Genetic characterization of three *qnrS1*-harbouring multidrug-resistance plasmids and *qnrS1*-containing transposons circulating in Ho Chi Minh City, Vietnam

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Plasmid-mediated quinolone resistance (PMQR) refers to a family of closely related genes that confer decreased susceptibility to fluoroquinolones. PMQR genes are generally associated with integrons and/or plasmids that carry additional antimicrobial resistance genes active against a range of antimicrobials. In Ho Chi Minh City (HCMC), Vietnam, we have previously shown a high frequency of PMQR genes within commensal *Enterobacteriaceae*. However, there are limited available sequence data detailing the genetic context in which the PMQR genes reside, and a lack of understanding of how these genes spread across the *Enterobacteriaceae*. Here, we aimed to determine the genetic background facilitating the spread and maintenance of *qnrS1,* the dominant PMQR gene circulating in HCMC. We sequenced three *qnrS1*-carrying plasmids in their entirety to understand the genetic context of these *qnrS1*-embedded plasmids and also the association of *qnrS1*-mediated quinolone resistance with other antimicrobial resistance phenotypes. Annotation of the three *qnrS1*-containing plasmids revealed a *qnrS1*-containing transposon with a closely related structure. We screened 112 *qnrS1*-positive commensal *Enterobacteriaceae* isolated in the community and in a hospital in HCMC to detect the common transposon structure. We found the same transposon structure to be present in 71.4 % (45/63) of *qnrS1*-positive hospital isolates and in 36.7 % (18/49) of *qnrS1*-positive isolates from the community. The resulting sequence analysis of the *qnrS1* environment suggested that *qnrS1* genes are widely distributed and are mobilized on elements with a common genetic background. Our data add additional insight into mechanisms that facilitate resistance to multiple antimicrobials in Gram-negative bacteria in Vietnam.

INTRODUCTION

Fluoroquinolones are among the current first line of drugs in Vietnam for treating infections ranging from pneumonia to diarrhoea to bacteraemia. Since the mid-1990s, resistance to quinolones has been increasing steadily in multiple organisms causing respiratory tract infections, diarrhoea and bacteraemia (Holt *et al.*, 2013; Nga *et al.*, 2012; Nhu *et al.*, 2014). The emergence of fluoroquinolone resistance

in these pathogens and other bacteria represents a clear threat to the effective treatment of common bacterial infections. Resistance to quinolones occurs commonly via mutations in the gene of the target enzyme, DNA gyrase, but can also be plasmid mediated. Plasmid-mediated quinolone resistance (PMQR), in the form of a *qnr* gene, was first described in 1998 in a *Klebsiella pneumoniae* isolate from a patient with urinary tract infection in North America (Martínez-Martínez *et al.*, 1998). Since this first report, multiple studies have described a range of PMQR determinants found within the *Enterobacteriaceae* and other bacterial families (Strahilevitz *et al.*, 2009). To date, five *qnr* genes have been described and are distinguished on the basis of their sequence homology: *qnrA*, *qnrB* and *qnrS1*, and more recently *qnrC* and *qnrD* (Cavaco *et al.*, 2009; Hata *et al.*, 2005; Jacoby *et al.*, 2006; Martínez-Martínez *et al.*, 1998; Wang *et al.*, 2009). The *qnr* genes appear to be highly promiscuous, having the capacity to become rapidly disseminated among related and unrelated hosts. The transmissibility of the *qnr* genes makes the genomic mechanisms facilitating their movement of considerable interest and of relevance in the community and in healthcare settings. Of the five *qnr* genes identified, only the genetic contexts of *qnrA* and *qnrB* have been comprehensively described. These genes are commonly located within complex *sul1*-type class 1 integrons (Garnier *et al.*, 2006; Poirel *et al.*, 2006). The context of *qnrS* is less well described, but there are reports of the gene being located within a gene cluster flanked by IS*26* transposases (Chen *et al.*, 2006; Hu *et al.*, 2008). However, it is currently unknown if the *qnrS* gene is always associated with IS*26* or if *qnrS* can be transferred and/or maintained by other, unrelated, mobile elements.

Several *qnrS1*-carrying plasmids have been described in the literature and have publicly available nucleotide sequences. These plasmids range in size and belong to various incompatibility groups including IncN (Dobiasova *et al.*, 2013; Literak *et al.*, 2010), IncI1 (Dobiasova *et al.*, 2013), IncX1 (Dobiasova *et al.*, 2013; Literak *et al.*, 2010) and IncX2 (Literak *et al.*, 2010; Sumrall *et al.*, 2014). These *qnrS1*-encoding plasmids have been identified in Asia and Europe and in a range of Gram-negative bacteria including *K. pneumoniae*, *Escherichia coli* and *Enterobacter aerogenes* (Dobiasova *et al.*, 2013; Hu *et al.*, 2008; Park *et al.*, 2009; Sumrall *et al.*, 2014). Two of the earliest and best-described *qnrS1*-encoding plasmids are pTPqnrS-1a and pK245. Plasmid pTPqnrS-1a is a 10 kb replicon, and was isolated from a multidrug-resistant (MDR) *Salmonella* Typhimurium DT193 in the UK (Kehrenberg *et al.*, 2007). The second, pK245, was characterized in a clinical isolate of *K. pneumoniae* originating in Taiwan (Chen *et al.*, 2006). In contrast to pTPqnrS-1a, pK245 is a large MDR plasmid of approximately 100 kb. The MDR phenotype of pK245 was demonstrated by transferring this plasmid into an antimicrobial-susceptible *Escherichia coli* strain by electrotransformation (Chen *et al.*, 2006). The pK245-positive transformant showed an increase in MICs to multiple

