Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104

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Abstract

The presence and genetic content of integrons was investigated in eight *Salmonella enterica* Typhimurium DT104 isolates from different pig herds in Denmark. Two different integrons were identified using PCR and sequencing. Each of the integrons carried a single resistance cassette in addition to the *sul1* and *qacEΔ1* genes characteristic of integrons. The first integron encoded the *ant* (β-Q)-Ia gene that specified resistance to spectinomycin and streptomycin. The second contained the *pse-1* β-lactamase gene. All the multiresistant strains contained both integrons. The presence of these two integrons did not account for the total phenotypic resistance of all the isolates and does not exclude the presence of other mobile DNA elements.

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Keywords: *Salmonella enterica* Typhimurium DT104; Integron; Antibiotic resistance

1. Introduction

Multiresistant *Salmonella enterica* serotype Typhimurium phage type DT104 has been reported with increased frequency in Europe and the United States [1–3] and since September 1996 multiresistant Typhimurium DT104 has been isolated from five pig herds in Denmark [4]. It has been inferred that the genes encoding resistance are chromosomally located in Typhimurium DT104 [2], but the exact location has not yet been defined nor the resistance genes identified.

A novel group of mobile DNA elements called integrons has been identified in Gram-negative bacteria. Integrons are able to incorporate single or groups of antibiotic resistance genes by site-specific recombination and are found in both chromosomal and extrachromosomal DNA [5,6]. Integrons comprise two conserved segments, the 5′ conserved segment (5′CS) and the 3′ conserved segment (3′CS), and an internal variable region. The latter is the target for integration of gene cassettes that encode antibiotic resistance with a recombination site termed the 59-base element [7]. The 5′ conserved segment contains the integrase gene (*intI*) integration site (*attI*) for the integron and promoters for expression of all downstream gene cassettes. The 3′ conserved segment encodes resistance to disinfectant (*qacEΔ1*) and sulfonamide (*sulI*) in the class 1 integrons. [8,9].

This study describes the genetic location of anti-
biotic resistance genes in different integrons in Danish Typhimurium DT104.

2. Materials and methods

2.1. Bacterial strains

Eight isolates of porcine Typhimurium DT104 from eight different herds were collected as a part of the Danish Salmonella surveillance programme between 1994 and 1997 (Table 1). All isolates were of the same PFGE type [4]. Two strains of Escherichia coli K12 that harboured plasmids R751 and R100,1 were kindly provided by L. Sundström [10] (Pharmaceutical Biosciences, Uppsala, Sweden) and were used throughout as controls of known integron sequences (GenBank accession numbers X72585 and X12870, respectively).

2.2. Antimicrobial susceptibility

Susceptibility to antimicrobial agents was determined by the two-fold dilution method using the Sensititre system (Accumed International Limited, East Grinstead, West Sussex, UK).

The antibiotics tested were: amoxicillin/clavulanic acid (A/Cl) 1/0.5–64/32 µg ml⁻¹, ampicillin (Ap) 0.5–32 µg ml⁻¹, apramycin (Ay) 0.5–64 µg ml⁻¹, carbadox (Ca) 2–256 µg ml⁻¹, ceftiofur (Ce) 0.25–16 µg ml⁻¹, chloramphenicol (Cm) 1–128 µg ml⁻¹, colistin (Ct) 1–64 µg ml⁻¹, enrofloxacin (En) 0.06–8 µg ml⁻¹, gentamicin (Gm) 0.25–32 µg ml⁻¹, kanamycin (Km) 0.25–32 µg ml⁻¹, nalidixic acid (Nal) 1–128 µg ml⁻¹, neomycin (Nm) 0.5–64 µg ml⁻¹, spectinomycin (Sp) 1–128 µg ml⁻¹, streptomycin (Sm) 2–256 µg ml⁻¹, sulfamethoxazole (Su) 2–256 µg ml⁻¹, tetracycline (Te) 0.5–32 µg ml⁻¹, trimethoprim (Tp) 0.25–32 µg ml⁻¹, trimethoprim/sulfamethoxazole (Tp/Su) 0.12/2.37–8/152 µg ml⁻¹.

