinants and their relations to ciprofloxacin resistance pattern in the *K. pneumoniae* isolates

PMQR	Total (43)	R (31)	I (1)	S (11)	r	p value
	No. (%)	No. (%)	No. (%)	No. (%)		
qnrB	36 (83.7%)	25 (80.6%)	1 (100%)	10 (90.9%)	-0.26	0.096
qnrS	35 (81.4%)	25 (80.6%)	1 (100%)	9 (81.8%)	0.15	0.350

R=Resistant I=Intermediate S=Sensitive
** Statistically significant correlation (p<0.01)

Co-existence of carbapenemases and PMQR determinants in *K. pneumoniae* isolates:

The prevalence of PMQR determinants amongst carbapenemase positive *K. pneumoniae* isolates (34) was 100%. Specifically, (87.5%), (81.3%) and (25%) of *bla$_{NDM1}$* positive isolates co-harbored *qnrS*, *qnrB* and *aac(6`)-Ib-cr*, respectively, while (90.5%), (85.7%) and (9.5%) of *bla$_{KPC}$* positive isolates co-harbored *qnrS*, *qnrB* and *aac(6`)-Ib-cr*, respectively. The presence of both *bla$_{NDM1}$* and *bla$_{KPC}$* was statistically significant higher in isolates containing *qnrS* or *qnrB* (Table 5).

Table 5: Co-existence of carbapenemases and PMQR determinants among *K. pneumoniae* isolates

Carbapenemases	PMQR No (%)				
	qnrS	p value	*qnrB*	p value	*aac(6`)-Ib-cr*
bla $_{KPC}$ (n=21)	19 (90.5%)	<0.001**	18 (85.7%)	<0.001**	2 (9.5%)
bla $_{NDM1}$ (n=32)	28 (87.5%)	<0.001**	26 (81.3%)	<0.001**	8 (25%)

** Statistically significant difference (p<0.01)

Conjugative transfer of resistance genes:

PMQR determinants (*qnrB*, *qnrS* and *aac(6`)-Ib-cr*) and carbapenemases (*bla$_{KPC}$* and *bla$_{NDM1}$*) were successfully transferred by conjugation from all *K. pneumoniae* isolates to the recipient (*E. coli J53*).

DISCUSSION

Serious infections caused by MDR *K. pneumoniae* are frequently treated by carbapenems or fluoroquinolones [19]. The co-existence of PMQR determinants together with the carbapenemases among *K. pneumoniae* isolates limits the treatment options for these drug-resistant strains [20]. In the present study, carbapenemases and PMQR coexisted in 34/43 of *K. pneumoniae* recovered from Assiut University Hospitals. As far as we know, the present study is the first to report the presence of statistically significant correlation between the occurrence of PMQR and carbapenemases as well as the co-existence of *qnrB*, *qnrS*, *aac(6`)-Ib-cr*, *bla$_{KPC}$* and *bla$_{NDM1}$* in the same *K. pneumoniae* isolate.

Carbapenem resistance resulting from production of carbapenemases is disseminating worldwide [21]. High

prevalence of carbapenemases (79.1%) among *K. pneumoniae* isolates was detected in our study. bla_{NDM1} was the most prevalent carbapenemase (74.4%), followed by bla_{KPC} (48.8%), while neither bla_{VIM1} nor bla_{IMP} was detected in any of *K. pneumoniae* isolates. In agreement with our results, previous studies reported similar prevalence of bla_{NDM1} and bla_{KPC} among *K. pneumoniae* [22,23]. However, in a recent Egyptian study, lower prevalence of bla_{NDM1} was reported in *K. pneumoniae* isolates [24]. The elevated frequency of carbapenemase positive isolates in the present study might be due to the excessive use of carbapenem in our hospitals.

Although bla_{KPC} and bla_{NDM1} were detected in (22.2%), (33.3%) of the imipenem sensitive *K. pneumoniae* isolates, both bla_{KPC} and bla_{NDM1} were statistically significant positively correlated to imipenem resistance pattern (r=0.44, p=0.004), (r=0.39, p=0.010), respectively (Table 2). This finding contradicts the previous assumption that bla_{NDM1} or bla_{KPC} alone mediate only reduced susceptibility to carbapenems and other mechanisms, as porin loss, are usually required for full resistance to appear [25].

In an attempt to understand the relation between the existence of PMQR and carbapenemases, we tested *K. pneumoniae* isolates for the presence of PMQR by PCR. Our study reported high prevalence of PMQR determinants (100%) in *K. pneumoniae* isolates which is similar to the results of a previous Egyptian study [12]. Interestingly, all *K. pneumoniae* isolates (even those isolated from PICU where FQs are contraindicated) have at least one PMQR, which implies that quinolone resistant plasmids are endemic and circulating in Assiut University Hospital. *qnrB* contributed to the majority (83.7%) of PMQR determinants detected in this study, followed by *qnrS*, which was detected in (81.4 %) of *K. pneumoniae* isolates, while *aac(6`)-Ib-cr* showed the lowest prevalence (23.3%).

Coexistence of resistance genes in the same isolate seemed to be the primary cause of the appearance of MDR or even PDR strains[26]**.** Co-occurrence of carbapenemases and PMQR in the same isolate was previously described in *Klebsiella pneumoniae*[27,28]**.** In the present study, (79.1%) of *K. pneumoniae* isolates co-harbored carbapenemases and PMQR determinants. bla_{KPC} and bla_{NDM1} co-existed with *qnrS* in (90.5%), (87.5%) of *K. pneumoniae*, respectively and with *qnrB* in (85.7%), (81.3%), of *K. pneumoniae* respectively. Excitingly, our results indicated statistically significant relations between different carbapenemases and PMQR determinants amongst *K. pneumoniae* isolates ($p<0.001$), which increase the possibility that the multiple resistance determinants might be co-expressed on the same plasmid.

The mobility of plasmid-mediated resistance carries additional risks of spread of resistance determinants between different species[29]. In this study, PMQR determinants and carbapenemases were successfully transferred via conjugation in all *K. pneumoniae* isolates. Our results are in accordance with what reported by previous studies [27,30]. This high transmissibility of PMQR genes and carbapenemases points to the risk of potential spread of these resistance determinants among other pathogens in the hospital, which intensify the need for implementation of strict infection control measures to limit their dissemination between strains.

It is prudent to say that all *K. pneumoniae* isolates recovered from PICU have the same combination of PMQR and carbapenemases ($bla_{KPC,}$ bla_{NDM1}, *qnrB*, *qnrS*) although they have different PFGE pattern [3]. Additionally, all the resistance genes were transferred successfully by conjugation, which is suggestive of the presence of an endemic plasmid harboring the four resistance genes circulating in the PICU.

CONCLUSION

The results of the current study revealed high prevalence of carbapenemases and PMQR determinants among *K. pneumoniae* isolates in Egypt, as well as the co-existence of multiple resistance determinants in various combinations in the same isolate. The presence of $bla_{KPC,}$ bla_{NDM1} is significantly related to the presence of both *qnrB* and *qnrS* which suggest a role of these PMQR in favoring selection of carbapenemases, however, this speculation needs further investigation.

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Conflicts of interest:

The authors declare no conflicts of interest.

Ethical Statement:

The Ethics Committee of the Faculty of Medicine, Assiut University approved the study according to the latest revision of the Declaration of Helsinki, and informed consent was obtained from participating patients.

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