classes of antimicrobials, including aminoglycosides, β-lactams and (fluoro)quinolones and had an extended-spectrum β-lactamase (ESBL) phenotype (Chen *et al.*, 2006). Comparative sequence analysis of available *qnrS1* plasmids revealed that the genetic architecture surrounding the *qnrS1* gene is identical between pTPqnrS-1a and pK245, and they additionally sharing a high sequence identity with the *qnrS1* genetic region in other partial plasmid sequences, including pAH0376 from *Shigella flexneri* (Hata *et al.*, 2005) and pINF5 from *Salmonella* Infantis (Kehrenberg *et al.*, 2006).

In a study investigating the distribution of PMQR determinants in *Enterobacteriaceae* isolated from hospitalized patients and healthy volunteers from Ho Chi Minh City (HCMC), Vietnam, we found an exceptionally high prevalence of the *qnrS1* genes in both hospital (63/139, 45 %) and community (49/413, 12 %) bacterial isolates (Vien *et al.*, 2009). We therefore hypothesized that *qnrS1* was embedded on a highly mobile and conserved genetic element, which was contributing to the spread and the apparent success of *qnrS1* across the *Enterobacteriaceae* in this setting. In this current study, we aimed to characterize the dominant *qnrS1*-containing elements circulating in *Enterobacteriaceae* isolated from hospital patients and community volunteers in HCMC to understand if *qnrS1* is being disseminated on one or more elements by defining their genetic context. To achieve this, we selected three *qnrS1*-containing plasmids, broadly representative of those found to be circulating in the hospital and community environments (Vien *et al.*, 2012), for DNA sequencing and analysis in their entirety. The resulting sequence analysis of the *qnrS1*-containing mobile elements has broadened our knowledge of the genetic architecture surrounding the *qnrS1* gene and added insight into MDR mechanisms that are circulating within these differing bacterial environments in Vietnam.

METHODS

Bacterial strains. A total of 115 *qnrS1*-positive *Enterobacteriaceae* strains (38 *Escherichia coli*, 69 *K. pneumoniae* and eight from other *Enterobacteriaceae* species) were selected for analysis in this study. All of the strains have been described previously and were isolated from patients admitted to the tetanus ward of the Hospital for Tropical Diseases (HTD) in HCMC, Vietnam, between May and October 2004 and between June and November 2005 or from healthy volunteers participating in a typhoid vaccine study in 2005 and 2006 (Tran *et al.*, 2010). The presence of the *qnrS1* gene in these strains has been previously confirmed and described (Vien *et al.*, 2009).

Three *qnrS1*-containing plasmids identified previously (Vien *et al.*, 2012) were selected to be broadly representative of *qnrS1*-containing plasmids circulating in our setting (i.e. harbouring strain, hospital/community infections and size) and were sequenced in their entirety, assembled and annotated gene by gene in comparison to sequences available in public databases. These plasmids (selected out of the 115 *qnrS1*-positive *Enterobacteriaceae* strains) were pE66An (*Escherichia coli* host), pK18An (*K. pneumoniae* host) and pK1HV (*K. pneumoniae* host). A summary of the basic features of these plasmids is given in Table 1.

Table 1. Features of the three sequenced *qnrS1*-containing plasmids

Characteristic	pE66An	pK18An	pK1HV
Original strain	*Escherichia coli*	*K. pneumoniae*	*K. pneumoniae*
Source	Hospital	Hospital	Community
Size (kb)	80.105	51.160	133.191
G+C (mol%)	51.25	51.32	52.5
Inc group	IncN	IncN	IncFII
Predicted coding sequences	109	72	167
Essential function genes	22	15	14
Conjugative system genes	16	6	21
Resistance genes	7	5	11
IS elements	IS*26*, IS*6100*, IS*6100*903D, IS*Ecp1*	IS*26*, IS*10*, IS*5*	IS*26*, IS*4*, IS*1414*
Integron	Integron 1	Integron 1	Integron 1

Plasmid sequencing and annotation. The three *qnrS1*-containing plasmids were sequenced at the Wellcome Trust Sanger Institute in the UK using conventional Sanger sequencing methods as described previously (Parkhill *et al.*, 2001). The plasmid sequences were annotated and analysed using Artemis (Rutherford *et al.*, 2000) and aligned and compared using the Artemis Comparison Tool (Carver *et al.*, 2008). Plasmid circularization and graphical representations were performed using DNA Plotter software (Carver *et al.*, 2009).

