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

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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 \( \text{bla}_{\text{VIM-4}} \) in six \( K. \text{pneumoniae} \) strains, one \( E. \text{coli} \), and one \( E. \text{cloacae} \) strain and compared it to that of VIM-4-producing isolates from other countries of the region. Five \( K. \text{pneumoniae} \) strains and the \( E. \text{coli} \) strain from Kuwait carried an \( \sim 165 \text{ kb Inca/C-type plasmid indistinguishable by restriction fragment length polymorphism. The complete sequence of one of them (pKKp4-VIM)} \) was established. pKKp4-VIM exhibited extensive similarities to episomes pKP-Gr642 carrying \( \text{bla}_{\text{VIM-19}} \) encountered in Greece and to the partially sequenced pCC416 harboring \( \text{bla}_{\text{VIM-4}} \) 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 \( \text{bla}_{\text{VIM-4}} \) 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. Group B enzymes, i.e., the metallo-beta-lactamases (MBLs), are especially worrisome, as recently introduced beta-lactamase inhibitors have no activity against them.

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-
and VIM-type enzymes. 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. 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<sub>VIM</sub>-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 (Ecl13) and one Escherichia coli (Ec7) were previously described as part of a VIM-producing Enterobacteriaceae outbreak in Kuwait. 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). 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. For the majority of antibiotics the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were used for interpretation, with the exception of Col, Tig and Fos whereby the The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria were used. Resistance genes (bla<sub>VIM</sub>, bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, bla<sub>PER</sub>, bla<sub>AmpC</sub>, bla<sub>NDM</sub>, bla<sub>OXA-48-like</sub>, bla<sub>KPC</sub>, bla<sub>VIM</sub>, bla<sub>IMP</sub>, armA, mrtA, mrtB, mrtC, mrtD, mrtE, mrtF, qnrS, qepA, aac6-1b-cr, mcr-1, mcr-2) were detected as described. The specific alleles of beta-lactamase genes were determined by direct sequencing of the respective amplicons performed with the BigDye Cycle Terminator V3.1 (Thermo Fisher Scientific, Waltham, MA, USA) using the 3130X Genetic Analyzer (Thermo Fisher Scientific).

Characterization of the genetic environment of bla<sub>VIM</sub>-4

The flanking region of the bla<sub>VIM</sub>-4 gene was determined by polymerase chain reaction (PCR) mapping and sequencing using primers designed (Table S1) according to the genetic surrounding of bla<sub>VIM</sub> published earlier (GenBank accession numbers AJ704863 and AY339625). 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 using E. coli 39R861 as plasmids molecular size standards. 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. If they were non-conjugative, heat shock transformation of the carbapenemase-bearing plasmids into E. coli DH5<alpha> was attempted. To prove the localization of genes, electrophoretically separated plasmids isolated from the wild-type strains by the alkaline lysis method 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. The incompatibility (Inc) groups of the plasmids transferred were identified by PCR and confirmed by hybridization as mentioned earlier. 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 HindII, HindIII and EcoRI restriction endonucleases. Furthermore, the complete sequence of pKKp4-VIM conjugally transferred from K. pneumoniae KKp4 into E. coli J53RAZ was established.
Unlike in the previous study, we could conjugally transfer \( \text{bla}_{\text{VIM-4}} \) among strains. All strains investigated carried a single carbapenemase, \( \text{bla}_{\text{CMY-4}} \), except for one (K. pneumoniae KW11) being resistant to all antibiotics tested (Tables 1 and S2).

