Plasmid-mediated quinoline resistance conferred by qnrS1 in Salmonella enterica serovar Virchow isolated from Turkish food of avian origin

M. D. Avsaroglu1, R. Helmuth2, E. Junker2, S. Hertwig2, A. Schroeter2, M. Akcelik3, F. Bozoglu1 and B. Guerra2*

1Middle East Technical University, Ankara, Turkey; 2Federal Institute for Risk Assessment (BfR), Berlin, Germany; 3Ankara University, Ankara, Turkey

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Objectives: To study the molecular characteristics of the quinolone and associated ampicillin resistance mechanisms present in Salmonella enterica serovar Virchow isolated from Turkish foods.

Methods: Nine epidemiologically unrelated Salmonella Virchow strains isolated from foods (chicken and minced meat) sold in different markets in Ankara were analysed for their susceptibility to 17 antimicrobials. The strains were typed by PFGE and plasmid profiling and investigated by molecular methods (PCR/sequencing) for the presence of several resistance genes, class 1 integrons and mutations in the quinolone resistance-determining regions. Plasmids conferring quinolone resistance were analysed by restriction fragment length polymorphism (RFLP) analysis, DNA hybridization, sequencing, replicon-typing PCR and mating experiments.

Results: All strains showed nalidixic acid resistance (MIC ≥ 128 mg/L) together with a decreased susceptibility to ciprofloxacin (three strains with an MIC of 1 mg/L and six with an MIC of 0.25 mg/L), associated with mutations within the gyrA gene (Asp-87 → Tyr-87). In three strains, qnrS1 genes were detected. Ampicillin resistance encoded by a blaCTX-M3 gene and/or blaTEM-1-like gene was found in four strains. Three of these strains carried an ~45 kb conjugative plasmid, designated pRQ2006, harbouring qnrS1 and a Tn3-like transposon. Partial sequencing and RFLP of pRQ2006 indicated its similarity to the qnrS1 plasmid pAH0376 found in a Japanese Shigella flexneri 2b isolate.

Conclusions: This is the first study describing the presence of qnrS1 genes in bacterial isolates from Turkey. The pRQ2006 plasmid seems to be more related to the S. flexneri 2b qnrS1 plasmid pAH0376 than to the Salmonella qnrS1-carrying plasmids pINF5 and TPqnrS-2.

Keywords: qnr, fluoroquinolones, antimicrobial resistance, PFGE, resistance plasmids

Introduction

Fluoroquinolones and β-lactams are among the most widely used antimicrobials categorized as drugs of ‘critical importance’ by the World Health Organization. Quinolones were introduced as antimicrobials in the 1960s and fluoroquinolones in the 1980s, and since then, bacterial resistance to these drugs has dramatically increased. Quinolone resistance is normally accompanied by a reduced susceptibility or resistance to fluoroquinolones and is mainly ascribed to mutations in the target genes gyrA (DNA gyrase) and parC (topoisomerase IV) and/or to active efflux. The combination of several mechanisms leads to high-level resistance. In most cases, the qnr genes were linked to genes encoding extended-spectrum or plasmid-mediated AmpC-type β-lactamases. In order to determine the prevalence of these genes, several studies on isolates of different species (i.e. Klebsiella, Enterobacter, Citrobacter, Escherichia coli and Salmonella), sources (human, animal or food) and countries (USA, India, Vietnam, France, UK, Germany, Turkey and so on) have been performed. In Salmonella, qnrA, B and S genes were detected with varying prevalence. The aim of the present study was the molecular characterization of the quinolone and

Plasmid-encoded quinolone/fluoroquinolone resistance conferred by qnr genes (qnrA, qnrB1–8, qnrS1–2) has been described as well. In most cases, the qnr genes were linked to genes encoding extended-spectrum or plasmid-mediated AmpC-type β-lactamases. In order to determine the prevalence of these genes, several studies on isolates of different species (i.e. Klebsiella, Enterobacter, Citrobacter, Escherichia coli and Salmonella), sources (human, animal or food) and countries (USA, India, Vietnam, France, UK, Germany, Turkey and so on) have been performed. In Salmonella, qnrA, B and S genes were detected with varying prevalence. The aim of the present study was the molecular characterization of the quinolone and

*Corresponding author. Tel: +49-30-8412-2057; Fax: +49-30-8412-2000; E-mail: beatriz.guerra@bfr.bund.de

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qnr genes in Salmonella

associated ampicillin resistance mechanisms present in Salmonella enterica serotype Virchow isolated from Turkish foods.