PCR amplification for the *qnrS1*-containing transposon and RFLP typing. Total genomic DNA from 112 *qnrS1* PCR amplification-positive *Enterobacteriaceae* isolates was extracted using a Wizard Genomic DNA purification kit (Promega), according to the manufacturer's specifications. PCR amplification for the *qnrS1*-containing transposon from the extracted genomic DNA was performed under the following condition: 94 °C for 10 s, 55 °C for 30 s and 68 °C for 6 min for 35 cycles. Amplification was performed using the Expand Long Template PCR System (Roche) using the primers Trans-qnrS-F (5′-CAGGAAGAGGCATTGTCAAAGG-3′) and Trans-qnrS-R (5′-GGTGCTTGTCAGCGTAAA-3′). These primers were designed using Primer Express 5 software (Applied Biosystems, Life Technologies) and their specificity was assessed *in silico* using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The resulting PCR amplicons were examined by electrophoresis and UV visualization on 2 % agarose gels containing 2 % ethidium bromide. The PCR amplicons containing the *qnrS1* gene were typed using RFLP with three different enzymes: *Eco*RV, *Hind*III and *Pvu*II (New England Biolabs). The restriction-digested PCR amplicons were analysed by gel electrophoresis for 2 h on a 1 % agarose gel, stained with 2 % ethidium bromide and examined under UV light. The restriction fragments were sized and compared for group typing using Bionumerics software (Applied Maths).

Primer-walking sequencing. The *qnrS1*-containing transposon from the K34N strain was sequenced using primer walking. The sequencing reaction was performed in a 20 µl reaction containing 4 µl Big Dye Terminator, 2 µl buffer, 20 ng genomic DNA and distilled water up to 20 µl. Each fragment was repeated four times using an ABI 3130XL machine (Applied Biosystems, Life Technologies). All sequences were assembled using Vector NTI software (Life Technologies).

Electrotransformation. PCR-negative isolates for the *qnrS1*-containing transposon were analysed for the presence of subfamilies of the known transposon. Plasmid DNA from these negative isolates was extracted using a Qiagen Midi Prep Plasmid DNA Extraction kit, as per the manufacturer's recommendations. *Escherichia coli* TOP10 cells (Invitrogen, Life Technologies) were transformed with isolated plasmid DNA by a Bio-Rad gene pulser, using conditions recommended by the manufacturer (Invitrogen, Life Technologies).

Transformants were selected on Luria–Bertani medium supplemented with 0.03 mg ciprofloxacin l^{-1}. Plasmid DNA from these transformants was extracted by the method of Kado & Liu (1981), examined on an agarose gel for the presence of only one plasmid and then subjected to PCR amplification for the *qnrS1* gene to ensure transformation of the appropriate plasmid.

Southern blot analysis. Isolates containing a subfamily of known *qnrS1*-containing transposons were detected by Southern blotting with two different probes: qnrS1 and bla$_{LAP-2}$, using the primers described for amplification of the qnrS1 region. Plasmid DNA from the transformants was extracted using a Qiagen Midi Prep Plasmid DNA Extraction kit, as per manufacturer's recommendations. These plasmids were then digested with *Eco*RI and duplicates were run on a gel. The gel was subsequently transferred to a membrane. The membrane was cut into two pieces and each was hybridized with one of the probes, qnrS1 or bla$_{LAP-2}$. If an isolate had signal with both probes binding to the same fragment, it was assigned as carrying a subfamily of a *qnrS1*-containing transposons.

RESULTS AND DISCUSSION

Global comparison of the three *qnrS1*-containing plasmids

Figure 1 shows a global DNA alignment of the three sequenced plasmids and a previously sequenced *qnrS1*-containing plasmid (pK245) identified in a *K. pneumoniae* isolate from Taiwan as a comparator (Chen *et al.*, 2006). These alignments showed that the two plasmids isolated independently from different bacterial genera within the hospital environment (pE66An and pK18An) exhibited substantial gene synteny with each other, but generally shared a lower degree of DNA homology with the plasmid identified in a community isolate (pK1HV).