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, \( \text{bla}_{\text{VIM-4}} \). 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). 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 (KEc3) 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 \( \text{bla}_{\text{VIM-4}} \)
Unlike in the previous study, 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 KEc3 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 \( \text{bla}_{\text{VIM-4}} \) on IncA/C Inc-type plasmids of ~165 kb. Beyond \( \text{bla}_{\text{VIM-4}} \), these plasmids also carried the \( \text{bla}_{\text{CMY-4}} \) gene (Table 1). The RFLP patterns of these plasmids were identical (Figure 1). E. cloacae ABC104 from the UAE (described earlier) also carried \( \text{bla}_{\text{VIM-4}} \) and \( \text{bla}_{\text{CMY-4}} \) 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 \( \text{bla}_{\text{VIM-4}} \) was located on a nontransferable IncA/C type, >300 kb plasmid, which also carried \( \text{bla}_{\text{CMY-4}} \). As shown in Table 1 and in Figure S3, the Kuwaiti, Saudi and Omani E. cloacae isolates all carried \( \text{bla}_{\text{VIM-4}} \) on smaller plasmids lacking \( \text{bla}_{\text{CMY-4}} \), which could not be identified by the PCR-based replicon typing (PBRT). In case of KKp1 and KKp2, the conjugal transfer of the \( \text{bla}_{\text{VIM-4}} \)-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 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 type plasmid of a Greek clinical K. pneumoniae isolate carrying \( \text{bla}_{\text{VIM-19}} \) (Figure 2A)\(^a\). Apart from the plasmid backbone, pKKp4-VIM harbored three resistance islands: RI-1, RI-2 and RI-3 (Figure 2A). On RI-1 \( \text{tet}(A) \), \( \text{strA} \), \( \text{strB} \) and \( \text{su}l2 \) genes are located (Figure 2B). The RI-2 consists of ISEcp1, \( \text{bla}_{\text{CMY-4}} \), \( \text{bIc} \) and \( \text{sugE} \) genes. The third resistance island RI-3 contains an \( \text{In416} \) with \( \text{bla}_{\text{VIM-4}} \), \( \text{aacA7} \), \( \text{dfra1} \), \( \text{AaadA1} \) and \( \text{smr} \) gene cassettes, a Tn8802 with arsenic resistance operon, an \( \text{Inr41}-\text{like} \) integron and a mercury resistance operon (Figure 2D).

Genetic surrounding of \( \text{bla}_{\text{VIM-4}} \) 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., \( \text{bla}_{\text{VIM-4}} \) was located on an \( \text{In416} \) 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 | Conjugative | Additional resistance genes | VIM-bearing integron structure |
|--------|--------------------------|----------------|----------------------------------|------|------------|-------------|-----------------------------|----------|--------------|-------------------------------|--------------------------------|
| KKp1   | Klebsiella pneumoniae    | Col            | blad_{TEM}, blb_{CTXM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-4 | ST1399     |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KKp2   | K. pneumoniae            | Col            | blad_{TEM}, blb_{CTXM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-4 | ST1399     |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KKp4   | K. pneumoniae            | Ak, Col, Fos   | blad_{TEM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-1 | ST138      |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KKp6   | K. pneumoniae            | Ak, Col, Fos   | blad_{TEM}, blb_{CTXM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-5 | ST1400     |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KEc7   | Escherichia coli         | Ak, Col, Fos   | blad_{TEM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr, qnrB | ND   | ST167      |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KKp8   | K. pneumoniae            | Ak, Col, Fos   | blad_{TEM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-2 | ST1401     |             | 165                         | A/C      | Yes          | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KW11   | K. pneumoniae            | None           | blad_{TEM}, blb_{CTXM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | KP-3 | ST147      | >300         | A/C                         | No       | None         | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021 |                                |
| KEcl3  | Enterobacter cloacae     | Ak, Tet, Tig, Fos | blad_{TEM} | ECL-4 | ST184 | 80       | NT                  | No       | None         | blad_{TEM}                              |                                |
| OM63   | E. cloacae               | Gn, Ak, Col, Chl, Tig, Fos | blad_{TEM}, blb_{CTXM}, blb_{SHV}, qnrB | ECL-1 | ST182 | 50       | NT                  | No       | None         | blad_{TEM}, aacA7, smr-ISP021                  |                                |
| OM69   | E. cloacae               | Gn, Ak, Chl, Col, Tig, Fos | blad_{TEM}, blb_{CTXM}, blb_{SHV}, qnrB | ECL-1 | ST182 | 50       | NT                  | No       | None         | blad_{TEM}, aacA7, smr-ISP021                  |                                |
| ABC104 | E. cloacae               | Ak, Col        | blad_{TEM}, blb_{CTXM}, blb_{SHV}, blb_{CMY}, aac-6'-Ib-cr | ECL-3 | ST182 | 165      | A/C                  | No       | None         | blad_{TEM}, blb_{SHV}                          |                                |
| SA4/2  | E. cloacae               | Azt, Gn, Tet, Chl, Col, Tig, Fos | qnrB | ECL-2 | ST183 | 50       | NT                  | No       | None         | blad_{TEM}, aacA7, dfIA1-ΔaadA1-smr-ISP021                  |                                |

