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ORIGINAL ARTICLE

pKpQIL-like plasmid contributes to the dissemination of *bla*$_{NDM-1}$ and plasmid mediated quinolone resistance determinants among multi drug resistant *Klebsiella pneumoniae* in Assiut university hospital, Egypt

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ABSTRACT

Background and Objectives: Concomitant carriage of *bla*$_{NDM-1}$ and plasmid mediated quinolone resistance determinants (PMQRs) by multi drug resistant (MDR) *Klebsiella pneumoniae (K. pneumoniae)* has increased globally, often related to their presence on transmissible plasmids. In this study, we hypothesized the presence of *bla*$_{NDM-1}$ and PMQRs on a single conjugative plasmid that circulates among *K. pneumoniae* strains isolated from Assiut University Hospital.

Materials and Methods: Twenty-two clinical MDR *K. pneumoniae* strains harboring both *bla*$_{NDM-1}$ and PMQRs were genotyped using pulsed field gel electrophoresis. Horizontal transfer of *bla*$_{NDM-1}$ and PMQRs was evaluated by conjugation and trans-conjugants were screened for the presence of both genes and integron by PCR. Trans-conjugant's plasmid DNA bands were purified using agarose gel electrophoresis and different DNA bands were screened for *bla*$_{NDM-1}$ and PMQRs. Plasmids carrying *bla*$_{NDM-1}$ and PMQRs were typed by PCR based replicon typing.

Results: All MDR *K. pneumoniae* contained class 1 integron and belonged to 15 pulsotypes. *Bla*$_{NDM-1}$ and PMQRs were co-transferred in each conjugation process. Multiple replicons (5-9 types) were detected in each trans-conjugant; with IncFIIK and IncFIB-KQ replicons being common among all trans-conjugants. Both *bla*$_{NDM-1}$ and PMQRs were detected on a pKpQIL-like multi-replicon plasmid that was present in all *K. pneumoniae* strains.

Conclusion: In view of these results, the presence of *bla*$_{NDM-1}$ and PMQRs on pKpQIL-like plasmid that existed in multiple unrelated *K. pneumoniae* isolates is highly suggestive of the circulation of pKpQIL-like MDR plasmids in our hospitals. Moreover, carriage of integrons by the-circulating MDR plasmids increases the risk of dissemination of antimicrobial resistance among pathogens.

Keywords: *Klebsiella pneumoniae*; Plasmids; New Delhi metalo beta-lactamase; Drug resistance; Polymerase chain reaction

INTRODUCTION

Klebsiella pneumoniae is a major cause of opportunistic nosocomial infections including; urinary tract infections, pneumonia, blood stream infections, wound infections and sepsis. It usually colonizes the gastrointestinal tract, nasopharynx and skin (1). Emergence of multidrug resistance (MDR) [defined

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as a lack of susceptibility to three or more antimicrobial categories (2)] among *K. pneumoniae* strains is considered an increasingly threatening problem globally. Infections caused by such organism are associated with major therapeutic problems resulting in increased morbidity and mortality (3).

The main mechanism associated with acquisition of MDR by bacteria is the dissemination of resistance genes by mobile genetic elements (insertion sequences, transposons and gene cassettes/integrons) carried by resistance plasmids. These resistance plasmids are the major players in the spread of MDR as they often carry multiple resistance elements and capable of transferring them simultaneously between different bacterial strains (4). Integrons are gene-capturing platforms that have an important role in the dissemination of antimicrobial resistance genes by plasmids (5). They are composed of two major components; the first is the integron-integrase gene (*intI*) and its promoter (*PintI*), an integration site named attachment site of the integron (*attI*), and a constitutive promoter (Pc) for integrated gene cassettes. The second component is a cluster of gene cassettes carrying antimicrobial resistance genes (6).

Carbapenems and/or fluoroquinolones (FQs) are often the best options for managing infections due to MDR *K. pneumoniae* (7). Unluckily, co-emergence of carbapenems and fluoroquinolone resistance has caused major difficulty in treating such infections (8). Plasmids play a main role in spreading of both carbapenem and FQs resistance. Plasmid-mediated quinolone resistance (PMQR) is conferred by *qnr* genes (*qnrA, qnrB, qnrS, qnrC, qnrD*) and *aac(6')-Ib-cr* (9). Likewise, many plasmid mediated carbapenemases (*IMP, VIM, SIM, SPM, GIM, KPC, SME*) have been detected in *K. pneumoniae*, with New Delhi metallo-β-lactamase-1 (NDM-1) has been accepted as the most widely disseminating carbapenemase worldwide (10).

