Infection and Drug Resistance

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ORIGINAL RESEARCH

Contribution of horizontal gene transfer to the emergence of VIM-4 carbapenemase producer Enterobacteriaceae in Kuwait

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Abstract: Carbapenem-resistant Enterobacteriaceae encountered in countries of the Arabian Peninsula usually produce OXA-48-like and New Delhi metallo-beta-lactamases (NDM) carbapenemases. However, a temporary increase in VIM-4-producing, clonally unrelated Enterobacteriaceae strains was described earlier in a Kuwaiti hospital. We investigated the genetic support of bla_{VIM-4} in six *Klebsiella pneumoniae* strains, one *Escherichia coli,* and one *Enterobacter cloacae* strain and compared it to that of VIM-4-producing isolates from other countries of the region. Five *K. pneumoniae* strains and the *E. coli* strain from Kuwait carried an ~165 kb IncA/C-type plasmid indistinguishable by restriction fragment length polymorphism. The complete sequence of one of them (pKKp4-VIM) was established. pKKp4-VIM exhibited extensive similarities to episomes pKP-Gr642 carrying bla_{VIM-19} encountered in Greece and to the partially sequenced pCC416 harboring bla_{VIM-4} detected in Italy. In other countries of the region, the only similar plasmid was the one detected in the isolate from the UAE. In all Kuwaiti strains, irrespective of the species and their VIM plasmids, the bla_{VIM-4} gene was located within the same integron structure (In416), different from those of other countries of the region. Our data show that the spread of this IncA/C plasmid and particularly that of the In416 integron caused a considerable, albeit temporary, increase in the rate of mostly clonally unrelated VIM-producing Enterobacteriaceae strains of multiple species. Monitoring of such events is of high importance as the interference with the spread of mobile genetic elements may represent a formidable challenge to infection control.

Keywords: Enterobacteriaceae, VIM carbapenemase, horizontal gene transfer, multidrug resistance, Middle East

Introduction

The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE) is a serious global threat that considerably limits therapeutic options available for life-threatening Gram-negative infections. Carbapenem-hydrolyzing enzymes have been described in the A, B and D classes of beta-lactamases.[1] Group B enzymes, i.e., the metallo-beta-lactamases (MBLs), are especially worrisome, as recently introduced beta-lactamase inhibitors have no activity against them.[2]

Although it has been observed that countries of the Arabian Peninsula are burdened by CPE, there are as yet no systematic surveillance-based data regarding the magnitude of the problem. However, studies from the region have shown that locally class D OXA-48-like enzymes and New Delhi metallo-beta-lactamases (NDM) are the most common carbapenemases in Enterobacteriaceae with sporadic occurrence of KPC-

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http://dx.doi.org/10.2147/IDR.S149321

and VIM-type enzymes.[3–7] An exception to this trend was a temporarily increased prevalence of VIM-producing strains in Kuwait between 2009 and 2011. Early investigations in Kuwait showed that a few of these strains were clonally related only.[8] In the current study, we investigate the role of mobile genetic elements in the increased number of VIM-positive isolates in Kuwait and compared the plasmids and integrons to other bla_{VIM}-bearing mobile genetic elements identified in other countries of the region.

Materials and methods

Bacterial strains

Five *Klebsiella pneumoniae* (KKp1, KKp2, KKp4, KKp6 and KKp8), one *Enterobacter cloacae* (KEcl3) and one *Escherichia coli* (KEc7) were previously described as part of a VIM-producing Enterobacteriaceae outbreak in Kuwait.[8] A further VIM producing *K. pneumoniae* (KW11) isolated in the same hospital during the same period was also included in the study. The characteristics of these isolates were compared to those of four VIM-producing *E. cloacae* (two [OM63 and OM69] from Oman, one [SA4/2] from the Kingdom of Saudi Arabia [KSA] and one [ABC104] from the UAE, respectively).[4,6] All isolates were recovered from individual patients and were considered clinically relevant. The strains were stored at −80°C in Tryptic Soy Broth (Mast, Merseyside, UK) containing 20% glycerol.

Antibiotic susceptibility assays

Susceptibility to cefotaxime, ceftazidime, aztreonam, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, trimethoprim/sulfamethoxazole, tetracycline, chloramphenicol and colistin (Col) was tested by broth microdilution, while susceptibility to fosfomycin (Fos) and tigecycline (Tig) was assessed by agar dilution.[9] For the majority of antibiotics the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were used for interpretation,[9] with the exception of Col, Tig and Fos whereby the The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.[10]

Molecular typing

The *XbaI*-digested genomic DNA's pulsed field gel electrophoresis (PFGE) pattern and the multi-locus sequence type of the isolates were established as described earlier.[6,7] The Kuwaiti *K. pneumoniae* isolates were also compared by repetitive element sequence-based polymerase chain reaction (rep-PCR; DiversiLab; bioMerieux, Marcy l'Etoile France) using the Klebsiella kit, according to the manufacturer's recommendation. Resistance genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{PER}, bla_{AmpC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{KPC}, bla_{VIM}, bla_{IMP}, *armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF, qnrS, qepA, aac6-1b-cr, mcr-1, mcr-2*) were detected as described.[4,7,11,12] The specific alleles of beta-lactamase genes were determined by direct sequencing of the respective amplicons performed with the BigDye Cycle Terminator V.3.1 (Thermo Fisher Scientific, Waltham, MA, USA) using the 3130X Genetic Analyzer (Thermo Fisher Scientific).