Plasmid pE66An

The *qnrS1*-encoding plasmid pE66An was extracted from an *Escherichia coli* strain isolated from a rectal swab taken from a patient admitted to the tetanus ward of the HTD in HCMC. Plasmid pE66An is 80 105 bp with an approximately neutral G+C content of 51.52 mol% (Fig. 2). After
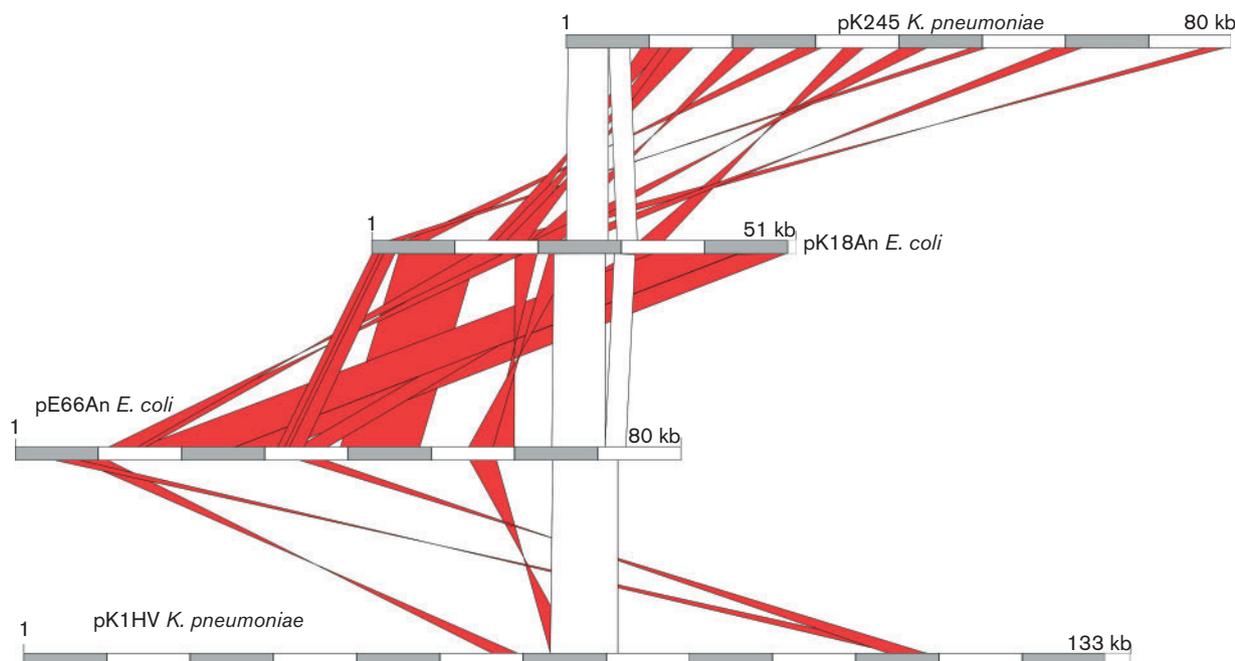
Fig. 1. Linear DNA sequence alignments of pE66An, pK18An and pK1HV against plasmid pK245 centred at the *qnrS1*-encoding region. Regions of DNA identity of 99 % or greater are linked by red blocks. The open box is the region containing the *qnrS1*-harbouring transposon, which is identical in all four plasmids except for a 980 bp insert in pK18An. The sizes of the plasmids are shown, with each shaded/open block representing 10 kbp.

complete annotation of the plasmid sequence, 109 predicted coding sequences (CDSs) were identified; the protein products of seven of these CDSs were predicted to be associated with resistance to a variety of antimicrobial classes. These antimicrobial resistance genes were: *aacC3* (gentamicin), *sulII* (sulfonamides), *tetR* and *tetA* (tetracyclines), *qnrS1* (quinolones), *bla*$_{LAP-2}$ (β-lactams) and *bla*$_{CTX-M-14}$ (third-generation cephalosporins). An association between the *qnrA* gene and ESBL-encoding genes has been reported previously (Castanheira *et al.*, 2007; Hamouda *et al.*, 2008; Lavigne *et al.*, 2006). The *bla*$_{CTX-M-14}$ gene within pE66An was adjacent to the element IS*Ecp1*. The? insertion element IS*Ecp1* has been shown previously to mediate the transfer of *bla*$_{CTX-M-14}$ (Bou *et al.*, 2002). As the *bla*$_{CTX-M-14}$ gene is in association with this IS*Ecp1* insertion element, the potential for dissemination of this gene to other plasmids or transposable elements is likely to be enhanced.

Comparative analysis showed that two regions within plasmid pE66An, a 16.7 and a 10.2 kb region, exhibited significant DNA sequence similarity to regions within the previously described *Klebsiella oxytoca* plasmid pKOX105 (Carattoli *et al.*, 2010) and three smaller regions (5.9, 5.2 and 3.5 kb) within plasmids pIP843 (Cao *et al.*, 2002), pRAx (Fricke *et al.*, 2009) and pKF3-94 (Zhao *et al.*, 2010) from HCMC, Madagascar and China, respectively (Fig. 2a). Plasmid pKOX105 was isolated from a *K. oxytoca* isolate present in the intestinal microbiota of an individual in a long-term care facility in Bolzano,

Italy, in 2005. Both pE66An and pKOX105 are IncN plasmids, with each containing the highly conserved IncN plasmid backbone (Carattoli *et al.*, 2010). Plasmid pE66An contains two regions of 10 280 and 5183 bp that encode sequences predicted to be responsible for the conjugal transfer. Indeed, our previous work has shown that pE66An has the capacity to be efficiently conjugated at high frequency into a suitable recipient strain. We therefore concluded that this conjugation system is functionally active (Vien *et al.*, 2009).

