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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 40 995 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 IS*26* remnant, a complete Tn*3* structure, a truncated IS*2* element and a *qnrS1* marker, followed by IS*26*. In contrast to pINF5, IS*26* 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 40 995 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 IS*26* element, (ii) a complete Tn*3* sequence containing bla_{TEM-1} flanked on the right side by an IS*2*-homologous sequence, and (iii) the *qnrS1* gene with a complete IS*26* element downstream. The latter sequence arrangement (located between positions 31 386 and 39 805) 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 31 386 and 32 298) proximal to Tn*3* 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 IS*26* was identified within this region (positions 32 255–32 298) and was identical to one found on pINF5 (positions 871–914). The IS*26* remnant was followed by the complete Tn*3*-like transposon. The region containing Tn*3*, IS*2* and *qnrS1* (positions 32 299–39 805) 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 Tn*3* was not found on R46, it does nonetheless contain an ORF (positions 50 134–50 790) sharing 82% nucleotide sequence identity with the Tn*3*-encoded resolvase.

The Tn*3*-like transposon (positions 32 299–37 248) exhibited 100% DNA sequence identity to that found in several plasmids in *E. coli* (accession number DQ390455.1) and showed 99% similarity to the Tn*3* present on the pAH0376 and pINF5. The Tn*3* 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 Tn*3*-like sequence was followed by a relic of an IS*2* element (positions 37 349–38 364), consisting of a defective resolvase gene and a transposase gene containing a premature stop codon that was indistinguishable from the one found adjacent to Tn*3* on pAH0376. The *qnrS1* gene (nt 38 668–39 324) located downstream of the truncated IS*2* 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 IS*26* element was identified in the DNA sequence flanking the *qnrS1* gene on the distal end (positions 39 803–40 622). The ORF identified therein encoded a transposase of 234 amino acids and was flanked by perfect 14 bp terminal inverted repeats. Two identical IS*26* elements were located in R46 (positions 1–820 and 40 612–41 431). In contrast, the corresponding IS*26*-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 IS*26* element.

The IS*26* element of pVQS1 was followed by a defective resolvase gene (Δ*resP*). This partial gene (positions 40 623–40 995) was identical at the nucleotide sequence level to part of the *resP* gene of plasmid R46. The latter was also located adjacent to IS*26* on pINF5 (positions 12 320–13 389). Further comparison