Characterization of the genetic environment of bla_{VIM-4}

The flanking region of the bla_{VIM-4} gene was determined by polymerase chain reaction (PCR) mapping and sequencing using primers designed (Table S1) according to the genetic surrounding of bla_{VIM} published earlier (GenBank accession numbers AJ704863 and AY339625).[13,14] Sequences were assembled with Clone Manager v9.0 (Sci-Ed Software, Cary, NC, USA) and annotated using Sequin (http://www.ncbi.nlm.nih.gov/Sequin) and submitted to GenBank.

Plasmid characterization

Plasmids were isolated and detected by the alkaline lysis method as described[6] using *E. coli* 39R861 as plasmids molecular size standards.[15] Mating out assays were performed with the clinical isolates using an azide-resistant derivative of rifampicin-resistant *E. coli* J53 (J53RAZ) as recipient. Transconjugants were selected on Tryptic Soy Agar containing 8 mg/L ceftazidime and 100 mg/L azide.[7] If they were non-conjugative, heat shock transformation of the carbapenemase-bearing plasmids into *E. coli* DH5α was attempted.[5] To prove the loclization of genes, electrophoretically separated plasmids isolated from the wild-type strains by the alkaline lysis method[6] and those of the transconjugants or transformants were capillary transferred to Hybond N+ membranes that were subsequently hybridized with the appropriate digoxigenin-labeled (Roche Diagnostics GmbH, Mannheim, Germany) probes.[6] The incompatibility (Inc) groups of the plasmids transferred were identified by PCR[16,17] and confirmed by hybridization as mentioned earlier.[7]

Plasmids were purified from single plasmid containing *E. coli* K-12 derivatives using the Plasmid Maxiprep Kit (Qiagen NV, Venlo, the Netherlands). Restriction patterns of similarly sized plasmids belonging to the same Inc type were visually compared after digesting with *Hinc*II, *Hind*III and *Eco*RI restriction endonucleases. Furthermore, the complete sequence of pKKp4-VIM conjugally transferred from *K. pneumoniae* KKp4 into *E. coli* J53RAZ was established

by next-generation sequencing on Illumina MiSeq platform (performed at the CCIB DNA Core Facility in Massachusetts General Hospital, Cambridge, MA, USA). The gaps between contigs assembled were closed by PCR and by direct sequencing of the amplicons. The complete plasmid sequence was assembled with Clone Manager v9.0, annotated using Sequin (http://www.ncbi.nlm.nih.gov/Sequin) and submitted to GenBank (MF582638).

Ethics approval

The ethics approval for the study was obtained from the Medical Ethics Committee of the Ministry of Health, Kuwait (288/MTT).

Results

Antibiotic susceptibility

The antibiotic susceptibility test results of the isolates are summarized in Table 1, while the respective minimal inhibitory concentration (MIC) values are provided in Table S1. All strains showed resistance to all beta-lactams tested. All Kuwaiti strains were multidrug resistant, with two being susceptible to Col only and one (*K. pneumoniae* KW11) being resistant to all antibiotics tested (Tables 1 and S2).

Molecular typing

All strains investigated carried a single carbapenemase, bla_{VIM-4}. The molecular characteristics of the clinical isolates are summarized in Table 1. Confirming the data of the previous study, i.e., of the Kuwaiti strains, only *K. pneumoniae* KKp1 and KKp2 exhibited similar PFGE patterns (KP-4; Figure S1).[8] With the exception of these two isolates, the sequence types (Table 1) and the rep-PCR patterns (Figure S2) of the other *K. pneumoniae* strains were all different. The sole *E. cloacae* (KEcl3) from Kuwait was different from the other four VIM-4-producing *E. cloacae* from the region both by PFGE and by multi-locus sequence typing (MLST) (Table 1 and Figure S1).

Comparison of the plasmids carrying bla_{VIM-4}

Unlike in the previous study,[8] we could conjugally transfer VIM-coding plasmids from six of the eight Kuwaiti strains as well as from the Saudi *E. cloacae* SA4/2. From the Omani *E. cloacae* isolates (OM63 and OM69), the VIM plasmids were transferred by transformation. From *K. pneumoniae* KW11 and *E. cloacae* KEcl3 and ABC104, neither conjugations nor transformations were successful.

As confirmed by PCR and by Southern hybridization (Figure S3), in addition to the two clonally related *K. pneumoniae* (KKp1 and KKp2), three unrelated *K. pneumoniae* (KKp4, KKp6 and KKp8) isolates and the *E. coli* (KEc7) from Kuwait harbored bla_{VIM-4} on IncA/C Inc-type plasmids of ~165 kb. Beyond bla_{VIM-4}, these plasmids also carried the bla_{CMY-4} gene (Table 1). The RFLP patterns of these plasmids were identical (Figure 1). *E. cloacae* ABC104 from the UAE (described earlier[6]) also carried bla_{VIM-4} and bla_{CMY-4} on similar-sized IncA/C Inc-type plasmid, but we were unable to compare the RFLP of this plasmid to the Kuwaiti ones as we could not generate a single VIM plasmid-containing derivative of this isolate. In *K. pneumoniae* KW11, the bla_{VIM-4} was located on a nontransferable IncA/C type, >300 kb plasmid, which also carried bla_{CMY-4}. As shown in Table 1 and in Figure S3, the Kuwaiti, Saudi and Omani *E. cloacae* isolates all carried bla_{VIM-4} on smaller plasmids lacking bla_{CMY-4}, which could not be identified by the PCR-based replicon typing (PBRT). In case of KKp1 and KKp2, the conjugal transfer of the bla_{VIM}-bearing plasmids was accompanied by their fusion with IncN-type plasmids (Figure S4). No attempts were made, within the frames of the current study, to clarify the molecular details of this fusion.

