Characterization of multidrug-resistant, qnrB2-positive and extended-spectrum-β-lactamase-producing Salmonella Concord and Salmonella Senftenberg isolates

Kees Veldman1*, Cindy Dierikx1, Alieda van Essen-Zandbergen1, Wilfrid van Pelt2 and Dik Mevius1,3

1Central Veterinary Institute (CVI) of Wageningen UR, PO Box 65, 8200 AB Lelystad, The Netherlands; 2National Institute for Public Health and the Environment, Bilthoven, The Netherlands; 3Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

*Corresponding author. Tel: +31-320238404; Fax: +31-320238153; E-mail: kees.veldman@wur.nl

Received 21 December 2009; returned 14 January 2010; revised 26 January 2010; accepted 30 January 2010

Objectives: To characterize plasmids and resistance genes of multidrug-resistant (MDR) Salmonella Senftenberg and Salmonella Concord isolated from patients in the Netherlands.

Methods: The resistance genes of four MDR Salmonella isolates (three Salmonella Concord and one Salmonella Senftenberg) were identified by miniaturized microarray, PCR and sequencing. Plasmids were characterized by S1 nuclease-PFGE and PCR-based replicon typing (PBRT). Linkage between plasmids and genes was determined by conjugation experiments and microarray analysis. The genetic relationship between the three Salmonella Concord isolates was determined by XbaI-PFGE.

Results: A large variety of resistance genes was detected, including qnrB2 and the β-lactamase genes blaTEM-1 and blaSHV-12 in all isolates; moreover all Salmonella Concord isolates also harboured blaCTX-M-15. Salmonella Senftenberg harboured a large IncHI2 plasmid. The three Salmonella Concord isolates harboured two large plasmids typed as IncHI2 and IncA/C.

Conclusions: We detected the first plasmid-mediated MDR Salmonella isolates in the Netherlands harbouring both qnr and extended-spectrum β-lactamase (ESBL) genes. In Salmonella Senftenberg one large plasmid (IncHI2) and in Salmonella Concord two large plasmids (IncHI2 and IncA/C) were responsible for the multidrug resistance.

Keywords: ESBLs, fluoroquinolones, microarray, plasmids

Introduction

Worldwide, Salmonella is one of the major causes of foodborne infections in humans. In the majority of the cases these infections are self-limiting. However, for patients at risk and for invasive or prolonged infections antibiotic treatment is indicated. Fluoroquinolones and third-generation cephalosporins are drugs of choice for these cases.1 Infections caused by multidrug-resistant (MDR) Salmonella will affect the available treatment options. This may result in treatment failure and an increase in complications.

Although extended-spectrum β-lactamase (ESBL)-producing Salmonella Concord isolates from adopted Ethiopian children have been reported previously from different European countries including the Netherlands,2–4 there is still scarce information about the genetic background of Salmonella Concord isolates carrying both qnr and ESBL genes. In addition, no information on the characterization of MDR Salmonella Senftenberg isolates is available to date.

The aim of the study was to characterize genes and plasmids of the first qnr-positive, ESBL-producing MDR Salmonella isolated from patients in the Netherlands.

Materials and methods

Susceptibility tests and detection of resistance genes

In 2007, four Salmonella isolates expressing a remarkable type of multidrug resistance were identified. The isolates were selected for further study, since all four strains showed resistance to third-generation cephalosporins and exhibited an unusual quinolone resistance phenotype; being low-level resistant to ciprofloxacin, but still susceptible to nalidixic acid. In addition, all isolates were resistant to most classes of antibiotics tested. Three Salmonella Concord isolates (199.69, 206.54 and 210.52) originated from adopted Ethiopian children and a Salmonella Senftenberg
isolate (200.27) was obtained from a male adult patient who had recently travelled to Egypt. Susceptibility to antimicrobials was tested by broth microdilution according to ISO standards (ISO 20776-1: 2006) in microtitre trays with a custom-made dehydrated panel of antibiotics (Sensititre®, Trek Diagnostic Systems, UK). The results were interpreted using epidemiological cut-off values as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org). The panel included the following antibiotics: ampicillin, cefotaxime, ceftazidime, tetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, nalidixic acid, chloramphenicol, florfenicol, gentamicin, kanamycin, streptomycin and colistin.

To detect antimicrobial resistance genes a miniaturized microarray (AMRO4, Identibac, Veterinary Laboratories Agency, UK) was used and the transconjugants were analysed by PCR-based replicon typing (PBRT). The PCR products were purified by the QIAquick PCR Product Purification Kit (Qiagen GmbH, Germany). Sequences were determined by using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA) on a 3100-Avant Genetic Analyzer (Applied Biosystems). Sequence data were analysed with the Sequencher 4.6 program. The BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search for gene sequences homologous to the nucleotide sequences found.