In addition to antimicrobial resistance and conjugal transfer functions, plasmid pE66An also encodes genes that suggest that it may be able to adapt to a variety of hosts and environments. For example, we identified a CDS with 99.5 % identity to *frmA*, a class III alcohol dehydrogenase identified in a *Pasteurella piscicida* isolate (Kim & Aoki, 1994), and 98.1 % identity to a class III alcohol dehydrogenase identified in an *Escherichia coli* isolate (Hochhut *et al.*, 2006). The function of the protein product encoded by *frmA* is involved in resistance to formaldehyde and other aldehydes. We predict that *frmA* provides a selective advantage for bacterial hosts in hospital environments where disinfectants containing aldehydes are used (Chen *et al.*, 2006).

Plasmid pK18An

Plasmid pK18An was carried by a *K. pneumoniae* isolate taken from a rectal swab of a tetanus patient in the HTD

Fig. 2. Annotated circular plasmid maps of pE66An, pK18An and pK1HV orientated from the origin of replication. (a) pE66An; (b) pK18An; (c) pK1HV. Identified and annotated ORFs are colour coded on each of the three plasmids: red, plasmid replication; dark blue, inorganic/metal/UV resistance; sky blue, conjugal transfer; dark pink, antimicrobial resistance; light green, unknown; light blue, regulators; orange, conserved hypothetical; brown, pseudogenes or partial genes; light pink, IS elements. The arrow annotation shows the strand on which the ORF is located. The inner circle shows the GC skew ([GC]/[G+C]) and the next outer circle shows G+C (mol%) plot. Fragments with substantial DNA homology to other sequenced plasmids and the *qnrS*-encoding region are highlighted.

in HCMC. The sequence of plasmid pK18An showed it was a circular replicon of 51 160 bp, with a G+C content of 51.32 mol% (Fig. 2). The annotation of pK18An identified 72 CDSs, the functions of six of which were predicted to be associated with resistance to antimicrobials, including *aacC3* (gentamicin), *sulII* (sulfonamides), *strA* and *strB* (streptomycins), *dhfrV* (trimethoprim) and *qnrS1* (quinolones). Plasmid pK18An was also found to harbour a *bla*$_{LAP-2}$ gene in close proximity to *qnrS1*, but that carried an IS5 element insertion and so was likely to have been inactivated. The *sulII–strA–strB* genes were located in close proximity to each other and adjacent to an IS26 element. The *strA–strB* genes are often linked with the *sulII* sulfonamide-resistance gene, commonly encoded on broad-host-range non-conjugative plasmids in a range of Gram-negative bacteria found in humans and animals. The usage of streptomycin in clinical and animal medicine has diminished dramatically over the last 10–20 years, yet the persistence of *sulII–strA–strB* implies that factors other than a direct selection pressure from the antimicrobial are important for the maintenance of these genes (Sundin & Bender, 1996).

Like pE66An, plasmid pK18An shared two large regions, of 10.2 and 9.4 kb, with extensive DNA homology to plasmid pKOX105 (Carattoli *et al.*, 2010) (Fig. 2b). Similarly, plasmid pK18An also contained two regions containing CDSs that are predicted to be responsible for the conjugal transfer, but these operons were disrupted by numerous IS26 elements. These sequence data probably explain why it was not possible to conjugate pK18An into a recipient *Escherichia coli* under laboratory conditions (Vien *et al.*, 2009). Whilst pK18An was not conjugative, the plasmid sequence was littered with multiple IS elements, particularly surrounding antimicrobial resistance genes, suggesting that such elements may facilitate the independent transfer of these genes to other plasmids.

Plasmids pK18An and pE66An both contained restriction modification systems; pK18An contained an *ecoRIIM* gene and pE66An contained the *ecoRIIR* and *ecoRIIM* genes. The *ecoRIIR* and *ecoRIIM* genes share 98.8 % nucleotide identity with the e*coRII* endonuclease gene (Bhagwat *et al.*, 1990), and have 100 % nucleotide identity with the *Escherichia coli* modification methylase gene, *ecoRII* (Som *et al.*, 1987). In addition to assisting with defence against bacteriophage infection, this restriction modification system has also been reported to contribute to the spread and maintenance of plasmids encoding these systems (Kobayashi, 2001).