Complete sequence of pKKp4-VIM

To obtain a more detailed picture on the conjugative IncA/C-type plasmids dominating the isolates from Kuwait, the entire sequence was determined from a transconjugant containing pKKp4-VIM derived from *K. pneumoniae* KKp4. The plasmid was a 162117 bp long, type 1 IncA/C_2 plasmid with respective regions for replication, conjugative transfer and plasmid maintenance (GenBank Accession No. MF582638). It was highly similar to pKP-Gr642, a type 1 IncA/C_2-type plasmid of a Greek clinical *K. pneumoniae* isolate carrying bla_{VIM-19} (Figure 2A)[18]. Apart from the plasmid backbone, pKKp4-VIM harbored three resistance islands: RI-1, RI-2 and RI-3 (Figure 2A). On RI-1 *tet(A), strA*, *strB* and *sul2* genes are located (Figure 2B). The RI-2 consists of IS*Ecp1*, bla_{CMY-4}, *blc* and *sugE* genes. The third resistance island RI-3 contains an In*416* with bla_{VIM-4}, *aacA7, dfrA1, ΔaadA1* and *smr* gene cassettes, a Tn*8802* with arsenic resistance operon, an In-*t4*-like integron and a mercury resistance operon (Figure 2D).

Genetic surrounding of bla_{VIM-4} in all isolates

PCR mapping and sequencing revealed that irrespective of the species or plasmid Inc type, the integron structure of all Kuwaiti isolates was identical to the one in pKKp4-VIM, i.e., bla_{VIM-4} was located on an In416 integron, which lacked the 3′ conserved sequences (CS). On the other hand, in *E. cloacae*

Table 1 Characteristics of VIM-producing Enterobacteriaceae isolated in countries of the Arabian Peninsula

Strain	Species	Susceptibility	Resistance genes detected by PCR	PFGE	MLST	VIM-plasmid				
						Size (approximately in kb)	Inc type[a]	Conjugative	Additional resistance genes[b]	VIM-bearing integron structure
KKp1	*Klebsiella pneumoniae*	Col	bla_{VIM-4}, bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$, bla_{CMY-4}, *aac-6'-Ib-cr*	KP-4	ST1399	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KKp2	*K. pneumoniae*	Col	bla_{VIM-4}, bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$, bla_{CMY-4}, *aac-6'-Ib-cr*	KP-4	ST1399	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KKp4	*K. pneumoniae*	Ak, Col, Fos	bla_{VIM-4}, bla_{CMY-4}, *aac-6'-Ib-cr*	KP-1	ST138	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KKp6	*K. pneumoniae*	Ak, Col, Fos	bla_{VIM-4}, bla_{TEM-1}, $bla_{CTX-M-15}$, bla_{CMY-4}, *aac-6'-Ib-cr*, *qnrB*	KP-5	ST1400	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KEc7	*Escherichia coli*	Ak, Col, Fos	bla_{VIM-4}, bla_{CMY-4}	ND	ST167	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KKp8	*K. pneumoniae*	Ak, Col, Fos	bla_{VIM-4}, bla_{CMY-4}	KP-2	ST1401	165	A/C	Yes	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrIA-ΔaadA1-smr-ISPa21*
KW11	*K. pneumoniae*	None	bla_{VIM-4}, bla_{TEM-1}, bla_{SHV-1}, $bla_{CTX-M-15}$, bla_{CMY-4}, *aac-6'-Ib-cr*	KP-3	ST147	>300	A/C	No	bla_{CMY}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
KEcl3	*Enterobacter cloacae*	Ak, Tet, Tig, Fos	bla_{VIM-4}	ECL-4	ST184	80	NT	No	None	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21*
OM63	*E. cloacae*	Gn, Ak, Chl, Col, Tig, Fos	bla_{VIM-4}, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	ECL-1	ST182	50	NT	No	None	bla_{VIM-4}-*aacA7-smr-ISPa21-qacEΔ1-sul1*
OM69	*E. cloacae*	Gn, Ak, Chl, Col, Tig, Fos	bla_{VIM-4}, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	ECL-1	ST182	50	NT	No	None	bla_{VIM-4}-*aacA7-smr-ISPa21-qacEΔ1-sul1*
ABC104	*E. cloacae*	Ak, Col	bla_{VIM-4}, bla_{TEM-1}, $bla_{CTX-M-15}$, bla_{CMY-4}, *aac-6'-Ib-cr*	ECL-3	ST182	165	A/C	No	bla_{CMY}, bla_{TEM}	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21-qacEΔ1-sul1*
SA4/2	*E. cloacae*	Azt, Gn, Tet, Chl, Col, Tig, Fos	*qnrB*	ECL-2	ST183	50	NT	No	None	bla_{VIM-4}-*aacA7-dfrA1-ΔaadA1-smr-ISPa21-qacEΔ1-sul1*

Notes: Features boxed by thick lines are identical. [a]As determined by PBRT. [b]As detected by hybridization.