NDM-1- encoding plasmids often co-carry other resistance determinants including PMQRs (9). Previous studies reported that bla_{NDM-1} carrying plasmids belongs to diverse incompatibility (*Inc*) groups, including IncN, IncC, IncFIB, IncFIB (K), IncR, IncFII, IncFIA, IncFII (K), IncHI1 and Col4401 (11). Recently, a pKpQIL-like plasmid was reported as a carrier of bla_{NDM-1} (12).

PKpQIL, a bla_{KPC-3}-encoding plasmid, was firstly isolated in 2006 from *K. pneumoniae* strain sequence type 258 (ST 258) (13). PKpQIL is a multi-replicon, self-transmissible plasmid with size of 113,637-bp, belongs to the IncFII group and carries two replicons IncFIIK and IncFIB-KQ (14). Sequencing of pKpQIL suggested that it was formed as a result of recombination between a pKPN4-like plasmid and a pNYC-like plasmid thus it carries 2 replicons. New variants of pKpQIL have emerged worldwide including; pKpQIL-IT isolated in Italy but mediated resistance to kanamycin that was nonexistent on the initial pKpQIL plasmid (15) and pKpQIL-UK that has few nucleotide substitutions from the original pKpQIL (16).

In Egypt, high prevalence of PMQRs and bla_{NDM-1} has been identified among MDR *K. pneumoniae* strains (17, 18). Nevertheless, the existence and genetic characterization of circulating plasmids co-carrying PMQRs and bla_{NDM-1} among *K. pneumoniae* is still not yet investigated. As far as we know, this is the first study to declare the role of pKpQIL-like plasmid in dissemination of PMQRs and bla_{NDM-1} among *K. pneumoniae* isolated from Assiut University Hospital, Egypt.

MATERIALS AND METHODS

The Ethics Committee of the Faculty of Medicine, Assiut University approved this study according to the latest revision of the Declaration of Helsinki, and informed consent was obtained from the participants (IBR no: 17200282).

Bacterial isolates. The current work is a retrospective study investigating 22 MDR *K. pneumoniae* strains collected from the Infection Control Laboratory at Assiut University Hospital from the period of January 2019 to January 2020. *K. pneumoniae* strains were retrieved from patients with hospital-acquired infections at different ICUs: chest (n= 10), paediatric (n= 8) and neurology (n= 4). Infections were considered as hospital-acquired when the symptoms and signs appeared 48 hrs. or more after hospitalization. All *K. pneumoniae* strains were chosen based on dual acquisition of bla_{NDM-1} and PMQR determinants (*qnrB, qnrS1* and/or *aac(6`)-Ib-cr*).

Resistance phenotype determination. Resistance phenotype was determined to all isolates using Kirby–Bauer disk diffusion test (19) with antibiotic discs (Oxoid, UK) for different classes of antibiotics in-

cluding; penicillin derivatives [amoxicillin (AML10 µg), amoxicillin/clavulanic acid (AMC 20/10 µg), and piperacillins (PI 100 µg)], cephalosporins [cefazolin (CZ 30 µg), cefpodoxime (CPD 30 µg), cefoperazone (CPZ 75 µg) and ceftriaxone (CTR 30 µg)], aminoglycosides [gentamicin (GE 10 µg) and amikacin (AK 30 µg)], tetracycline (TE 30 µg), chloramphenicol (C 30 µg) and trimethoprim-sulfonamide (SXT 1.25 /23.75 µg). The minimum inhibitory concentrations (MICs) of imipenem and ciprofloxacin were determined by E-tests (BioMérieux, Solna, Sweden). Results were interpreted based on the Clinical and Laboratory Standard Institute guidelines (CLSI) 2019 (20).