Plasmid pK1HV

Plasmid pK1HV was isolated from a *K. pneumoniae* strain cultured from a healthy child, resident in HCMC. Plasmid pK1HV was the largest of the three sequenced plasmids at 133 191 bp with a G+C content of 52.5 mol% (Fig. 2). Plasmid pK1HV contained 167 predicted CDSs, of which the overwhelming majority were of unknown function. However, pK1HV was also found to carry 11 genes that are associated with resistance to various classes of

antimicrobials, including *aadA2* (streptomycin), *ble* (bleomycin), *aphA1* (gentamicin), *aac(3′)-IV* (gentamicin), *hph* (hygromycin), *sulII* (sulfonamides), *forR* (chloramphenicol), *tetR* and *tetA* (tetracyclines), *bla*$_{LAP-2}$ (β-lactams) and *qnrS1* (quinolones). Similar to pE66An, pK1HV also carried an IS26–*tetR*–*tetA* complex, which is a common mechanism facilitating the transfer of tetracycline resistance. Plasmid pK1HV also harboured a type 1 integron containing the *dfrA12–orfF–aadA2* cassette, an antimicrobial resistance region that remains common in contemporarily isolated MDR Gram-negative organisms (Gestal *et al.*, 2005). The presence this *dfrA12–orf–aadA2*-containing type 1 integron in pK1HV (isolated from the community) again poses questions regarding the ongoing selection of genes encoding resistance to streptomycin.

Comparative sequence analysis of pK1HV with pKOX105 revealed a large contiguous fragment of ~84 kb shared between both plasmids (Carattoli *et al.*, 2010) (Fig. 2c). All of the predicted genes on this extended fragment of plasmid DNA were mostly conserved but functionally unknown, or were genes proposed to encode components required for conjugal transfer. The remaining regions of pK1HV carried the identified antimicrobial resistance genes and an array of IS elements (Fig. 2c). Whilst pK1HV did not contain the *mucAB* operon, like pE66An and pK18An, it did contain the *imp* operon, which had 85 % nucleotide identity to *impA* and *impB* on the IncI1 plasmid TP110 from a *Salmonella* Typhimurium strain isolated in the UK in 1968 (Lodwick *et al.*, 1990).

Characterization of *qnrS1*-containing transposons

Annotation of the three sequenced *qnrS1*-containing transposons extracted from the plasmid sequences of pE66An, pK18An and pK1HV and their alignments with other described *qnrS1*-containing fragments are shown in Fig. 3. The alignment of *qnrS1*-containing fragments from pE66An, pK18An and pK1HV showed that they were identical, except for pK18An, which contained a 980 bp insertion. The *qnrS1* gene in all of the three plasmids was located within a transposon structure composed of two identical IS26 elements at either terminal portion of the transposon. In addition to containing the *qnrS1* gene, these transposons also carried the *bla*$_{LAP-2}$ gene, which confers resistance to narrow-spectrum β-lactams. Additionally, this transposon shared a common backbone with other available *qnrS* sequences and contained an IS2 element, a putative resolvase (*ydaA*) predicted to belong to a family of stress proteins (Beliaev *et al.*, 2002), and three other proteins of unknown function (Park *et al.*, 2009; Wu *et al.*, 2008) (Fig. 3). The G+C content of the *qnrS*-containing transposons was ~50 mol%, which was slightly lower than the mean G+C content of the sequenced plasmids in their entirety, consistent with the notion that the *qnrS1*-encoding transposons have been inserted into these plasmids via horizontal gene transfer.
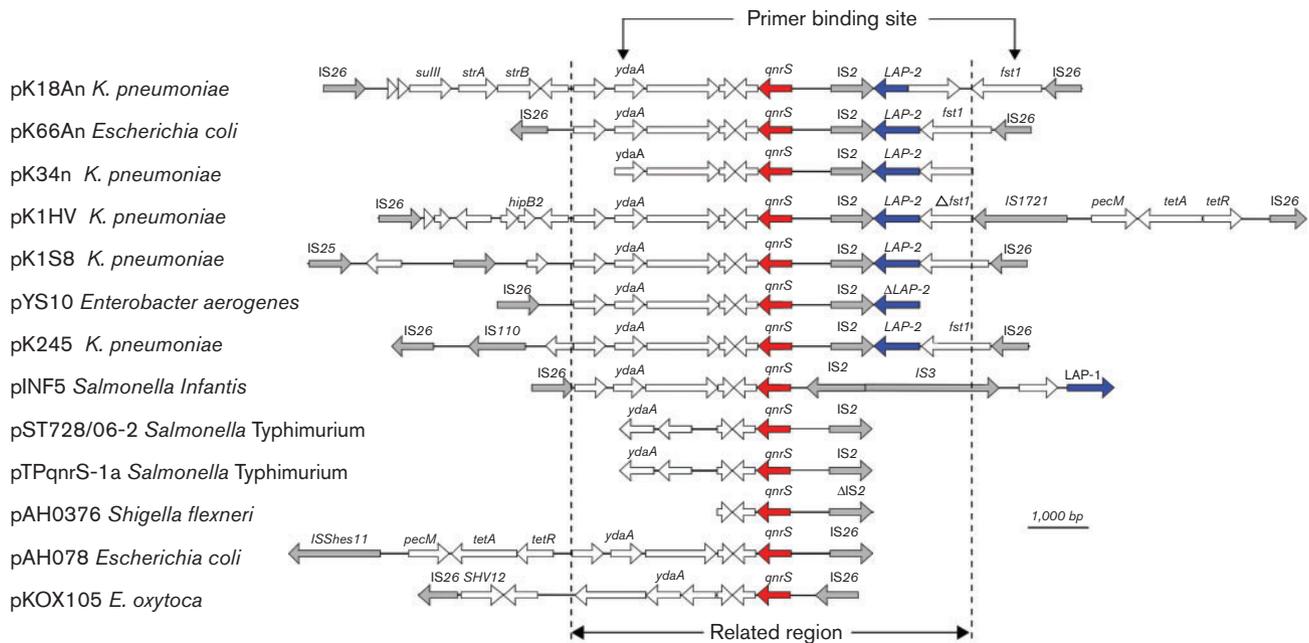
Fig. 3. A schematic representation of sequenced *qnrS1*-containing transposons. Graphical representation of the synteny between the *qnrS*-containing transposons between the three plasmids sequenced here (pK18An, pE66An and pK1HV) and other sequenced fragments containing the *qnrS*-encoding region. The plasmids and the host organism in which they were first identified are given. The region with the greatest DNA homology is identified and includes the highlighted ORFs for *qnrS* (red), a putative IS2 element (grey), a gene encoding a putative resolvase protein (*ydaA*) and three other ORFs encoding hypothetical proteins of unknown function. Additional genes are colour coded: blue, bla_{LAP-2}; grey, IS elements; white, ORFs without a name encoding hypothetical uncharacterized proteins. The locations of the binding sites for PCR amplification of the transposon are highlighted.