Notes: Features boxed by thick lines are identical. *As determined by PBRT. **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; CH, chloramphenicol; Azt, aztreonam; PBRT, PCR-based replicon typing; MLST, multi-locus sequence typing; ND, not detected.
ABC104 described earlier from the UAE (GenBank Accession No. JX275775) and in E. cloacae SA4/2 from Saudi Arabia, the qacED1-sul1-orf5 structure was present downstream of the ISPa21. In the two Omani isolates, the integron lacked the dfrA1 and DaadA1 cassettes, and the 3′ CS was present downstream of ISPa21 (GenBank Accession No. MF178139; Table 1 and Figure 2C).

Discussion

VIM-producing Enterobacteriaceae have only been sporadically encountered in countries of the Arabian Peninsula. Between April 2009 and February 2011, a higher prevalence of mostly unrelated VIM-4 producer Enterobacteriaceae strains was observed in Kuwait. As shown by our current data, this increased rate of blaVIM-carrying strains was mostly due to local horizontal gene transmission, leading to the uniform presence of the same blaVIM-containing In416 integron in all Kuwaiti isolates, irrespective of the species and plasmids carried, and to the wide distribution of a 162 kb IncA/C2-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 blaVIM-4 in Kuwait was found in Greece. It bore a single amino acid variant of this enzyme, i.e., blaVIM-19. The slight differences between this latter plasmid and pKKp4-VIM are highlighted in Figure 2. The RI-1 of pKP-Gr642 contains an additional ISCR2-driven floR gene (Figure 2B), and its RI-3 lacks the In-t4-like integron containing aadB, cmlA7, qacEA1 and sul1 genes (Figure 2D).

Furthermore, pKP-Gr642 carries two insertion elements (ISEc23 and ISYpa4) in the plasmid backbone.

Moreover, features of pCC416, the conjugative IncA/C plasmid known to transfer blaVIM-4 between E. cloacae and K. pneumoniae clinical isolates of a patient in Italy, 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. 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 blaVIM-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.
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.

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**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 blaVIM-bearing integron variants. (D) Comparison of RI-3 of pKKp4-VIM to RI-3 of pKP-Gr642. Gray areas represent ≥95% similarity.
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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\textsubscript{VIM} gene