Genotyping of *K. pneumoniae* isolates by PFGE. Clonal relatedness of *K. pneumoniae* isolates was determined by pulsed field gel electrophoresis (PFGE) following the PulseNet protocol of the Centres for Disease Control (CDC) 2017 (21). Briefly, total genomic DNA from each strain was digested with *XbaI* (New England Biolabs, Beverly, MA, USA) for 16 h at 37°C. Fragments separation was performed using 1% certified Mega base agarose in a CHEF-DR III system (Bio-Rad). Size was estimated by Lambda Ladder PFGE Marker (New England Biolabs, Beverly, MA, USA). The subsequent Conditions were selected: Initial switch time: 6.7 Sec., Final switch time: 35.3 Sec., Voltage: 6 V/cm, included angle: 120° and Run time: 22 hours. The PFGE patterns were analyzed by a transient BioNumerics software evaluation license (Version 7.6, Applied Maths, Belgium), and agreement to publish was received. The dendrogram was created using the un-weighted pair group method with arithmetic mean (UPGMA) clustering method with Dice's Similarity coefficient. A similarity coefficient of 80% or more was used as the threshold of a cluster.

Conjugation experiment. Conjugal transfer of plasmids carrying bla_{NDM-1} and PMQR genes from *K. pneumoniae* strains was implemented according to the method modified by Miller (22) using *E. coli* J53 (*recA*$^-$, Az^r) as the recipient. Trans-conjugants were selected on Luria–Bertani (LB) agar plates to which sodium azide (200 µg/mL) and nalidixic acid (20 µg/mL) were added. Trans-conjugants were Replica-plated on 2 LB agar plates; one with meropenem (0.5 µg/mL) and the other containing nalidixic acid (20 µg/mL). Colonies that showed growth on both replica LB agar plates were selected.

Screening for PMQRs, bla_{NDM-1} and integrons among trans-conjugants. Plasmids from trans-conjugants were extracted using PureLinkTM Quick Plasmid Miniprep Kit (Thermo Fisher Scientific, Massachusetts, USA) following the manufacturer's instructions. The existence of PMQRs and bla_{NDM-1} in trans-conjugants was determined by PCR. To assess the capability of plasmids to capture new antibiotic resistance gene cassettes, the three classes of integrons (*Int1, Int2* and *Int3*) were screened among trans-conjugants by conventional PCR. PCR amplification was done in a thermal cycler (BioRadT100, USA) using GoTaq® Flexi DNA Polymerase Kit (Promega, USA) in a 50-µl volume containing 100 pmol of each primer, 1×PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 U of Taq polymerase and 100 ng of DNA template. The amplification programs were conducted as follows: an initial denaturation step at 95°C for 5 min, followed by 30 cycles of DNA denaturation at 95°C for 30 sec, primer annealing for 30 sec, and primer extension at 72°C, then a final extension step was done at 72°C for 5 min. Annealing temperatures, time required for primer extension and sequences of oligonucleotide primers used for PCR amplification are listed in Table (1).

Plasmid typing from trans-conjugants. PCR based replicon typing (PBRT) was used for detection of plasmid replicons in trans-conjugants, using PBRT 2.0 kit (DIATHEVA, Italy). This new kit affords a set of eight specific PCR tests adjusted to do eight multiplex PCRs for the amplification of 30 replicons: HI1, HI2, I1 alpha, I1 gamma, I2, X1, X2, X3, X4, L, M, N, N2, FIA, FIB, FII, FIIS, FIIK, FIB-KN, FIB-KQ, W, P1, A/C, T, K, U, R, B/O, HIB-M and FIB-M, that are representatives of the main plasmid incompatibility groups among *Enterobacteriaceae.* Moreover, FIB-KN recognizes the pKPN3- like plasmids; FIB-KQ recognizes the pKpQIL-like plasmid which are common in *K. pneumoniae* (29). The kit included positive control plasmid's DNA for each mix (supplementary Table 1). The eight multiplex PCRs were performed in a 50 µL volume containing the reaction mix, 1.25 U of Taq polymerase and 100 ng of DNA template. The amplification program was conducted as follows: an initial denaturation step at 95°C for 10 min, followed by 30 cycles of DNA denaturation at 95°C for 60 sec, primer annealing at 60°C for 30 sec, and primer extension at 72°C for 60 sec, then a final extension step was done at 72°C for 5 min. The result-

Table 1. Annealing temperatures, time required for primer extension and sequences of oligonucleotide primers used in PCR amplification of *bla*$_{NDM-1}$, PMQRs and integrons