Abbreviations: PCR, polymerase chain reaction; PFGE, pulsed field gel electrophoresis; Inc, incompatibility; Col, colistin; Ak, amikacin; Fos, fosfomycin; Tet, tetracycline; Tig, tigecycline; NT, non typable; Gn, gentamicin; Chl, chloramphenicol; Azt, aztreonam; PBRT, PCR-based replicon typing; MLST, multi-locus sequence typing; ND, not detected.

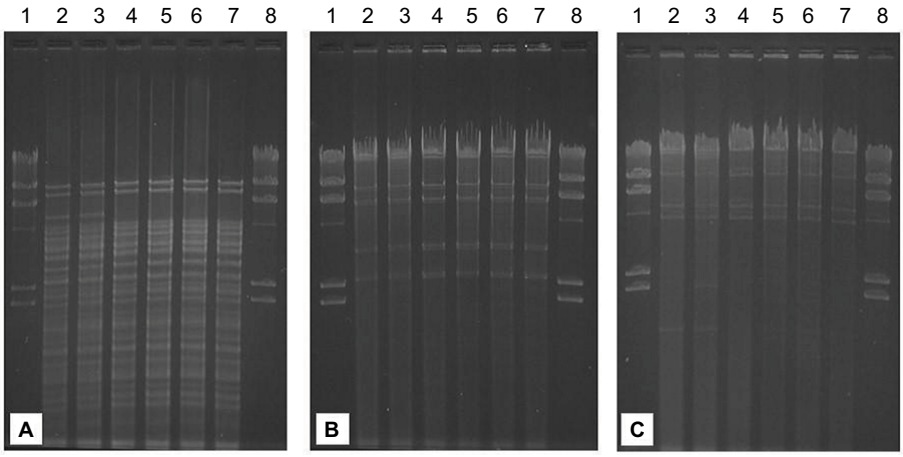

Figure 1 Restriction fragment length polymorphism of conjugative IncA/C plasmids of the Kuwaiti isolates.
Notes: (**A**) Digestion with HincII. (**B**) Digestion with EcoRI. (**C**) Digestion with HindIII. Lanes 1 and 8, lambda phage DNA digested with HindIII; Lane 2, pKKp1-VIM; Lane 3, pKKp2-VIM; Lane 4, pKKp4-VIM; Lane 5, pKKp6-VIM; Lane 6, pKEc7-VIM and Lane 7, pKKp8-VIM.

ABC104 described earlier from the UAE (GenBank Accession No. JX275775)[6] and in *E. cloacae* SA4/2 from Saudi Arabia, the *qacEΔ1-sul1-orf5* structure was present downstream of the IS*Pa21*. In the two Omani isolates, the integron lacked the *dfrA1* and *ΔaadA1* cassettes, and the 3′ CS was present downstream of IS*Pa21* (GenBank Accession No. MF178139; Table 1 and Figure 2C).

Discussion

VIM-producing Enterobacteriaceae have only been sporadically encountered in countries of the Arabian Peninsula.[4,6,19,20] Between April 2009 and February 2011, a higher prevalence of mostly unrelated VIM-4 producer Enterobacteriaceae strains was observed in Kuwait.[8] As shown by our current data, this increased rate of bla_{VIM}-carrying strains was mostly due to local horizontal gene transmission, leading to the uniform presence of the same bla_{VIM}-containing In416 integron in all Kuwaiti isolates, irrespective of the species and plasmids carried, and to the wide distribution of a 162 kb IncA/C_2-type plasmid. It is noteworthy that this type of integron and plasmid was characteristic to the Kuwaiti isolates, whereas in strains from other countries of the region, there was much heterogeneity of episomes and integrons (Table 1 and Figure 2C).

It is of interest that a plasmid (pKP-Gr642) very similar to the one spreading bla_{VIM-4} in Kuwait was found in Greece. It bore a single amino acid variant of this enzyme, i.e., bla_{VIM-19}.[18] The slight differences between this latter plasmid and pKKp4-VIM are highlighted in Figure 2. The RI-1 of pKP-Gr642 contains an additional IS*CR2*-driven *floR* gene (Figure 2B), and its RI-3 lacks the In-*t4*-like integron containing *aadB, cmlA7, qacEΔ1* and *sul1* genes (Figure 2D). Furthermore, pKP-Gr642 carries two insertion elements (IS*Ec23* and IS*Vpa4*) in the plasmid backbone.

Moreover, features of pCC416, the conjugative IncA/C plasmid shown to transfer bla_{VIM-4} between *E. cloacae* and *K. pneumoniae* clinical isolates of a patient in Italy,[14,21] were closely similar to the endemic pKKp4-VIM of Kuwait. Both RI-2 and RI-3 of pKKp4-VIM were 99% identical to the two fragments sequenced of pCC416 (GenBank Accession Nos. AJ875405 and AJ704863). Furthermore, pCC416 was also reported to carry a *sul2* gene, which is located on RI-1 of pKKp4-VIM. After detecting its in vivo transfer in a patient, it was speculated that this particular IncA/C plasmid could play a role in the spread of carbapenem resistance.[14,21] Our study confirmed this hypothesis by showing that such plasmids are indeed able to spread, over a year-long period, between strains carried by different patients, not even respecting species' barriers.