| Primer name         | 5'-3' sequence | Annealing to AJ704863 | Size of products (bp) | Comment                                                                 |
|---------------------|----------------|------------------------|-----------------------|--------------------------------------------------------------------------|
| AS\_ClassI\_int\_L  | TGT CGT TTT CAG ACG ACG GCT GC | 5250                   | 434                   | For amplification and sequencing the 5' end of class I integron          |
| AS\_ClassI\_int\_R  | CAA ACG TGC CGT AGA ACA AGA | 5683C                  |                       |                                                                          |
| AS\_intI\_L         | GGG AGG ACT TTC CGC AAC CG | 5363                   | 1084                  | For amplification and sequencing the bla\textsubscript{VIM} upstream region |
| AS\_VIM\_R          | CGT TAC CAC CGC TGC GTT GC | 6446C                  |                       |                                                                          |
| AS\_VIM\_GS\_S1     | GCC TGG ATG TTA CCC GAG AG | 5937C                  | NA                    |                                                                          |
| AS\_VIM4\_G\_S-f    | GAT GCG TGG AGG AGG AAA CC | 6228                   | 1295                  | For amplification and sequencing the bla\textsubscript{VIM} and its immediate surroundings |
| AS\_VIM4\_G\_S-r    | TGC CTA ACG CCT GAG TGG AG | 7522C                  |                       |                                                                          |
| AS\_VIM\_L          | AAT CGC TCA GTC GCC GAG TA | 7412                   | 3750                  | For amplification and sequencing the bla\textsubscript{VIM} downstream region |
| AS\_ISPa21\_R       | CTA TAA GAC ACG ACG 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                   |                       |                                                                          |
| AS\_dhfr\_R         | ACC CTT TTG CCA GAT TGG GT | 8597C                  | NA                    |                                                                          |
| AS\_aacA7\_R        | GAG CAA CTC CGG TGC ATC CA | 7955C                  | NA                    |                                                                          |
| AS\_VIM\_dn\_LS1    | TTC GGT CAA GCC GAA CCT GC | 8010                   | NA                    |                                                                          |
| AS\_VIM\_dn\_LS2    | AAT AGA CAT CGA GCC GGA AG | 8477                   | NA                    |                                                                          |
| AS\_VIM\_dn\_LS3    | AAT AGA CAT CGA GCC GGA AG | 9030                   | NA                    |                                                                          |
| AS\_orf5\_R         | TTA GAT TTC GAG TTC TAG GCG TTC TG | NA                  | 3647 bp (if 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 ACG 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\_sul\_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\_sul\_R2         | CACAACCTGGTCGATATCAC      | 13311C                 | NA                    |                                                                          |
| AS\_orf5\_L          | ATGGACAGCGAGGAGG          | 13249                  | NA                    |                                                                          |
| AS\_qacED1\_L        | GCG AAG TAA TCG CAA CAT CC | 11978                  | NA                    |                                                                          |
| AS\_VIM\_dn\_LS4     | GAT CAG ATG CAC CGT GTC TTG | 12501                  | NA                    |                                                                          |

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

Figure S1 PFGE comparison of VIM-producing Enterobacteriaceae.

**Klebsiella pneumoniae**

| Dice (Tol 1.5%–1.5%) (H >0.0% S >0.0%) [0.0%–100.0%] | PFGE | PFGE |
|--------------------------------------------------------|------|------|
| [0.0%–100.0%]                                           |      |      |

**Enterobacter cloacae**

| Dice (Tol 1.5%–1.5%) (H >0.0% S >0.0%) [0.0%–100.0%] | PFGE | PFGE |
|--------------------------------------------------------|------|------|
| [0.0%–100.0%]                                           |      |      |