Gene	Sequence (5'-3')	Size (bp)	Annealing temperature	Time for primer extension	Reference
bla$_{NDM1}$	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621 bp	55°C	30 Sec.	(23)
qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469 bp	54°C	30 Sec.	(24)
qnrS	F: CAATCATACATATCGGCACC R: TCAGGATAAACAACAATACCC	641 bp	56°C	30 Sec.	(25)
aac(6`)-Ib	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482 bp	58°C	30 Sec.	(26)
Int1	F: CTG CGT TCG GTC AAG GTT CT R: GGA ATG GCC GAG CAG ATC CT	882 bp	58°C	50 Sec.	(27)
Int2	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	788 bp	54°C	40 Sec.	(28)
Int3	F: GCCTCCGGCAGCGACTTTCAG R: ACGGATCTGCCAAACCTGACT	979 bp	56°C	60 Sec.	(28)

Bp = base pair

ing PCR products were analyzed in a 2% agarose gel with ethidium bromide staining and visualized under ultraviolet light.

Replicon typing of plasmids carrying *bla*$_{NDM-1}$ and PMQRs. Gel electrophoresis of each trans-conjugant's plasmid DNA- using a 1Kb DNA ladder (New England BioLabs, USA) - was employed thus plasmids of different sizes separate into distinct bands. In an attempt to identify which plasmid/s carry *bla*$_{NDM-1}$ and PMQRs, each plasmid DNA band was purified from the gel using PureLink™ Quick gel extraction Kit (Thermo Fisher Scientific, Massachusetts, USA) according to the manufacturer's instructions. Each band was screened for the presence of *bla*$_{NDM1}$ and PMQR genes by PCR. Determination of replicon type/s of the *bla*$_{NDM-1}$, PMQRs containing plasmid was performed by PBRT using the previously mentioned kit.

RESULTS

Resistance phenotypes. All 22 *K. pneumoniae* isolates were found to be MDR. Moreover, seventeen (77.3%) *K. pneumoniae* isolates were imipenem resistant, while 12 (54.5%) isolates were ciprofloxacin resistant detected by E-test®. Ciprofloxacin and imipenem-MICs as well as resistance phenotype of *K. pneumoniae* isolates are shown in Table (2).

Genotyping of *K. pneumoniae* strains by PFGE. PFGE analyses of the 22 *K. pneumoniae* isolates identified 15 different clusters (A-O) (Supplementary Fig. 1) and a dendrogram was constructed using BioNumerics software (Version 7.6, Applied Maths, Belgium) (a transient evaluation license) with isolates sharing ≥ 80% of the bands were assigned to the same cluster (Fig. 1). Five clusters comprised more than one *K. pneumoniae* strain; cluster A (No.= 4; KP10, KP15, KP18 and KP19), KP10 was isolated from chest ICU while the others were isolated from paediatric ICU, cluster C (No.= 2; KP16, KP20) both were isolated from paediatric ICU, cluster D (No.= 2; KP2, KP6) both were isolated from chest ICU, cluster F (No= 2; KP21, KP22) both were isolated from paediatric ICU and cluster I (No.= 2; KP11, KP12) both were isolated from chest ICU. The remaining isolates (No.= 10) were clonally unrelated and were categorized into different clusters.

Screening for *bla*$_{NDM-1}$, PMQRs and integrons among trans-conjugants. Both *bla*$_{NDM-1}$ and PMQRs were co-transferred in each conjugation event from all *K. pneumoniae* isolates. All the 22 trans-conjugants were found to be (*Int1*) positive, while (*Int2* and *Int3*)