**Results**

**Susceptibility tests and detection of resistance genes**

All *Salmonella* isolates were resistant to ampicillin, cefotaxime, ceftazidime, tetracycline, sulfamethoxazole, trimethoprim, chloramphenicol, gentamicin and streptomycin; the *Salmonella* Concord isolates were also resistant to florfenicol and the *Salmonella* Senftenberg isolate was resistant to kanamycin. Furthermore, all isolates were low-level resistant to ciprofloxacin (MIC: 0.12–0.5 mg/L), but still susceptible to nalidixic acid (MIC: 8–16 mg/L). The resistance genes *qnrB2, bla_TEM-1, bla_SHV-12, sul1, dfrA19, tet(D), strA and strB* were detected in all isolates. Some resistance genes were only detected in *Salmonella* Concord, including *bla_CTX-M-15, floR, sul2 and tet(A)*. The aminoglycoside resistance gene *aac(6’)-1b* was only detected in *Salmonella* Senftenberg. This classical variant of the gene was confirmed by sequencing the amplicon. In addition, the resistance genes *qnrC, qnrD and qepA* (not included in the microarray) were not detected by PCR in any of the four isolates.

**Table 1.** Replicon types of plasmids and resistance genes detected in donor strains and transconjugants

<table>
<thead>
<tr>
<th>Isolates</th>
<th>IncHI2</th>
<th>IncA/C</th>
<th>intI1</th>
<th>TEM</th>
<th>SHV</th>
<th>CTX-M</th>
<th>qnrB</th>
<th>tet(A)</th>
<th>sul1</th>
<th>sul2</th>
<th>dfrA19</th>
<th>floR</th>
<th>strA</th>
<th>strB</th>
<th>aac(6’)-1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>200.27 (D)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>200.27-T1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>199.69 (D)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>199.69-T1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>199.69-T2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>206.54 (D)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>206.54-T1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>210.52 (D)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>210.52-T1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>210.52-T7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Replicon types of plasmids and resistance genes detected in donor strains (D) and transconjugants (-T) of the *Salmonella* Senftenberg isolate (200.27) and *Salmonella* Concord isolates (199.69, 206.54 and 210.52).
(200 kb) and IncA/C (230 kb). Transconjugant 199.69-T1 harboured both plasmids including all 12 resistance genes identified in the donor strain. Transconjugants harbouring IncA/C lacked two resistance genes identified in the donor strain [bla<sub>SHV</sub> and tet(D)]. Transconjugants with only IncHI2 were not obtained. Salmonella Concord 210.52 also harboured IncHI2 and IncA/C plasmids, but of different sizes: 170 kb (IncA/C); and 290 kb (IncHI2).

Conjugation experiments with Salmonella Concord 210.52 resulted in transconjugants with either IncHI2 or IncA/C plasmids. Transconjugant 210.52-T1 harbouring an IncHI2 plasmid lacked bla<sub>CTX-M-15</sub>, tet(A) and floR, whereas transconjugant 210.52-T7 harbouring an IncA/C plasmid lacked bla<sub>SHV-12</sub>, qnrB2, tet(D) and dfrA19 (Table 1). All IncHI2 plasmids in the three Salmonella Concord isolates were characterized as R478-like plasmids. However, all plasmids lacked three genes present in R478 (arsB, smr136 and tnsD) and harboured the O1R_160 locus as in pAPEC-O1-R.

Southern blot hybridization experiments demonstrated that the bla<sub>CTX-M-15</sub> gene was only located on an IncA/C plasmid in all three Salmonella Concord isolates (results not shown). Finally, XbaI-PFGE revealed a unique digestion pattern for all three Salmonella Concord isolates indicative of the genetic variation of MDR Salmonella Concord strains originating from Ethiopia (results not shown).

**Discussion**

Salmonella Senftenberg is a common serotype in the Netherlands; in the last decade, a total of 581 isolates (3%), originating from different sources, were tested for antibiotic susceptibility. Until 2007, all Salmonella Senftenberg isolates were susceptible to third-generation cephalosporins. On the contrary, Salmonella Concord is a very rare serotype; in the last decade, only nine Concord isolates indicative of the genetic background of ESBL-producing, qnr-positive Salmonella Concord and Salmonella Senftenberg isolates. The potential human health impact of infections with such MDR Salmonella emphasizes the need to monitor these resistance patterns in Salmonella carefully.

**Acknowledgements**

Part of this work was presented at the ASM conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, Copenhagen, Denmark, 2008 (abstract number B102).

We would like to thank Dr Hilde Smith for carefully reading our manuscript and Dr Alessandra Carattoli for providing us with the R478-positive control strain.

**Funding**

This work was supported by the Ministry of Agriculture, Nature and Food Quality (WOT-01-002-03.02).

**Transparency declarations**

None to declare.

**References**