A limitation of our study is the lack of epidemiological data linking the cases to each other. No details were available to us regarding possible routes of transmission. Furthermore, only eight strains (seven of the originally described outbreak set of 11 plus one further isolate) were available for the investigation. However, even the data on this set of strains showed the heterogeneity of strains being in sharp contrast with the near uniformity of plasmids and the complete identity of bla_{VIM-4} containing integrons in all Kuwaiti isolates. Importantly, this increased rate of VIM-positive isolates seems to be a temporary event, as strains collected subsequently from Kuwait and even from the same hospital expressed mostly NDM- and OXA-type enzymes, otherwise characteristic of the region.[4,22]

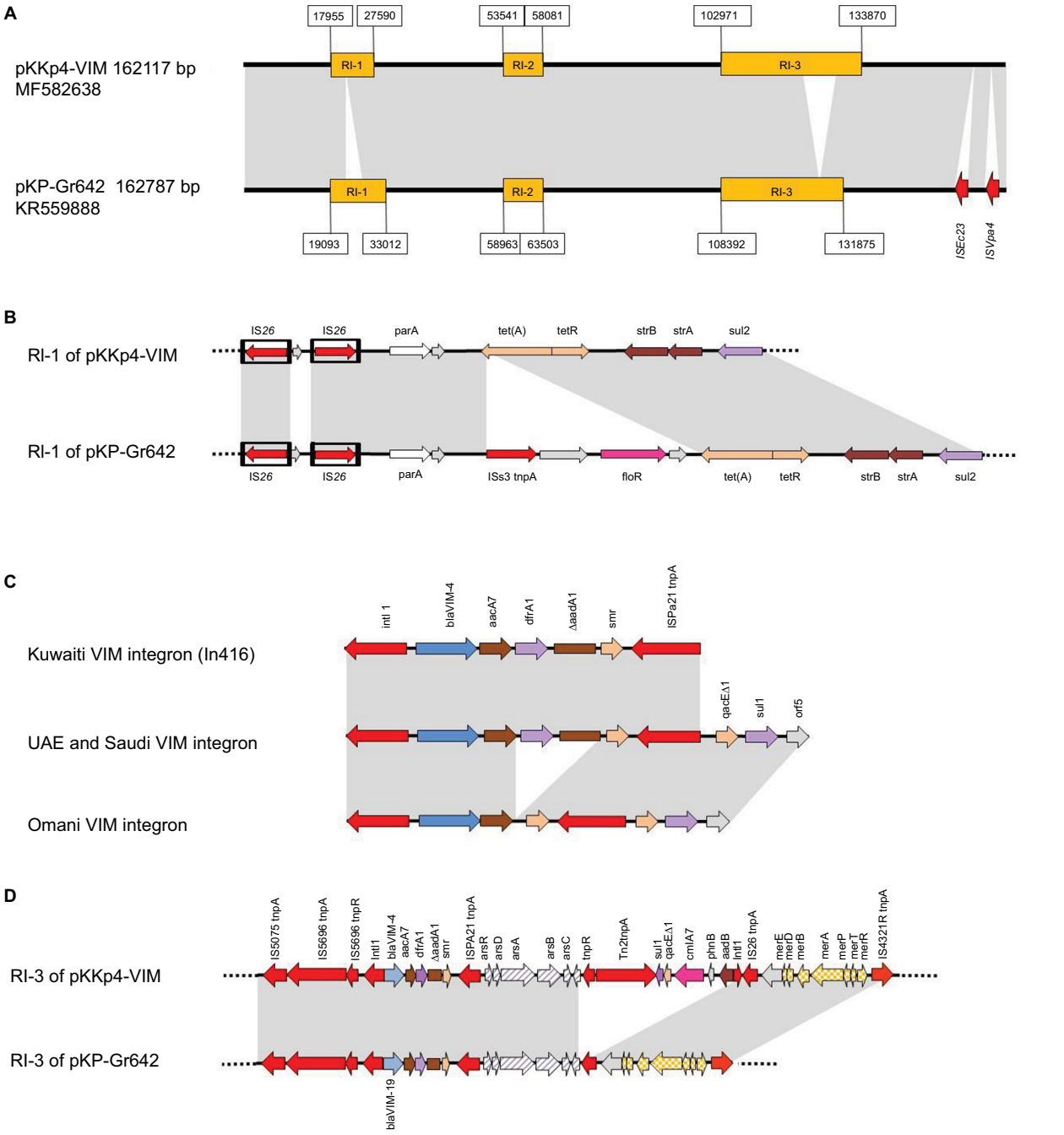

Figure 2 Structure of pKKp4-VIM.

Notes: (**A**) Comparison of the complete pKKp4-VIM to pKP-Gr642. (**B**) Comparison of RI-1 of pKKp4-VIM and pKP-Gr642. (**C**) Comparison of the three bla_{VIM-4}-bearing integron variants. (**D**) Comparison of RI-3 of pKKp4-VIM to RI-3 of pKP-Gr642. Gray areas represent ≥95% similarity.