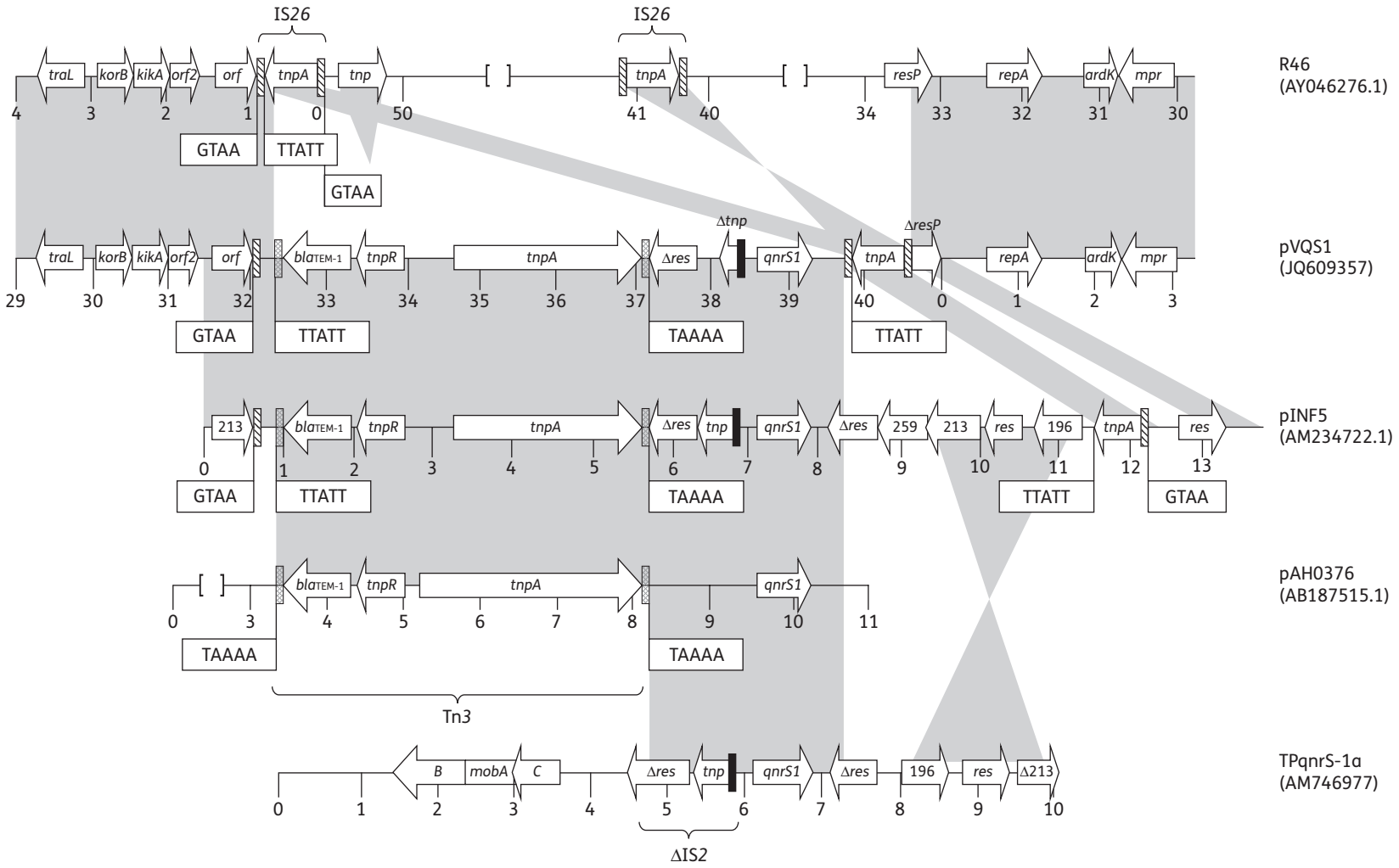

Figure 1. Genetic organization of the antimicrobial resistance region of pVQS1 in comparison with R46, pINF5, pAH0376 and TPqnrS-1a. Arrowheads represent ORFs and the direction of transcription. The hatched, light-grey cross-hatched and black boxes indicate terminal inverted repeats of the IS*26*-like elements, Tn*3* and the IS*2* 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 Tn*3*-mediated transposition. Numbers below each sequence represent a distance scale expressed in kb.

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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 Tn*3*, an IS*2* relic and the *qnrS1*-containing region. In support of this hypothesis, the sequence preceding the Tn*3*-like transposon, including a hypothetical ORF and an IS*26* remnant, was indistinguishable from the corresponding sequence in R46, while showing several differences when compared with pINF5. The Tn*3* transposon itself showed differences in comparison with pINF5, while being indistinguishable from the Tn*3* 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 Tn*3* whilst the second copy of the corresponding TTATT was identified in the left terminal repeat of the IS*26* element, suggesting that the acquisition of the Tn*3*–*qnrS1* region occurred via multiple steps. The presence of the direct repeat sequence TAAAA flanking the Tn*3* on the right side, identical to those flanking Tn*3* in pAH0376, supports this view. An 8 bp duplication, typically generated upon IS*26* integration, was not identified. The remnant of IS*26* was preceded by a 4 bp duplication (GTAA) previously identified in pINF5; however, no target sequence duplication was present adjacent to the complete IS*26* 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 Tn*3* transposon, an IS*2* relic and IS*26*, and all were associated with a highly mobile IncN plasmid. Although several studies have described the linkage of Tn*3* and *qnrS1*, a novel gene arrangement was identified in pVQS1.

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Transparency declarations

None to declare.

References

1 Lee HY, Su LH, Tsai MH *et al*. High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoid *Salmonella* clinical isolates in Asia. *Antimicrob Agents Chemother* 2009; **53**: 2696–9.

2 Bonalli M, Stephan R, Kappeli U *et al*. *Salmonella enterica* serotype Virchow associated with human infections in Switzerland: 2004–2009. *BMC Infect Dis* 2011; **11**: 49.

3 Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**: 797–9.

4 Jacoby GA, Chow N, Waites KB. Prevalence of plasmid-mediated quinolone resistance. *Antimicrob Agents Chemother* 2003; **47**: 559–62.

5 Hata M, Suzuki M, Matsumoto M *et al*. Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. *Antimicrob Agents Chemother* 2005; **49**: 801–3.

6 Wang M, Guo Q, Xu X *et al*. New plasmid-mediated quinolone resistance gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob Agents Chemother* 2009; **53**: 1892–7.

7 Cavaco LM, Hasman H, Xia S *et al*. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother* 2009; **53**: 603–8.

8 Veldman K, Cavaco LM, Mevius D *et al*. International collaborative study on the occurrence of plasmid-mediated quinolone resistance in *Salmonella enterica* and *Escherichia coli* isolated from animals, humans, food and the environment in 13 European countries. *J Antimicrob Chemother* 2011; **66**: 1278–86.

9 Karczmarczyk M, Martins M, McCusker M *et al*. Characterization of antimicrobial resistance in *Salmonella enterica* food and animal isolates from Colombia: identification of a *qnrB19*-mediated quinolone resistance marker in two novel serovars. *FEMS Microbiol Lett* 2010; **313**: 10–9.

10 Kehrenberg C, Friederichs S, de Jong A *et al*. Identification of the plasmid-borne quinolone resistance gene *qnrS* in *Salmonella enterica* serovar Infantis. *J Antimicrob Chemother* 2006; **58**: 18–22.