Materials and methods

One hundred S. enterica isolates were obtained from foods bought in several markets in Ankara, Turkey, during 2005–06. Salmonella Virchow (15 isolates) predominated and 9 of these isolates (7 isolates from chicken and 2 from minced meat) were considered as epidemiologically unrelated (different isolation date or place, see Table 1) and selected for further studies. The strains were phage-typed following the scheme used at the Health Protection Agency (HPA), London, UK and tested for their susceptibility to 17 antimicrobials by broth microdilution (CLSI), as described previously.7

PFGE with XhoI was carried out following the PulseNet protocol (www.pulsenet-europe.org). Patterns were defined considering bands ≥ 50 kb. Plasmid profiling was performed by the method described by Kado and Liu.8

The detection of antimicrobial resistance genes, class 1 integrons and mutations in the quinolone resistance-determining regions (QRDRs) was performed by PCR amplification/sequencing, as described previously.7

Plasmid characterization was carried out by cloning, primer walking and/or amplification using primers deduced from published sequences. Determination of the incompatibility group, restriction fragment length polymorphism (RFLP) analysis using several endonucleases and Southern hybridization were carried out as described previously.7,9 Conjugation experiments using an E. coli J53 rifampicin-resistant strain as recipient were performed at 22°C and 37°C on filters and in liquid broth. To avoid the selection of J53 gyrA mutants, selection was made on Eosin Methylene Blue (EMB) agar plates with ampicillin and rifampicin (200 and 100 mg/L, respectively).

For information about the primers used and their references, please see Table S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

Results and discussion

Phenotypical and molecular characterization of the strains

The nine Salmonella Virchow isolates were phage type PT26. All strains showed nalidixic acid resistance (MIC ≥ 128 mg/L) together with decreased susceptibility to ciprofloxacin (three with an MIC of 1 mg/L and six with an MIC of 0.25 mg/L). Four strains also showed ampicillin resistance. Only one of these isolates showed a multiresistant phenotype (ampicillin–cefotiofur–nalidixic acid–streptomycin–sulfamethoxazole–trimethoprim–sulfamethoxazole/trimethoprim).

PFGE with XbaI (Figure 1) defined three similar PFGE patterns (X1 and X2, shown by four strains, and X3 by one strain). By plasmid profiling, four patterns were defined: PP0, no plasmid (two strains); PP1, one ~45 kb plasmid (three strains); PP2, one ~45 kb plasmid and three small plasmids (~<5 kb, one strain) and PP3, three small (~<10 kb) cryptic plasmids (three strains) (Figure 1).

Molecular characterization of quinolone and ampicillin resistance determinants

The isolates were tested for the presence of mutations in the QRDR of both gyrA and parC genes. All strains exhibited mutations in the gyrA gene resulting in the amino acid substitution Asp-87→Tyr-87. They were further screened for the presence of the plasmid-encoded quinolone resistance genes qnrA, qnrB and qnrS. Three strains that showed resistance to ampicillin and nalidixic acid (ciprofloxacin MIC = 1 mg/L) gave PCR products of 417 bp with the qnrS primers.4 Sequences of these products were identical to qnrS (GenBank accession no. AB187515). Further PCR amplifications/sequencing of the flanking regions of one positive isolate (DMC9) using the Pre-qnrS primers9 and primers designed for the present work confirmed the presence of the qnrS1 gene (656 bp) found in different Enterobacteriaceae.2 In the three qnrS1 carriers, blaTEM-1 genes coding for ampicillin resistance were detected. The fourth ampicillin-resistant isolate carried both blaTEM-1-like and blaCTX,M3 extended-spectrum β-lactamase genes (Table 1).

Mapping of the qnrS1 and blaTEM-1 genes on pRQ2006

To examine whether the qnrS1 gene was localized on the chromosome or on a plasmid, Southern hybridization of total (XbaI PFGE patterns) and plasmid (plasmid patterns) DNA was performed. Both the qnrS1 and the blaTEM-1-like probes hybridized with an ~45 kb PFGE band of pattern X1 that corresponded to a plasmid, designated pRQ2006, present in the three qnrS1-positive isolates (two with plasmid profile PP1 and one with PP3) (Figures 1 and 2). Conjugation experiments were performed. Six selected transconjugants showed low resistance to nalidixic acid (8–16 mg/L) and a decreased susceptibility to ciprofloxacin (0.5 mg/L). They were positive for the qnrS1 and blaTEM-1 genes and did not show any chromosomal mutation affecting the QRDR of the gyrA gene.

