

Complete nucleotide sequence of pVQS1 containing a quinolone resistance determinant from *Salmonella enterica* serovar Virchow associated with foreign travel

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Objectives: Nalidixic acid-resistant *Salmonella enterica* serovars Kentucky ($n=5$) and Virchow ($n=6$) cultured from individuals were investigated for the presence of plasmid-mediated quinolone resistance (PMQR) determinants.

Methods: PMQR markers and mutations within the quinolone resistance-determining regions of the target genes were investigated by PCR followed by DNA sequencing. Conjugation, plasmid profiling and targeted PCR were performed to demonstrate the transferability of the *qnrS1* gene. Subsequently, a plasmid was identified that carried a quinolone resistance marker and this was completely sequenced.

Results: A *Salmonella* Virchow isolate carried a *qnrS1* gene associated with an IncN incompatibility group conjugative plasmid of 40995 bp, which was designated pVQS1. The latter conferred resistance to ampicillin and nalidixic acid and showed sequence similarity in its core region to plasmid R46, whilst the resistance-encoding region was similar to pAH0376 from *Shigella flexneri* and pINF5 from *Salmonella* Infantis and contained an IS26 remnant, a complete Tn3 structure, a truncated IS2 element and a *qnrS1* marker, followed by IS26. In contrast to pINF5, IS26 was identified immediately downstream of the *qnrS1* gene.

Conclusions: This is the first known report of a *qnrS1* gene in *Salmonella* spp. in Switzerland. Analysis of the complete nucleotide sequence of the *qnrS1*-containing plasmid showed a novel arrangement of this antibiotic resistance-encoding region.

Keywords: *Salmonella* spp., *qnrS*, plasmids

Introduction

Salmonellosis is a major foodborne disease worldwide. While the fluoroquinolone ciprofloxacin is the recommended antimicrobial for the treatment of severe human cases of infection, the occurrence of bacterial resistance to nalidixic acid coupled with reduced susceptibility to fluoroquinolones has been increasing among salmonellae.¹ In the years 2004–09 in Switzerland, *Salmonella* Virchow ranked between the top four to eight serovars and the percentage of nalidixic acid resistance among these reached 62%.²

Resistance to quinolones and fluoroquinolones in enteric bacteria develops mainly through target gene mutations in the type II (DNA gyrase) and type IV DNA topoisomerases. However, a number of plasmid-mediated quinolone resistance (PMQR) markers have been reported. The discovery of the first such

marker, *qnrA*, was reported in a *Klebsiella pneumoniae* in 1994,³ followed by the description of *qnrB*,⁴ *qnrS*,⁵ *qnrC*⁶ and *qnrD*.⁷ Worldwide dissemination of these markers has been reported in recent years in Enterobacteriaceae. According to a recent European study, including 13 countries, *qnrS1* and the more diverse *qnrB* were the most frequent PMQR markers identified in *Salmonella* spp.⁸

In this study, the complete nucleotide sequence of a plasmid which was responsible for transferable ampicillin and quinolone resistance was determined and analysed.

Materials and methods

MICs of nalidixic acid and ciprofloxacin were determined using Etest strips (AB Biodisk, Solna, Sweden). Purification of genomic DNA, amplification of the quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*,

parC and *parE* genes and PCR-based detection of the PMQR markers [*qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr* and *qepA*] were conducted as described previously.⁹ Published PCR primers were used for the identification of *qnrC*⁶ and *qnrD*.⁷

Conjugation experiments were conducted with a rifampicin-resistant *Escherichia coli* 26R793 at 37°C in liquid broth overnight. Mueller–Hinton agar (Oxoid, Basingstoke, UK) plates supplemented with 100 mg/L rifampicin (Sigma, Dublin, Ireland) and 0.4 mg/L ciprofloxacin (Sigma) were used for the selection of the transconjugants. Plasmid profiling of the donor and transconjugants was carried out using the Wizard® Plus SV DNA Purification System (Promega, Madison, WI, USA) following separation in a 0.7% agarose gel (SeaKem® LE Agarose, Lonza Wokingham Ltd, UK) stained with GelRed™ (Biotium, Hayward, CA, USA).