Screening for *qnrSI* transposons in commensal *Enterobacteriaceae* isolated in HCMC

Using the newly generated DNA sequences of the *qnrS1*-containing transposons from the three sequenced plasmids (Fig. 3), we designed PCR primers to amplify and compare *qnrS1*-containing transposons from DNA extracted from 112 *qnrS1*-positive hospital- and community-acquired *Enterobacteriaceae* that have been described previously (Vien *et al.*, 2011, 2012; see Methods). The locations of the primer-binding sites are shown in Fig. 3 and the predicted sizes of PCR amplicons were 6859 bp (pE66An *qnrS1* transposon, type A) and 8059 bp (pK18An *qnrS1* transposon, type B). Seventy-one of the 112 isolates (63.4 %) were PCR amplicon-positive for the *qnrS1*-containing transposon and were of sizes consistent with those described in pE66An and pK18An. The 71 PCR amplicons with known *qnrS1*-containing transposons were subjected to RFLP analysis with *Eco*RV, *Hin*dIII and *Pvu*II. In addition to the two described *qnrS1*-containing transposons that were identified in the three sequenced plasmids, the RFLP mapping patterns from these 71 amplicons also revealed a transposon with a third structure (K34N strain, type C). Using a primer-walking sequencing method, we found that the *qnrS1*-containing transposon

in K34N strain was 6652 bp and identical to the *qnrS1*-containing transposon from E66An strain, except for a 200 bp deletion in a gene of unknown function (Fig. 3). We were therefore able to distinguish three related yet distinct *qnrS1*-containing transposons of 6859, 8059 and 6652 bp, which we arbitrarily named types A, B and C, respectively.