Our results also highlight the importance of the detailed molecular typing of CPE to obtain a realistic picture of the complexity of the spread of carbapenem resistance. The spread of plasmids and integrons represents a considerable challenge to infection control. Horizontal gene transfer is difficult to prevent by routine infection control measures, and only limiting the selective antibiotic pressure in the human body and in the environment may possibly mitigate its efficacy.

Acknowledgments

The kind help of Ms Geraldine Kershaw (CMHS, UAE University) in language editing the manuscript is highly

appreciated. This work was supported by grants UPAR-25143 (31R061) and UAEU FMHS grants NP/12/13 and NP-10-11/1019 awarded to TP and UAEU UPAR-31M235 grant awarded to AS.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Primers used in sequencing the molecular structures carrying the bla_{VIM} gene

Primer name	5′-3′ sequence	Annealing to AJ704863	Size of products (bp)	Comment
AS_Classlint_L	TGT CGT TTT CAG AAG ACG GCT GC	5250	434	For amplification and sequencing the 5′ end of class I integron
AS_Classlint_R	CAA ACG TGC CGT AGA ACA AG	5683C		
AS_intI1_L	GGG AGG ACT TTC CGC AAC CG	5363	1084	For amplification and sequencing the bla_{VIM} upstream region
AS_VIM_R	CGT TAC CAC CGC TGC GTT CG	6446C		
AS_VIM_GS_S1	GCC TTG ATG TTA CCC GAG AG	5937C	NA	
AS-VIM4GS-f	GAT GCG TGG AGA CCG AAA CC	6228	1295	For amplification and sequencing the bla_{VIM} and its immediate surroundings
AS-VIM4GS-r	TGC CTA ACG CCT GAG TTG AG	7522C		
AS_VIM_L	AAT CGC TCA GTC GCC GAG TA	7412	3750	For amplification and sequencing the bla_{VIM} downstream region
AS_ISPa21_R	CTA TAA GAC ACG AGG TGT CTG	11161C		
AS_ISPa21_L	CAC CAC AAC CGC AAG AAA TA	10034	NA	
AS_ISPa21_seq	CGC GCA TCG ATT GTT CGT AG	10549	NA	
AS_smr_f	GCT GGA CTC TTT GAG ATT GG	9507	NA	
AS_dhfrI_R	ACC CTT TTG CCA GAT TTG GT	8597C	NA	
AS_aacA7_R	GAG CAA CCT CCG TGA ATC CA	7955C	NA	
AS_VIMdn_LS1	TTC GTT CAA GCC GAA CTT GC	8010	NA	
AS_VIMdn_LS2	AAT AGA CAT CGA GCC GGA AG	8477	NA	
AS_VIMdn_LS3	ACA TAG CGT TGC CTT GGT AG	9030	NA	
AS_orf5_R	TTA GAT TTC GAG TTC TAG GCG TTC TG	NA	3647 bp (if class I integron classical 3′ end is present)	Primer AS_orf5_R anneals to the 3′ end of the class I integron; the two primers amplify the 3′ region of class I integron if present
AS_smr_f	GCT GGA CTC TTT GAG ATT GG	9507		
AS_ISPa21_R	CTA TAA GAC ACG AGG TGT CTG	11161C	NA	Sequencing the amplicon produced by the PCR above
AS_ISPa21_L	CAC CAC AAC CGC AAG AAA TA	10034	NA	
AS_ISPa21_seq	CGC GCA TCG ATT GTT CGT AG	10549	NA	
AS_sul1_R1	TTG CCG ATC GCG TGA AGT TC	13000C	NA	Sequencing the amplicon produced by the PCR using primer AS_orf5_R and AS_smr_f
AS_sul1_R2	CACAACCTGGTCGATATCAC	13311C	NA	
AS_orf5_L	ATGGACAGCGAGGAGC	13249	NA	
AS_qacED1_L	GCG AAG TAA TCG CAA CAT CC	11978	NA	
AS_VIMdn_LS4	GAT CAG ATG CAC CGT GTT TC	12501	NA	

Abbreviations: NA, not applicable; PCR, polymerase chain reaction.