Table 2. Antimicrobial susceptibility of *K. pneumoniae* isolates

Code	MIC IMP	MIC CIP	Resistance phenotype *
KP 1	>32 (R)	>32 (R)	MDR[1,2,3,6,7]
KP 2	>32 (R)	>32 (R)	MDR[1,2,3,4,5, 6,7,8]
KP 3	4 (R)	0.75 (I)	MDR[1,2,3,4,5,7]
KP 4	4 (R)	0.75 (I)	MDR[1,2,3,4,5,7]
KP 5	8 (R)	0.125 (S)	MDR[1,2,3,5,7,8]
KP 6	12 (R)	>32 (R)	MDR[1,2,3,5,6,7]
KP 7	16 (R)	>32 (R)	MDR[1,2,3,5,6,7]
KP 8	1 (S)	>32 (R)	MDR[1,2,4,5,6,7,8]
KP 9	0.75 (S)	>32 (R)	MDR[1,2,4,5,6,7,8]
KP 10	4 (R)	0.75 (I)	MDR[1,2,3,7,8]
KP 11	4 (R)	>32 (R)	MDR[1,2,3,4,5, 6,7,8]
KP 12	4 (R)	>32 (R)	MDR[1,2,3, 6,7,8]
KP 13	>32 (R)	>32 (R)	MDR[1,2,3,4, 6,7,8]
KP 14	0.125(S)	4 (R)	MDR[1,2,4,5,6,7,8]
KP 15	4 (R)	.064 (S)	MDR[1,2,3,7,8]
KP 16	>32 (R)	>32 (R)	MDR[1,2,3,6,7,8]
KP 17	12 (R)	>32 (R)	MDR[1,2,3,6,7,8]
KP 18	3 (I)	0.75 (I)	MDR[1,2,7,8]
KP 19	4 (R)	0.38 (I)	MDR[1,2,3,7,8]
KP 20	1.5 (I)	0.125 (S)	MDR[1,2,5,7,8]
KP 21	4 (R)	.047 (S)	MDR[1,2,3,7,8]
KP 22	4 (R)	.064 (S)	MDR[1,2,3,7,8]

*Key: 1=Penicillins; 2=Cephalosporins; 3= Carbapenems; 4=Tetracyclines; 5=Chloramphenicol; 6=Fluoroquinolone; 7= Trimethoprim sulfonadmide; 8 = Aminoglycosides.

were negative among all trans-conjugants.

PBRT of trans-conjugant's plasmid DNA. PBRT revealed the presence of multiple plasmid replicons (5-9 types) in each trans-conjugant as shown in Table 3. IncFIIK and IncFIB-KQ were the most common replicon types identified among all trans-conjugants, indicating that each trans-conjugant harbored a plasmid with pKpQIL-like backbone. Moreover, 10 *K. pneumoniae*– belonging to 8 pulsotypes- were isolated from chest ICU between January 2019 and May 2019, and all contained IncI1gamma and IncFII replicons as well. Similarly, four *K. pneumoniae*- comprised different pulsotypes- isolated from neurology ICU between January 2019 and January 2020 also contained IncI1gamma and IncFII replicons.

Replicon typing of plasmids carrying bla_{NDM-1} and PMQRs. Gel electrophoresis revealed that each trans-conjugant harbored multiple plasmids appeared as discrete DNA bands on the gel. Screening the various plasmid DNA bands for bla_{NDM-1} and PMQRs, revealed that- in all trans-conjugants- a single plasmid DNA band was found to be positive for both bla_{NDM-1} and PMQRs. PBRT of the plasmid's DNA co-harboring bla_{NDM-1} and PMQRs revealed that in all trans-conjugants, each plasmid carried 2 replicons (IncFIIK and IncFIB-KQ) signifying that bla_{NDM-1} and PMQRs might be carried by a multi-replicon pKpQIL-like plasmid.

DISCUSSION

Global emergence and dissemination of MDR *K. pneumoniae* pose a serious public health burden worldwide (30). Various genes entangled in multiple drug resistances are plasmid mediated; including PMQRs and bla_{NDM-1} genes (4). Horizontal transfer of plasmids coding multiple resistance determinants is associated with dissemination of MDR among bacterial pathogens (31). In this study, we hypothesized