The 41 isolates with an undetermined *qnrS1*-containing mobile element were further investigated for the presence of a subfamily of the known transposon. There have been multiple reports regarding the association between *qnrS1* and bla_{LAP-2} genes (Cano *et al.*, 2009; Dahmen *et al.*, 2010; Huang *et al.*, 2008; Park *et al.*, 2009; Poirel *et al.*, 2006, 2007). Yet, due to a lack of a PCR amplicon, we hypothesized that some isolates contain both the *qnrS1* and the bla_{LAP-2} sequence, but the adjacent regions demonstrate a different structure. A laboratory *Escherichia coli* strain was successfully transformed with a *qnrS1*-encoding plasmid extracted from 27 of the 41 isolates with an undefined *qnrS1*-containing transposon structure. Plasmid DNA from these 27 transformants was extracted and digested with *Eco*RI and individually probed by Southern blotting targeting the *qnrS1* and bla_{LAP-2} genes. With the resulting plasmid hybridization of the plasmid DNA extracted from the 27 isolates, only two
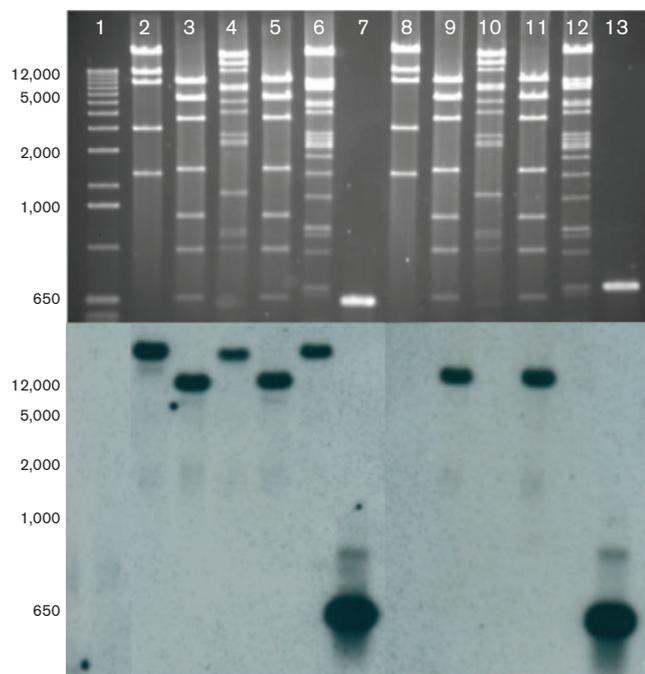
Fig. 4. EcoRI digestion of qnrS-encoding plasmids hybridized with the qnrS1 and bla$_{LAP-2}$ probes. Upper panel: agarose gel electrophoresis of qnrS-encoding plasmids after digestion with EcoRI. The resulting patterns are duplicate digestions from five plasmids after conjugation. Lanes: 2 and 8, isolate LTMV18; 3 and 9, isolate LTMV33; 4 and 10, isolate LTMV6; 5 and 11, isolate LTMV30; 6 and 12, isolate LTMV1. The ladder (lane 1) is 1 kb Plus (Invitrogen) with sizes shown in kb. Lanes 7 and 13 contain the PCR amplicons of qnrS and bla$_{LAP-2}$ as positive controls. Bottom panel (left): hybridization against the qnrS1 probe after Southern blotting. All five plasmids contained fragments corresponding to probe qnrS1. Bottom panel (right): hybridization against the bla$_{LAP-2}$ probe after Southern blotting.

produced a detectable signal with both qnrS1 and bla$_{LAP-2}$ probes on the same digestion fragment, implying that these two plasmids (and their corresponding hosts) also carry a qnrS1-containing transposon of the same subfamily as described in the sequenced plasmids (Fig. 4).

We stratified the proportion of the three qnrS1-containing transposons across all the hospital- and community-acquired isolates that were compared (Table 2). Forty-five of the 63 hospital isolates (71.4 %) harboured a qnrS1-containing transposon that was identical to pE66An (type A), one isolate (1.6 %) carried a qnrS1-containing transposon identical to pK18An (type B) and three isolates (4.8 %) carried a qnrS1-containing transposon identical to K34N (type C). Similarly, in the 49 community isolates, there were 18 (36.7 %), one (2 %) and three (6.1 %) isolates carrying type A, B and C qnrS1-containing transposons, respectively. However, 14 isolates (22.2 %) from the hospital and 27 isolates (55.1 %) from the community qnrS1 gene-positive isolates were negative by PCR targeting the qnrS1-containing transposon, suggesting that the qnrS1 gene in these strains is embedded on a different genetic element, which was undeterminable by the described PCR amplification methods used here.

CONCLUSIONS

Hospital-acquired infections with antimicrobial-resistant organisms can be dangerous, and we have recently shown the problems that can be associated with highly virulent clones of K. pneumoniae (Chung The et al., 2015). Here, we have shown that there is a dominant qnrS1-containing transposon circulating in qnrS1-positive Enterobacteriaceae in HCMC, Vietnam, which we found to be present in 71.4 % of qnrS1-positive isolates from a hospital and in 36.7 % of qnrS1-positive isolates from the community. Moreover, we determined the complete nucleotide sequences of three qnrS1-encoding plasmids. These sequences permitted a description of the circulating genes contributing to an MDR phenotype in three bacterial isolates from a hospital and the community, and also provided insights into the means of adaptation of these plasmids within a variety of hosts and environments. Notably, the DNA sequences of the two plasmids isolated from two different bacterial genes in a hospital setting exhibited substantial homology, thus presenting evidence of genetic transfer among nosocomial commensal bacteria. Finally, the annotation of plasmid pE66An provides further evidence for the association of the PMQR gene qnrS and the ESBL gene bla$_{CTX-M-14}$.

Table 2. Distribution of the various qnrS1-containing transposons in 122 Enterobacteriaceae isolated in HCMC

Strain	Type A	Type B	Type C	Subtype	Unknown
Hospital strains (n=63)					
Escherichia coli (n=5)	3 (60 %)	0	0	0	2 (40 %)
K. pneumoniae (n=52)	40 (77 %)	1 (2 %)	3 (5.7 %)	0	8 (15.3 %)
Others (n=6)	2 (33.3 %)	0	0	0	4 (66.7 %)
Community strains (n=49)					
Escherichia coli (n=32)	13 (40.6 %)	0	0	1 (3.1 %)	18 (56.3 %)
K. pneumoniae (n=15)	5 (33.3 %)	1 (6.7 %)	3 (20 %)	1 (6.7 %)	5 (33.3 %)
Others (n=2)	0	0	0	0	2 (100 %)

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