2.3. Polymerase chain reaction

One bacterial colony was suspended in 1.0 ml phosphate-buffered saline (PBS), centrifuged, the pellet resuspended in 100 µl 10 mM Tris, 1 mM EDTA buffer pH 8.0 and boiled for 10 min. After lysis the suspension was stored at −20°C. For each reaction 2 µl of the lysis suspension was used. The PCR amplification was performed according to Aarestrup et al. [11], using the PCR primers shown in Table 2. The annealing temperature was set 5°C below the calculated melting temperature (Tm) [12]. All strains were tested for the content of the genes intI [9], ant (3')-Ia [13], pse-1 [14], qacEΔ1 and sul1 [8]. The PCR method was optimised to detect amplicons in the range of 300–1500 bp. The 5′CS and 3′CS primers (int1F and int1B) were used in combination with the primers for the antibiotic resistance genes to determine the content of integrons. All amplicons positive with the primers for antibiotic resistance genes were sequenced.

2.4. DNA sequencing

After PCR amplification the DNA was purified

| Table 1 |
| Genotypic and phenotypic characteristics of Salmonella Typhimurium DT104 isolates |
| Organism | Designation | Date and source | PFGE type | Antibiotic resistance | Integrons detected |
| S. Typhimurium DT104 | 9412445 | 17-11-1994, Porcine | XI | Sensitive | None |
| S. Typhimurium DT104 | 9521676 | 31-07-1994, Porcine | XI | Sensitive | None |
| S. Typhimurium DT104 | 9423245 | 13-12-1994, Porcine | XI | Sp,Sm,Su | InC-like |
| S. Typhimurium DT104 | 9616368 | 20-11-1996, Porcine | XI | Ap,Cm,Sp,Sm,Su,Te | InC-like and InD |
| S. Typhimurium DT104 | 9621927 | 05-07-1996, Porcine | XI | Ap,Cm,Sp,Sm,Su,Te | InC-like and InD |
| S. Typhimurium DT104 | 9622121 | 06-08-1996, Porcine | XI | Ap,Cm,Sp,Sm,Su,Te | InC-like and InD |
| S. Typhimurium DT104 | 9620179 | 19-01-1996, Porcine | XI | Ap,Cm,Sp,Sm,Su,Te | InC-like and InD |
| S. Typhimurium DT104 | 9720921 | 10-04-1997, Porcine | XI | Ap,Cm,Sp,Sm,Su,Te | InC-like and InD |

aBaggesen and Aarestrup [4].

bAp: ampicillin; Cm: chloramphenicol; Sp: streptomycin; Sm: spectinomycin; Su: sulfamethoxazole; Te: tetracycline.

cInC-like integron: the integron containing the ant (3')-Ia gene cassette.

dInD integron: the integron containing the pse-1 gene cassette.
using a QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) and the nucleotide sequence was determined by cycle sequencer using AmpliTaqFS dye terminator kit and a 373A automatic sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA, USA) [14]. For analysis of data DNAsis software was used (Hitachi Software Engineering Co., Ltd).

3. Results

3.1. Antimicrobial resistance

The resistance patterns of the eight Danish Typhimurium DT104 isolates are shown in Table 1. Two of the eight isolates were sensitive to all the antimicrobial agents tested. One isolate (No. 9423245) was resistant to Sp, Sm and Su and the five isolates were multiresistant and had the resistance pattern: Ap, Cm, Sp, Sm, Su, Te.

3.2. PCR mapping and sequencing of antibiotic resistance gene cassettes and integrons

Two distinct integrons were detected by the use of PCR. From the first integron, a PCR product of 1008 bp was obtained (Fig. 1A) using the int1F and int1B primers. The sequence of the 1008-bp fragment confirmed the presence of the aminoglycoside-resistant gene cassette \textit{ant (3′)-Ia}, which conferred resistance to streptomycin and spectinomycin. The amplicon from the second integron was 1133 bp when the same primers were used and corresponded to the conserved regions of the integron together with the \textit{pse-1} gene cassette that encoded a β-lactamase (Fig. 1B).

![Diagram A](image)

![Diagram B](image)

Fig. 1. Relation between integron structure and PCR products obtained using a range of primers for InC and InD detected in Danish Typhimurium DT104. The lines below the integron structure represent amplicons and the black boxes regions that were sequenced. The primers were used in different combinations to reveal the arrangement and content of the integrons. The numbers above the PCR products refer to the primers used (see Table 2). 5′CS and 3′CS represents the 5′ and 3′ conserved segments of the integron; \textit{qacE} and \textit{sulI} encode resistance to disinfectant and sulfonamide respectively. Numbers correspond to sequence positions in GenBank accession number D43625 [13].
Both integrons were detected in five of the eight Danish Typhimurium DT104 isolates whereas the first integron with the 1008-bp amplicon was found in the one isolate resistant to Sp, Sm and Su only (Table 1). From this isolate six different PCR products were obtained using primers in different combinations as shown in Fig. 1A. No amplicon was obtained using primers 9 and 10 (Table 2). The same six amplicons were also obtained from the five multiresistant strains. However, from each of these strains four additional PCR products were also obtained, which corresponded to regions of the \( \text{pse-1} \) gene cassette. No PCR product was obtained from the two sensitive isolates with any primer combination indicating that no integrons were present. The amplicons and sequenced regions of the two integrons are illustrated in Fig. 1A,B.