The complete nucleotide sequence of the plasmid was determined using shotgun cloning and Next-Generation Sequencing (Eurofins MWG Operon, Ebersberg, Germany).

Results and discussion

Transferability of the *qnrS* determinant

Isolates of *Salmonella* serovars Kentucky ($n=5$) and Virchow ($n=6$) cultured from individuals from 2005 to 2009 demonstrating resistance to nalidixic acid were received from the Swiss National Centre for Enteropathogenic Bacteria (NENT). The isolates were selected for this study on the basis of their quinolone resistance profiles and represented different PFGE clusters (data not shown). A *qnrS1* determinant was identified in a single *Salmonella* Virchow isolate (number 07N0368) cultured from a patient with a history of recent travel to Thailand. Liquid broth mating assays with the latter *qnrS1*-positive *Salmonella* Virchow isolate as the donor resulted in high-frequency transfer, measured at 3×10^{-2} transconjugants per donor cell. *In vitro* susceptibility testing of the transconjugants revealed that a plasmid of 40995 bp mediated resistance to ampicillin and nalidixic acid and decreased susceptibility to ciprofloxacin. Upon acquisition of the plasmid by the *E. coli* recipient, the MIC of nalidixic acid increased from 2 to 32 mg/L and the MIC of ciprofloxacin increased from 0.064 to 0.75 mg/L. The presence of the *qnrS1* marker was confirmed by PCR in the presumptive transconjugants. The isolate possessed a mutation within the *gyrA* sequence resulting in Asp-87 → Tyr substitution. No mutations were present in the QRDRs of *gyrB*, *parC* and *parE*.

Analysis of the *qnrS1*-harbouring plasmid pVQS1

The sequence of the plasmid was deposited in GenBank under accession number JQ609357. This plasmid contained 49 annotated open reading frames (ORFs), with an overall GC content calculated at 50.01%. The largest region, involved in replication, maintenance and conjugative transfer, demonstrated 99% sequence similarity to plasmids of the IncN type, including R46 (accession number AY046276.1) and pMUR050 (accession number AY522431.4).

Figure 1 shows the structure of the resistance gene region of pVQS1, comparing it with R46 and several partially sequenced plasmids containing the *qnrS1* determinant. The most significant difference observed between pVQS1 and R46 was the presence of the ampicillin and quinolone resistance gene region, consisting

of (i) a remnant of an IS26 element, (ii) a complete Tn3 sequence containing *bla*_{TEM-1} flanked on the right side by an IS2-homologous sequence, and (iii) the *qnrS1* gene with a complete IS26 element downstream. The latter sequence arrangement (located between positions 31386 and 39805) showed 99% identity to those sequences of the pINF5 from *Salmonella* Infantis (GenBank accession number AM234722.1) cultured from a chicken sample in Germany.¹⁰ A short DNA region (located between positions 31386 and 32298) proximal to Tn3 corresponded to a similar sequence in pINF5 (between positions 1 and 914 bp), demonstrating 99% nucleotide sequence identity with pINF5 and 100% identity with a region found in R46 (nucleotides 777–1689). A 44 bp remnant of IS26 was identified within this region (positions 32255–32298) and was identical to one found on pINF5 (positions 871–914). The IS26 remnant was followed by the complete Tn3-like transposon. The region containing Tn3, IS2 and *qnrS1* (positions 32299–39805) showed 99% similarity to pAH0376 from *Shigella flexneri*, the carrier of the original *qnrS1* marker (accession number AB187515.1).⁵ It was interesting to note that, although Tn3 was not found on R46, it does nonetheless contain an ORF (positions 50134–50790) sharing 82% nucleotide sequence identity with the Tn3-encoded resolvase.