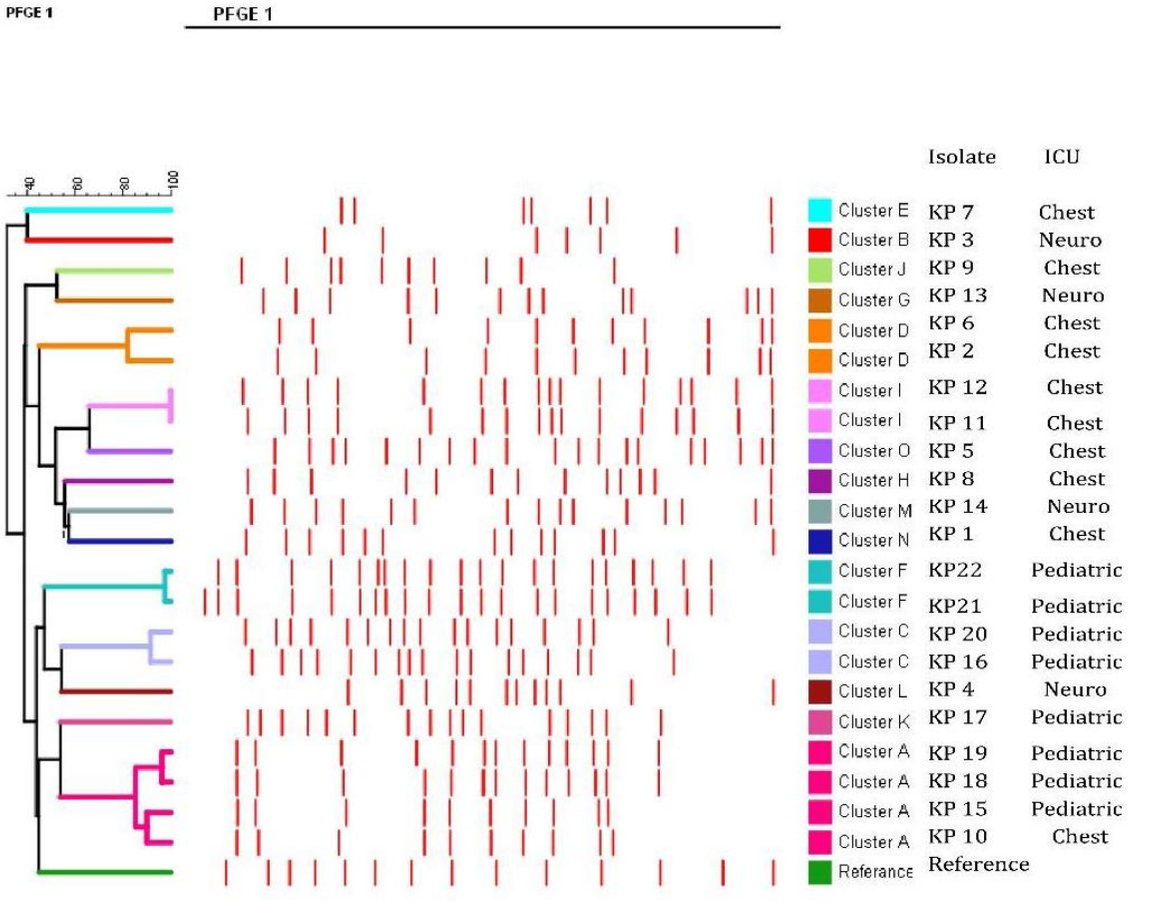

Fig. 1. Dendrogram comparing PFGE profiles of *K. pneumoniae* isolates co-harboring bla_{NDM1} and PMQRs

the existence of bla_{NDM-1} and PMQRs on a single transmissible plasmid that circulates among MDR *K. pneumoniae* isolated from Assiut University Hospital, Egypt. To test this hypothesis, we performed plasmid typing of 22 clinical *K. pneumoniae* isolated from 3 different ICUs; chest ICU, paediatric ICU, and neurology ICU; all contained one or more of the PMQRs as well as bla_{NDM-1}.

Twenty-two MDR *K. pneumoniae* were included in this study. These MDR *K. pneumoniae* possessed at least resistance to four drug classes, with 2 *K. pneumoniae* found to be resistant to the all tested drug classes. These results suggest that ICUs in Assiut University Hospitals serve as a source for MDR *K. pneumoniae* probably due to increased consumption of antibiotics (32). Previous studies investigating *K. pneumoniae* isolated from Assiut University Hospitals reported multidrug resistance among *K. pneumoniae* strains, however, the resistance rates are higher in this study (18, 33). The predominance of MDR bacteria in the community is of great concern, as it is associated with increased length of hospital stay, deaths, healthcare charges, and antimicrobial use (34).

In the present study, the resistance rate against imipenem in bla_{NDM} positive *K. pneumoniae* isolates, as detected by E-test, was 77.3%. However, Huang et al. (2018) reported higher imipenem resistance rates (100%) among bla_{NDM} positive *K. pneumoniae* isolates (35). On the other hand, the resistance rate against ciprofloxacin in PMQRs positive *K. pneumoniae* isolates, as detected by E-test, was 54.5%. However, Xue et al. (2017) reported that only 8% of PMQRs positive *K. pneumoniae* isolates were ciprofloxacin resistant (36). The variation in the resistance determinants rates could be attributed to the differences in the geographic localities.