To determine the incompatibility group of the pRQ2006 plasmid, the 18 incompatibility group replicon-typing PCR9 together with IncQ detection (repB of RS1010, accession no. M28829 and repA of the pGNB2-qnrS2 positive plasmid, accession no. DQ460733) was applied. Although the scheme was useful to characterize other resistance plasmids in our laboratory (data not shown), no positive product was obtained using the DMC9 isolate or plasmid DNA as templates.

Restriction analysis of pRQ2006 was performed using several endonucleases (Figure 2). Restriction fragments generated with HincII, HindIII, EcoRV and EcoRI were chosen for DNA hybridization withblaTEM-1 and qnrS1 probes. Only in the case of restriction with EcoRI, both probes hybridized to the same fragment (~20 kb) (Figure 2).

The presence of a Tn3-like transposon (accession no AB187515.1), which contained theblaTEM-1 gene, was confirmed by partial sequencing of HindIII (8 kb fragment) and EcoRV (2.7 kb fragment) restriction fragments that had been inserted into the vector pV2 and introduced into the E. coli strain NEB5 (New England Biolabs).

Relationship of pRQ2006 to other known qnrS1-blaTEM-1 plasmids

Only a few qnrS1-carrying plasmids have been described so far. They differ in their molecular sizes, resistance genes (i.e. for β-lactamases) and bacterial host. The 47 kb plasmid pAH0376 (accession no. AB187515.1) was the first qnrS1-carrier plasmid described. It was found in a human clinical isolate of Shigella
### Table 1. Strains used in the present study

<table>
<thead>
<tr>
<th>Strain/isolate ref. no.</th>
<th>Species/serotype</th>
<th>Origin</th>
<th>Isolation date</th>
<th>Phage type</th>
<th>PFGE pattern</th>
<th>Plasmid profile</th>
<th>Resistance phenotypes</th>
<th>MIC (mg/L)</th>
<th>Resistance genotypes</th>
<th>Class 1 or 2 integrons</th>
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<td><em>Salmonella</em> Virchow</td>
<td>chicken</td>
<td>15 November 2005</td>
<td>PT26</td>
<td>X3</td>
<td>PP3</td>
<td>NAL</td>
<td>&gt;128</td>
<td>0.25</td>
<td><em>gyrA</em>&lt;sup&gt;Tyr-87&lt;/sup&gt;</td>
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<td>15 November 2005</td>
<td>PT26</td>
<td>X1</td>
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<td>AMP–NAL</td>
<td>&gt;128</td>
<td>1</td>
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</tr>
<tr>
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<td>4 October 2005</td>
<td>PT26</td>
<td>X1</td>
<td>PP2</td>
<td>AMP–NAL</td>
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<td>1</td>
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<td>15 October 2005</td>
<td>PT26</td>
<td>X1</td>
<td>PP0</td>
<td>NAL</td>
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<td>PP1</td>
<td>AMP–EFT–NAL–STR–SUL–TMP–SXT</td>
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<td>0.25</td>
<td>(bla&lt;sub&gt;TEM-1&lt;/sub&gt;–bla&lt;subCTX,M3&lt;/sub&gt;)–<em>gyrA</em>&lt;sup&gt;Tyr-87&lt;/sup&gt;–<em>strA</em>–<em>B</em>–*sul2–<em>dfrA14</em> none</td>
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<td>NAL</td>
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<td>NAL</td>
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<td>PP1</td>
<td>AMP–NAL</td>
<td>&gt;128</td>
<td>1</td>
<td><em>bla&lt;sub&gt;TEM-1&lt;/sub&gt;</em>(<em>gyrA</em>&lt;sup&gt;Tyr-87&lt;/sup&gt;–<em>qnrS1</em>) none</td>
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<td>X2</td>
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<td>0.25</td>
<td><em>gyrA</em>&lt;sup&gt;Tyr-87&lt;/sup&gt; none</td>
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<tr>
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<td>conjugation</td>
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<tr>
<td>Tj3</td>
<td><em>E. coli</em> J53</td>
<td>conjugation</td>
<td>—</td>
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<td>—</td>
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<td>—</td>
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</tbody>
</table>

AMP, ampicillin; EFT, ceftiofur; CIP, ciprofloxacin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfamethoxazole; SXT, sulfamethoxazole/trimethoprim; TMP, trimethoprim.