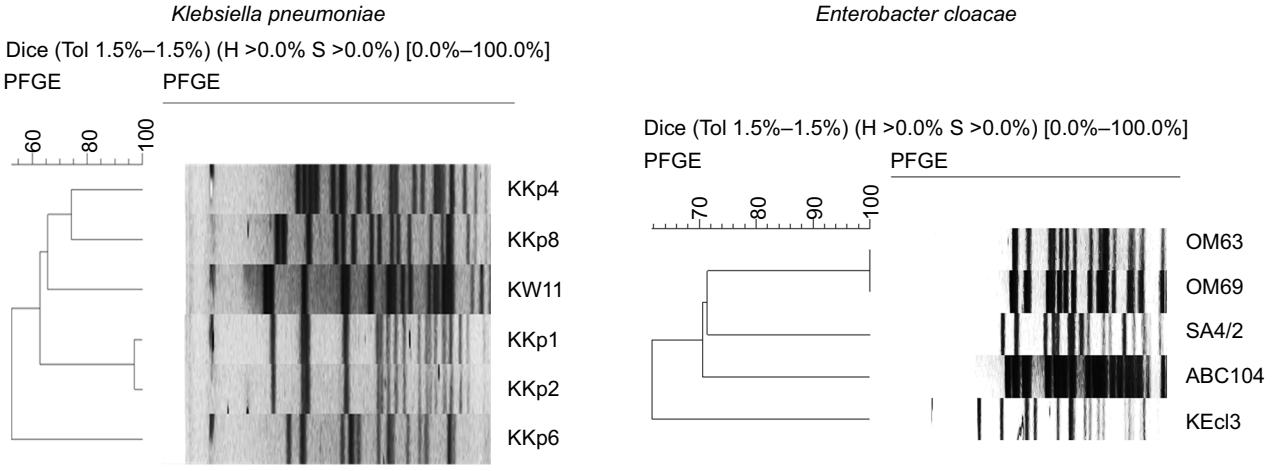

Figure S1 PFGE comparison of VIM-producing Enterobacteriaceae.
Abbreviation: PFGE, pulsed field gel electrophoresis.

Table S2 MIC values of antibiotics against VIM-producing strains and their derivatives

Strain	Type	Ceftazidime	Cefotaxime	Azt	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Gn	Ak	Co-trimoxazole	Tet	Chl	Col	Tig	Fos
KKp1	W	>128	>128	>128	>64	128	128	2	256	32	>256/4864	>256	>256	<0.5	2	64
J53RAZ(pKKp1-VIM)	TC	>128	128	128	16	8	2	<0.125	32	8	128/2432	32	256	<0.5	0.25	0.5
KKp2	W	>128	>128	>128	>64	128	128	2	256	32	>256/4864	>256	>256	<0.5	2	64
J53RAZ(pKKp2-VIM)	TC	128	128	128	4	2	2	<0.125	32	8	128/2432	32	256	<0.5	0.25	0.5
KEcl3	W	>128	>128	>128	>64	64	32	2	>256	16	>256/4864	4	16	4	0.5	32
KKp4	W	128	128	32	64	16	16	0.5	64	16	>256/4864	>256	256	<0.5	8	16
J53RAZ(pKKp4-VIM)	TC	64	32	32	4	1	<0.25	<0.125	16	4	128/2432	32	16	<0.5	0.25	2
KKp6	W	128	>128	>128	64	16	8	16	128	8	>256/4864	>256	>256	<0.5	8	4
J53RAZ(pKKp6-VIM)	TC	128	64	64	4	2	1	<0.125	16	8	128/2432	64	128	<0.5	0.25	2
KEc7	W	>128	>128	64	64	8	8	>64	64	16	>256/4864	128	256	<0.5	0.5	0.5
J53RAZ(pKEc7-VIM)	TC	64	64	64	16	2	0.5	<0.125	16	8	256/4864	128	256	<0.5	0.25	1
KKp8	W	>128	>128	>128	64	16	8	>64	128	16	>256/4864	>256	>256	<0.5	0.5	16
J53RAZ(pKKp8-VIM)	TC	64	64	32	4	2	<0.25	<0.125	16	8	128/2432	64	128	<0.5	0.25	2
KW11	W	>128	>128	>128	>64	>128	128	>64	256	32	>256/4864	>256	16	64	2	128
SA4/2	W	64	>128	1	64	64	16	1	2	32	>256/4864	4	8	<0.5	1	4
J53RAZ(pSA4/2-VIM)	TC	32	64	0.5	32	4	2	0.25	1	8	<0.5/9.5	2	8	<0.5	<0.125	1
OM63	W	128	>128	>128	32	4	4	64	2	16	>256/4864	>256	8	<0.5	0.5	32
DH5α(pOM63-VIM)	TF	16	32	<0.25	2	0.5	<0.25	<0.125	1	8	<0.5/9.5	<0.5	1	<0.5	<0.125	<0.25
OM69	W	128	>128	128	64	64	4	32	2	16	>256/4864	256	8	<0.5	0.5	32
DH5α(pOM69-VIM)	TF	16	32	<0.25	2	0.5	<0.25	<0.125	1	8	<0.5/9.5	<0.5	1	<0.5	<0.125	<0.25
ABC104	W	>128	>128	>128	>64	32	4	32	64	8	>256/4864	>256	128	<0.5	8	64
J53RAZ	R	<0.25	<0.25	<0.25	<0.125	<0.25	<0.25	<0.125	1	<0.5	<0.5/9.5	<0.5	8	<0.5	<0.125	0.5
DH5α	R	<0.25	<0.25	<0.25	<0.125	<0.25	<0.25	<0.125	1	1	<0.5/9.5	<0.5	1	<0.5	<0.125	<0.25

Abbreviations: MIC, minimal inhibitory concentration; Azt, aztreonam; Gn, gentamicin; Ak, amikacin; Tet, tetracycline; Chl, chloramphenicol; Col, colistin; Tig, tigecycline; Fos, fosfomycin; W, wild; TC, transconjugant; TF, transformant; R, recipient.