Plasmids circulate between different bacterial species and are capable of acquiring resistance genes through small mobile elements such as integrons and transposons. Integrons carrying resistance genes, when jump on a plasmid, bring about acquisition

Table 3. Resistance genes, replicon types, source and PFGE type of *K. pneumoniae* isolates.

Code	NDM	PMQRs	*Int1*	Replicon types	ICU	Pulsotype
KP 1	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncFIB, IncFII, IncI1gamma	Chest	N
KP 2	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIB-M, IncFII, IncI1gamma	Chest	D
KP 3	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncL, IncFIB-M, IncFII, IncI1gamma, IncFIA	Neuro	B
KP 4	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncFIB, IncFIB-M, IncFII, IncI1gamma	Neuro	L
KP 5	+	qnr B, qnrS, aac(6`)-Ib-cr	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncL, IncFIB-M, IncFII, IncI1gamma	Chest	O
KP 6	+	qnr B, qnrS, aac(6`)-Ib-cr	+	IncFIIK, IncFIB-KQ, IncHI1, IncFIB-M, IncFII, IncI1gamma	Chest	D
KP 7	+	qnr B	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIA, IncL, IncFIB-M, IncFII, IncI1gamma	Chest	E
KP 8	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIA, IncFII, IncI1gamma	Chest	H
KP 9	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFII, IncI1gamma	Chest	J
KP 10	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFII, IncI1gamma	Chest	A
KP 11	+	qnr B, qnrS, aac(6`)-Ib-cr	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIA, IncFII, IncI1gamma	Chest	I
KP 12	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncM, IncFIA, IncFII, IncI1gamma	Chest	I
KP 13	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIB, IncFII, IncI1gamma	Neuro	G
KP 14	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ IncM, IncFIA, IncFII, IncI1gamma	Neuro	M
KP 15	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncFIB, IncFIB-M, IncFII, IncI1gamma	Pediatric	A
KP 16	+	qnr B, qnrS, aac(6`)-Ib-cr	+	IncFIIK, IncFIB-KQ, IncFIA, IncFIB, IncFII	Pediatric	C
KP 17	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncFIA, IncFIB, IncFII, IncL IncFIIK, IncFIB-	Pediatric	K
KP 18	+	qnr B, qnrS	+	KQ, IncHI1, IncFIA, IncFII, IncI1gamma, IncFIB-M IncFIIK, IncFIB-KQ,	Pediatric	A
KP 19	+	qnr B, qnrS, aac(6`)-Ib-cr	+	IncHI1, IncM, IncFIA, IncFIB, IncI1gamma, IncFIB-M IncFIIK, IncFIB-KQ,	Pediatric	A
KP 20	+	qnr B, qnrS	+	IncHI1, IncFIB, IncFIB-M, IncFII	Pediatric	C
KP 21	+	qnr B, qnrS	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncFIB, IncFIB-M, IncFII	Pediatric	F
KP 22	+	qnr B	+	IncFIIK, IncFIB-KQ, IncHI1, IncM, IncL, IncI1gamma	Pediatric	F

and dissemination of MDR (37). In this study, Int1 was identified in all *K. pneumoniae* isolates, which is in consistent with previous studies reporting *Int1* as the most prevalent type of integrons associated with bla_{NDM-1} and PMQRs in *K. pneumoniae* (17, 38). The high frequency of integrons in plasmids extracted from *K. pneumoniae* isolated from our hospital increases the danger of acquisition of more resistance determinants by those plasmids and thus spread of resistance to more antibiotics.

PFGE typing of the 22 *K. pneumoniae* isolates displayed that these strains comprised 15 pulsotypes; hence they were polyclonal in origin. Nevertheless, a number of *K. pneumoniae* sharing the same pulsotype were recovered from different patients admitted to the same ICU at different times, denoting that some *K. pneumoniae* strains are endemic and circulate between patients admitted to such ICU. Surprisingly, one isolate (KP10) recovered from chest ICU and 3 isolates recovered from paediatric ICU (KP15, KP18 and KP19) were found to belong to the same cluster. The presence of pathogens of the same pulsotypes in different hospital wards emphasizes the circulation of *K. pneumoniae* in the hospital environment (39).