*Carried out following the scheme used at the HPA, London, UK.*
flexneri in Japan and carries a Tn3-like transposon. The 58 kb pINF5 plasmid (accession no. AM234722.1) also carries the Tn3-like transposon and was isolated from Salmonella Infantis recovered from poultry in Germany. The pK245 plasmid (accession no. JQ449578.1) is a 98 kb plasmid, which carries a blaSHV-2 (ampicillin resistance) gene found in a clinical isolate of Klebsiella pneumoniae. In Enterobacter cloacae isolated in France and Vietnam, several plasmids with sizes between 50 (p287) and 100 kb (i.e. pS3–5) have been described and some of them carry a new blaLAP-1 gene. Recently, Hopkins et al. identified several qnrS1 plasmids in the UK, including the 44 kb IncN plasmid TPqnrS-2a/b, which also carries the blaTEM-1 gene, in Salmonella Virchow PT8 isolated from Thai cooked chicken.

The plasmid pRQ2006 was compared with pINF5 from Salmonella Infantis and pAH0376 from S. flexneri by primer walking and/or amplification using primers deduced from the published sequences of pINF5 (13 389 bp) and pAH0376 (11 002 bp), as well as by comparison of RFLP hybridization patterns.

First, a region of ~3430 bp of pRQ2006 was sequenced. This region matched with nucleotides 915–3093 from pINF5 and nucleotides 2114–5545 from pAH0376 and contained the blaTEM-1 gene, the tnpR gene (encoding the resolvase) and part of the tnpA gene (encoding the transposase) of the Tn3-like transposon. The region located upstream of Tn3 only matched with pAH0376 from S. flexneri (nucleotides 2114–2909). This region included a TAAAA direct repeat at the boundaries of the Tn3 element, which differs from the TTATT repeat part of an IS26 relic found in pINF5.

Secondly, a pRQ2006 region of ~1677 bp containing qnrS1 was sequenced. This sequence was similar to the sequences described for pINF5 and pAH0376 (nucleotides 6871–8547 from pINF5 and 9661–10 132 from pAH0376). On the basis of the sequence of pINF5, amplification experiments targeting other genes/sequences located downstream of qnrS1 were performed. Using primers designed for the CS12 fimbrial gene cluster of E. coli present in pINF5 (nucleotides 9661–10 143), a 100% homologous PCR product of 482 bp could be amplified. In contrast, no product targeting the tnp gene encoding the transposase of IS26 of pINF5 (expected size of 429 bp corresponding to nucleotides 11 591–12 020) was obtained, showing the lack of the IS26 relic in pRQ2006.

Kehrenberg et al. reported that pINF5 and pAH0376 showed different HindIII restriction patterns. RFLP hybridization with qnrS1 and blaTEM-1 probes had been carried out. The

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**Figure 2.** Restriction and hybridization analysis of the plasmid pRQ2006. (a) Plasmid restriction profiles generated by PstI (Ps), HindII (Hc), HindIII (Hd), EcoRI (EI) and EcoRV (EV). (b) Hybridizations of digested DNA with qnrS1 and blaTEM-1 probes. U, undigested pRQ2006. Molecular weight marker used: phage lambda DNA digested PstI (I) and phage lambda DNA digested with HindIII (II; Molecular weight marker DNA II, digoxigenin labelled, Roche Applied Science).
results are similar to those obtained with pRQ2006: qnrS1 is located on a 2.6 kb HindIII-fragment of pRQ2006 (as described for pINF5 and pAH0376) and the blatem.1 gene is located on an 8.4 kb fragment (as described for pAH0376). Furthermore, both genes are located on an ~20 kb EcoRI fragment (as described for pAH0376). The pRQ2006 plasmid yielded a PstI-RFLP (Figure 2) similar to those shown by Hopkins et al.5 for the S. flexneri plasmid, whereas the TPqnrS-2 plasmids found in the Salmonella Virchow isolates yielded very different PstI-RFLPs.5

This is the first study describing the presence of qnrS1 genes in bacterial strains from Turkey. The Salmonella Virchow strains were isolated from chicken and minced meat sold for human consumption. The pRQ2006 qnrS1 plasmid differs from the other described plasmids found in Salmonella and seems to be related to the plasmid pAH0376 found in S. flexneri isolated from human clinical samples in Japan. The presence of these plasmids in Salmonella isolates from food-producing animals is another worrying step in the increasingly observed fluoroquinolone resistance.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