### 4. Discussion

In the present study two integrons with different resistance gene cassettes were identified in Typhimurium DT104. The 1008-bp amplicon showed complete homology to the integron InC described by Kazama et al. [13] (GenBank accession number D43625) when the sequences were compared. In comparison with \( \text{aadA2} \) from plasmid pSa this gene cassette differed by a single nucleotide [9].

The second integron encoded the \( \beta \)-lactamase gene \( \text{pse-1} \) (Fig. 1B). Although the sequence published by Zuhldorf and Wiedemann [15] contained the antibiotic resistance gene cassette, 5' conserved segment and 59 bp, the presence of the integron was not acknowledged. In this study we have demonstrated that \( \text{pse-1} \) is located within an integron. The presence of an integron and the \( \text{pse-1} \) gene as a single cassette has to our knowledge not been described. We therefore suggest InD as a designation.

Both integrons detected were of class I which is the most prevalent type among clinical isolates [9]. Sallen et al. [16] reported a high incidence of an integron that contained the \( \text{ant (3')-Ia} \) gene among clinical isolates of antibiotic-resistant Enterobacteriaceae but they did not find two different integrons in the same isolate. In contrast to these authors we have identified two distinct integrons within the same isolate.

The recognition of the two integrons does not account for the entire phenotypic resistance of the Typhimurium DT104 isolates. It is possible that larger integrons are present but were not detected or that the two observed integrons are positioned in a larger mobile DNA element that contains additional resistance genes.

Since the PFGE of all the eight isolates indicated a close relationship between the Typhimurium DT104 isolates it is possible that all isolates came from a common sensitive ancestor and that the resistant isolates have accumulated integrons as a result of selection pressure. Further investigation of both location of the integrons and the additional resistance genes is in progress.

### Table 2

<table>
<thead>
<tr>
<th>Number, name of PCR primer</th>
<th>Accession no.</th>
<th>Position of primer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 int1 F (5'CS): GGC ATC CAA GCA GCA AGC</td>
<td>U12338</td>
<td>1416→1433</td>
<td>Collis and Hall [17]</td>
</tr>
<tr>
<td>2 int1 B (3'CS): AAG CAG ACT TGA CCT CAT</td>
<td>U12338</td>
<td>4831→4814</td>
<td>Hall et al. [7]</td>
</tr>
<tr>
<td>3 sul1 F: CTT CGA TGA GAG CCG GCC GC</td>
<td>X12869</td>
<td>924→943</td>
<td>Sundström et al. [18]</td>
</tr>
<tr>
<td>4 sul1 B: GCA AGG CGG AAA CCC GCG CC</td>
<td>X12869</td>
<td>1360→1341</td>
<td></td>
</tr>
<tr>
<td>5 qacE\text{v} \text{I} F: ATC GCA ATA GTT GGC GAA GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 qacE\text{v} \text{I} B: CAA GCT TTT GCC CAT GAA GC</td>
<td>X15370</td>
<td>211→230</td>
<td>Stokes and Hall [5]</td>
</tr>
<tr>
<td>7 \text{ant (3')-Ia} F: GTG GAT GGC GGC GTG CTC AAG CC</td>
<td>M10241</td>
<td>514→533</td>
<td>Hollingshead and Vapnek [19]</td>
</tr>
<tr>
<td>8 \text{ant (3')-Ia} B: ATT GCC CAG TCG GCA GCG</td>
<td>M10241</td>
<td>1040→1023</td>
<td></td>
</tr>
<tr>
<td>9 \text{pse-1} F: CGC TTC CCG TTA ACA AGT AC</td>
<td>M69058</td>
<td>323→342</td>
<td>Huovinen and Jacoby [20]</td>
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<tr>
<td>10 \text{pse-1} B: CTG GTC CAT TTC AGA TAG CG</td>
<td>M69058</td>
<td>742→723</td>
<td></td>
</tr>
</tbody>
</table>

F: nucleotide sequence forward 5'→3'; B: nucleotide sequence backward 3'→5'.

Accession numbers are from the published sequences in GenBank database.
Acknowledgments

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References