**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 | Erapermen | Imipenem | Menpenem | Ciprofloxacin | Gn | Ak | Cm | Tet | Chi | Col | Tig | Fos |
|-----------------|------|-------------|------------|-----|-----------|----------|----------|---------------|----|----|----|-----|-----|-----|-----|-----|
| KKp1            | W    | >128        | >128       | >64 | 128       | 128      | 2        | 256           | 32 | >256/4864 | >256 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| J53RAZ(pKKp1-VIM) | TC   | >128        | 128        | 16  | 8         | 2        | <0.125   | 32            | 8  | 128/432   | 32   | 256       | >0.5    | 2    | 64 |
| KKp2            | W    | >128        | >128       | >64 | 128       | 2        | 2        | 256           | 32 | >256/4864 | >256/4864 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| J53RAZ(pKKp2-VIM) | TC   | 128         | 128        | 4   | 2         | 2        | <0.125   | 32            | 8  | 128/432   | 32   | 256       | >0.5    | 2    | 64 |
| KEc7            | W    | >128        | >128       | >64 | 64        | 32       | 2        | >256/4864   | 4  | 16         | 16    | 4         | 0.5     | 32   |
| J53RAZ(pKKp4-VIM) | TC   | 64          | 32         | 4   | 1         | <0.125   | 16        | 4             | 128/432 | 32         | 16    | >0.5    | 2    |
| KKp4            | W    | 128         | 128        | 32  | 64        | 16       | 16       | 64            | 16 | >256/4864 | >256/4864 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| J53RAZ(pKKp6-VIM) | TC   | 128         | 64         | 4   | 2         | 1        | <0.125   | 16            | 8  | 128/2432  | 64   | 128       | >0.5    | 2    |
| KEc7            | W    | >128        | >128       | 64  | 8         | 8        | >64       | 64            | 16 | >256/4864 | >256/4864 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| J53RAZ(pKEc7-VIM) | TC   | 64          | 64         | 16  | 2         | 0.5      | <0.125   | 16            | 8  | 256/4864  | 128   | 256       | >0.5    | 2    |
| KKp8            | W    | >128        | >128       | >64 | 16        | 8        | >64       | 128           | 16 | >256/4864 | >256/4864 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| J53RAZ(pKKp8-VIM) | TC   | 64          | 64         | 32  | 4         | 2        | <0.25    | <0.125        | 16 | 8          | 128   | >0.5    | 2    |
| KW11            | W    | >128        | >128       | >64 | >128      | 128      | >64       | 256           | 32 | >256/4864 | >256/4864 | >256/4864 | >256/4864 | >256 | >256/4864 | >256/4864 |
| SA4/2           | W    | 64          | >128       | 4   | 2         | 0.25     | 1         | 8             | <0.5  | 9.5     | 2     | 8        | >0.5  | 1    |
| J53RAZ(pSA4/2-VIM) | TC   | 32          | 64         | 0.5 | 32        | 4         | 2         | 0.25          | 1   | 8        | >0.5 | 125 |
| OM63            | W    | 128         | >128       | 32  | 4         | 4         | 64        | 2             | 16 | >256/4864 | >256 | 8        | >0.5  | 32   |
| DHC58(pOM63-VIM) | TF   | 16          | 32         | 0.5  | <0.25     | 2         | 0.5       | <0.25         | 0.125 | 1     | 8     | >0.5 | 9.5 | 0.5 |
| OM69            | W    | 128         | >128       | 64  | 4         | 4         | 32        | 2             | 16 | >256/4864 | 256  | 8        | >0.5  | 32   |
| DHC58(pOM69-VIM) | TF   | 16          | 32         | 0.5  | <0.25     | 2         | 0.5       | <0.25         | 0.125 | 1     | 8     | >0.5 | 9.5 | 0.5 |
| ABC104          | W    | >128        | >128       | >64 | 32        | 4         | 32        | 64            | 8   | >256/4864 | >256 | 128      | >0.5  | 8    |
| J53RAZ          | R    | <0.25       | <0.25      | <0.125 | <0.25 | <0.25     | <0.25 | <0.125 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 |
| DHC58          | R    | <0.25       | <0.25      | <0.125 | <0.25 | <0.25     | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 |

Abbreviations: MIC, minimal inhibitory concentration; Azt, aztreonam; Gn, gentamicin; Ak, amikacin; Tet, tetracycline; Chi, 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 \textit{bla}_{VIM} probe. (C) Membrane hybridized with \textit{bla}_{CMY} probe. (D) Membrane hybridized with IncA/C probe. Lane 1, \textit{Escherichia coli} 39R861; Lane 2, \textit{E}. coli J53RAZ; Lane 3, \textit{Klebsiella pneumoniae} KKp1; Lane 4, \textit{K}. pneumoniae KKp2; Lane 5, \textit{K}. pneumoniae KKp4; Lane 6, \textit{K}. pneumoniae KKp6; Lane 7, \textit{K}. pneumoniae KKp8; Lane 8, \textit{K}. pneumoniae KW11; Lane 9, \textit{E}. coli K67; Lane 10, \textit{Enterobacter cloacae} KEcl3; Lane 11, \textit{E}. cloacae OM63; Lane 12, \textit{E}. cloacae OM69; Lane 13, \textit{E}. cloacae SA4/2; Lane 14, \textit{E}. cloacae ABC104; Lane 15, \textit{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, \textit{Klebsiella pneumoniae} KKp1; Lane 2, \textit{K}. pneumoniae KKp2; Lane 3, \textit{Escherichia coli} J53RAZ(pKKp1-VIM); Lane 4, \textit{E}. coli J53RAZ(pKKp2-VIM).