The Tn3-like transposon (positions 32299–37248) exhibited 100% DNA sequence identity to that found in several plasmids in *E. coli* (accession number DQ390455.1) and showed 99% similarity to the Tn3 present on the pAH0376 and pINF5. The Tn3 element of pVQS1 consisted of 38 bp inverted terminal repeats, a *bla*_{TEM-1} gene encoding a 287 amino acid protein, a *tnpR* resolvase gene of 186 amino acids and a *tnpA* gene encoding an 850 amino acid transposase. The Tn3-like sequence was followed by a relic of an IS2 element (positions 37349–38364), consisting of a defective resolvase gene and a transposase gene containing a premature stop codon that was indistinguishable from the one found adjacent to Tn3 on pAH0376. The *qnrS1* gene (nt 38668–39324) located downstream of the truncated IS2 was 100% identical at the nucleotide sequence level to the *qnrS1* gene found on both pINF5 and pAH0376.

A small region of similarity common to pVQS1 and pINF5 included 318 nt located distal to the *qnrS1* gene and this region was followed by a partial sequence corresponding to the final 163 nt of a truncated ORF (denoted as Δ *res*) in pINF5 (nt 8259–8421). Interestingly, pVQS1 lacked the CS12 fimbrial gene cluster derived from *E. coli*, consisting of four genes located downstream of a truncated resolvase gene (Δ *res*) within pINF5, while a classic IS26 element was identified in the DNA sequence flanking the *qnrS1* gene on the distal end (positions 39803–40622). The ORF identified therein encoded a transposase of 234 amino acids and was flanked by perfect 14 bp terminal inverted repeats. Two identical IS26 elements were located in R46 (positions 1–820 and 40612–41431). In contrast, the corresponding IS26-like element found in pINF5 and downstream of the CS12-like fimbrial genes was disrupted by the insertion of the antibiotic resistance region and showed differences compared with the classic IS26 element.

The IS26 element of pVQS1 was followed by a defective resolvase gene (Δ *resP*). This partial gene (positions 40623–40995) was identical at the nucleotide sequence level to part of the *resP* gene of plasmid R46. The latter was also located adjacent to IS26 on pINF5 (positions 12320–13389). Further comparison