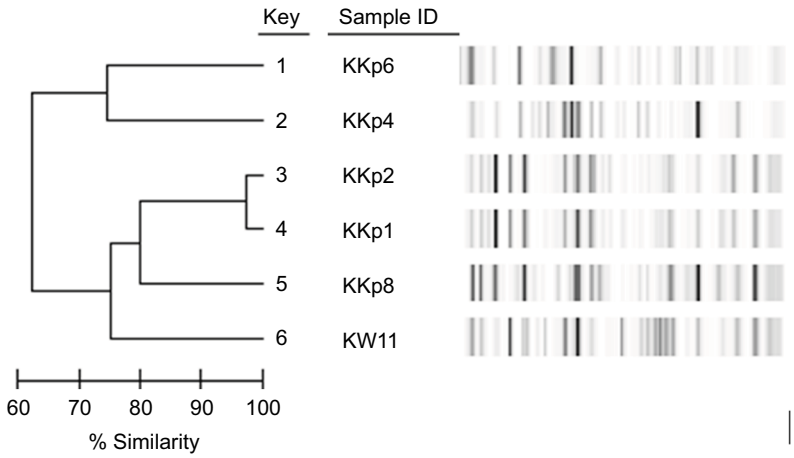

Figure S2 rep-PCR comparison of Kuwaiti *Klebsiella pneumoniae* strains.
Abbreviation: rep-PCR, repetitive element sequence-based polymerase chain reaction.

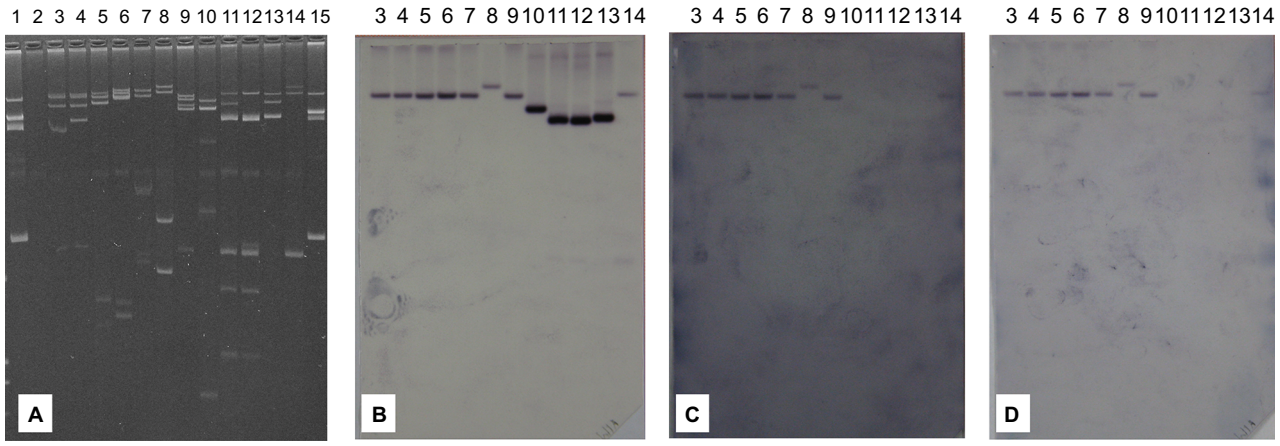

Figure S3 Plasmid profiles of VIM-producing Enterobacteriaceae.
Notes: (**A**) Plasmid gel. (**B**) Membrane hybridized with bla_{VIM-4} probe. (**C**) Membrane hybridized with bla_{CMY-4} probe. (**D**) Membrane hybridized with IncA/C probe. Lane 1, *Escherichia coli* 39R861; Lane 2, *E. coli* J53RAZ; Lane 3, *Klebsiella pneumoniae* KKp1; Lane 4, *K. pneumoniae* KKp2; Lane 5, *K. pneumoniae* KKp4; Lane 6, *K. pneumoniae* KKp6; Lane 7, *K. pneumoniae* KKp8; Lane 8, *K. pneumoniae* KW11; Lane 9, *E. coli* KEc7; Lane 10, *Enterobacter cloacae* KEcl3; Lane 11, *E. cloacae* OM63; Lane 12, *E. cloacae* OM69; Lane 13, *E. cloacae* SA4/2; Lane 14, *E. cloacae* ABC104; Lane 15, *E. coli* 39R861.

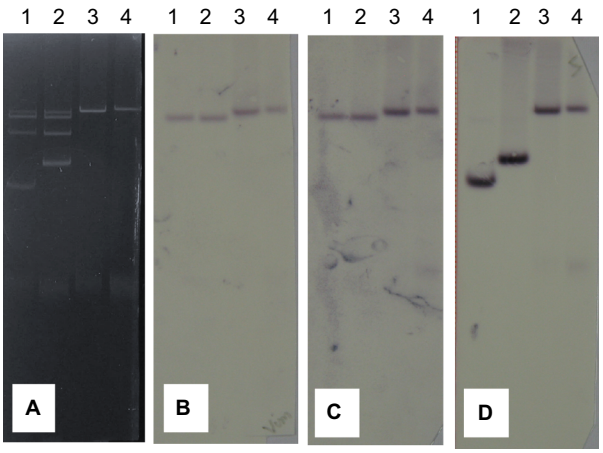

Figure S4 Fusion of IncA/C-VIM and IncN plasmids.
Notes: (**A**) Plasmid gel. (**B**) Membrane hybridized with VIM probe. (**C**) Membrane hybridized with Inc A/C probe. (**D**) Membrane hybridized with Inc N probe. Lane 1, *Klebsiella pneumoniae* KKp1; Lane 2, *K. pneumoniae* KKp2; Lane 3, *Escherichia coli* J53RAZ(pKKp1-VIM); Lane 4, *E. coli* J53RAZ(pKKp2-VIM).