Transmissible plasmids are capable of causing major changes in bacterial populations by simultaneously disseminating resistances to multiple antibacterial agents (40). Both PMQRs and bla_{NDM-1} were reported to be present on transmissible plasmids (18, 41). In the present study, bla_{NDM-1} and PMQRs were successfully transferred at a rate of 100% to the recipient (*E. coli* J53) through conjugation. The high rate of transfer of bla_{NDM-1} and PMQRs contributes to the fast spread of those genes among clonally unrelated bacterial strains (42).

PBRT was used for typing of plasmids isolated from trans-conjugants; PBRT is a method for plasmid typing which helps understanding their distribution and relationships (43). Multiple plasmid replicons (5-9 types) were detected in every trans-conjugant denoting the existence of several transmissible plasmids in each *K. pneumoniae* pathogen. The most common detected replicon type was IncFIIK, which was found in all trans-conjugants and hence implying its existence in all of the 22 *K. pneumoniae* isolates as well. This finding is in accordance with previously published data reporting IncFIIK as being the most prevalent replicon type in *Klebsiella* species (44-46). Moreover, all trans-conjugants were also positive for IncFIB-KQ replicon signifying that each of the 22 *K. pneumoniae* pathogens contained a plasmid with pKpQIL-like backbone (29). Sharing pKpQIL-like plasmids (and other plasmid replicons) between clonally unrelated *K. pneumoniae* strains isolated from different ICUs throughout the whole duration of the study is highly suggestive for the circulation and the endemicity of such plasmid in the hospital environment.

In order to determine whether bla_{NDM-1} and PMQRs are co-carried by a single plasmid, we purified plasmid DNA bands- after gel electrophoresis- from all trans-conjugants and screened them for the presence of such resistance determinants. For all trans-conjugants, both bla_{NDM-1} and PMQRs were detected on a single plasmid DNA band. PBRT of such plasmid revealed that it is multi-replicon plasmid carrying 2 replicons; IncFIIK and IncFIB-KQ. These findings imply that bla_{NDM-1} and PMQRs are highly suggested to be co-carried by a multi-replicon pKpQIL-like plasmid that was shared among the 22 *K. pneumoniae* strains. Similarly, a recent study reported the discovery of pKpQIL-like plasmid co-harboring bla_{NDM1} and PMQR (*qnrS1*) and carried by 98% of the *K. pneumoniae* isolates (12).

In agreement with our results, a study implemented in USA revealed that (35.6%) of *K. pneumoniae* isolates were harboring pKpQIL-like plasmids (47). Furthermore, several studies established the major role of pKpQIL-like plasmid in the dissemination of carbapenem resistance among *K. pneumoniae* strains. Interestingly, in 2010, a study reported that loss of pKpQIL plasmid from *K. pneumoniae* isolates resulted in complete loss of carbapenem resistance demonstrating its fundamental role in disseminating carbapenem resistance (48). Doumith et al. stated that pKpQIL-like plasmids participated by a main role in the propagation of KPC enzymes in the UK (16). Similarly, Reyes et al. reported the presence of the bla_{KPC-2} gene on the Tn*4401a* transposon carried by pKpQIL-like plasmid (49).

CONCLUSION

Findings of this study suggest that MDR pKpQIL-like plasmid is among the major players in the spread of bla_{NDM-1} and PMQRs among *K. pneumoniae* strains in Assiut University Hospital. The presence of pKpQIL-like plasmid together with other plasmid replicons in many clonally unrelated *K. pneumoniae* isolates is strongly suggestive of the presence of

circulating MDR plasmids in the hospital environment. As far as we know this is the first report of pKpQIL-like plasmid co-carrying PMQRs and bla_{NDM-1} in *K. pneumoniae* isolated from Egypt. Plasmid sequencing is recommended for better understanding the genetic aspects of circulating plasmids and the relatedness of plasmids discovered in this study with the worldwide plasmid epidemiology.

Determining the Sequence Type of *K. pneumoniae* using multi locus sequence typing (MLST) would further identify the clonal linage and the relatedness of the isolates. Also, plasmid sequencing would have supported the results of this study. Unfortunately, due to the restricted funding resources we were not able to perform either of these tests.

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