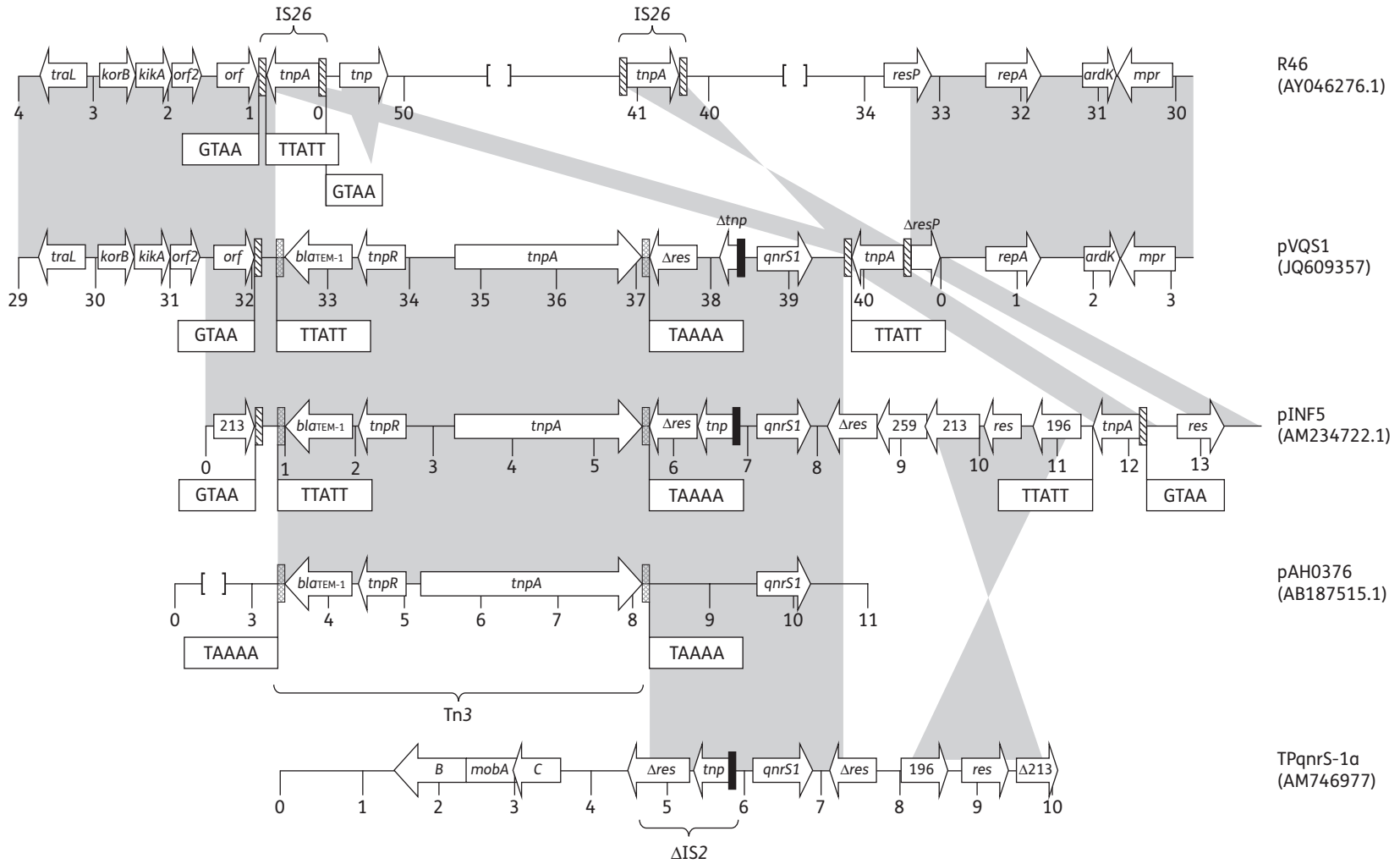


Figure 1. Genetic organization of the antimicrobial resistance region of pVQS1 in comparison with R46, pINF5, pAH0376 and TPqnrS-1a. Arrowheads represent ORFs and the direction of transcription. The hatched, light-grey cross-hatched and black boxes indicate terminal inverted repeats of the IS26-like elements, Tn3 and the IS2 remnant element, respectively. Homologous regions are indicated with grey shading. Sequences of 5 bp shown in boxes indicate duplicated nucleotides of the target site characteristic of Tn3-mediated transposition. Numbers below each sequence represent a distance scale expressed in kb.

of these plasmids was not possible due to the lack of available sequence.

Concluding remarks

Several features of pVQS1 suggested that this plasmid emerged along an independent evolutionary path, possibly involving recombination events between an R46-like progenitor and one harbouring Tn3, an IS2 relic and the *qnrS1*-containing region. In support of this hypothesis, the sequence preceding the Tn3-like transposon, including a hypothetical ORF and an IS26 remnant, was indistinguishable from the corresponding sequence in R46, while showing several differences when compared with pINF5. The Tn3 transposon itself showed differences in comparison with pINF5, while being indistinguishable from the Tn3 associated with other plasmids found in Enterobacteriaceae. Another feature supporting our hypothesis was based on the identification of the deletion of the fimbrial gene cluster. A 5 bp target sequence duplication, TTATT, was identified at the left boundary of the Tn3 whilst the second copy of the corresponding TTATT was identified in the left terminal repeat of the IS26 element, suggesting that the acquisition of the Tn3–*qnrS1* region occurred via multiple steps. The presence of the direct repeat sequence TAAAA flanking the Tn3 on the right side, identical to those flanking Tn3 in pAH0376, supports this view. An 8 bp duplication, typically generated upon IS26 integration, was not identified. The remnant of IS26 was preceded by a 4 bp duplication (GTAA) previously identified in pINF5; however, no target sequence duplication was present adjacent to the complete IS26 element.

In conclusion, this study described the first known report of a *qnrS1* marker identified in *Salmonella* spp. in Switzerland. The gene was located to a region containing a complete Tn3 transposon, an IS2 relic and IS26, and all were associated with a highly mobile IncN plasmid. Although several studies have described the linkage of Tn3 and *qnrS1*, a novel gene arrangement was identified in pVQS1.

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Transparency declarations

None to declare.

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