The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products

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Objectives: To investigate the distribution of, associations between and the transferability of antimicrobial resistance genes in resistant *Escherichia coli* strains isolated from Norwegian meat and meat products.

Methods: The 241 strains investigated were collected within the frame of the Norwegian monitoring programme for antimicrobial resistance in bacteria from feed, food and animals (NORM-VET) during the years 2000–2003. PCR was carried out for detection of resistance genes. Conjugation experiments were carried out with the resistant isolates from meat as donor strains and *E. coli* DH5α as the recipient strain.

Statistical analyses were performed with the SAS-PC-System version 9.1 for Windows.

Results: Resistance genes common in pathogenic *E. coli* were frequently found among the isolates investigated. Strains harbouring several genes encoding resistance to the same antimicrobial agent were significantly (\(P < 0.0001\)) more frequently multiresistant than others. Strong positive associations were found between the tet(A) determinant and the genetic elements sul1, dfrA1 and aadA1. Negative associations were found between resistance genes encoding resistance to the same antimicrobial agent: tet(A)/tet(B), sul1/sul2 and strA-strB/aadA1. The resistance genes were successfully transferred from 38% of the isolates. The transfer was more frequent from resistant isolates harbouring class 1 integrons (\(P < 0.001\)).

Conclusions: Acquired resistance played a major role in conferring resistance among the isolates investigated. The possibility of transferring resistance increases both by increased multiresistance and by the presence of class 1 integrons. The conjugation experiments suggest that tet(A) and class 1 integrons are often located on the same conjugative plasmid.

Keywords: commensals, food, integrons, conjugation, NORM-VET

Introduction

The increase in antimicrobial resistance and the impact on human health is an emerging problem worldwide (http://www.who.int/mediacentre/factsheets/fs194/en/). Exposure to resistant bacteria through the food chain has gained increased attention, as the presence of resistant bacteria in food and water might have an impact on the development and dissemination of resistance to antimicrobial agents in human bacterial pathogens. A recent study conducted by Johnson et al.\(^1\) suggests that retail foods, and especially meat and meat products, may be an important vehicle for community-wide dissemination of antimicrobial-resistant *Escherichia coli* and extraintestinal pathogenic *E. coli*.

The presence of acquired antimicrobial resistance among certain bacterial species of the normal enteric microflora such as *E. coli* and enterococci may represent a reservoir of transferable resistance elements from which antimicrobial resistance can be spread to other bacteria, including those responsible for infections in animals or humans.

The EU member states have to implement monitoring programmes that provide comparable data on the occurrence of antimicrobial resistance in zoonotic agents and of other agents insofar as they present a threat to public health.\(^2\) Further monitoring of indicator bacteria is still voluntary but is expected to be implemented in the near future.\(^2\)

The Norwegian monitoring programme for antimicrobial resistance, NORM (for humans) and NORM-VET (for food,
feed and animals), has been running since the year 2000. The occurrence of antimicrobial resistance in indicator bacteria (E. coli and enterococci) isolated from meat and meat products of Norwegian origin is examined in the annual NORM-VET programmes. The collection of meat samples for the NORM-VET programme is performed in order to obtain a representative random sample of meat from each of the species included. The number of samples taken is based on the slaughter volume of the particular species. The sampling procedures, which differ slightly from year to year, have been described thoroughly in the annual NORM/VET reports. From each meat sample, one E. coli isolate is chosen and subjected to further analysis. The isolates included in the NORM-VET programme may be regarded as representing a stratified random sample of the respective populations and products. The occurrence of resistance provides an estimate of the true occurrence in the populations. This material constitutes a valuable source for further studies on the occurrence of the various genetic elements responsible for the expression of resistance among E. coli present in meat and meat products of Norwegian origin.

It is well known that genes encoding antimicrobial resistance are often linked to mobile genetic elements. Transferable drug resistance was first discovered in the late fifties, and since then numerous plasmids and transposons containing resistance genes have been characterized. For the past 15 years, integrons and the inserted gene cassettes have also enabled the spread and maintenance of resistance genes among bacteria. Selection for one resistance feature may lead to co-selection of other resistance genes even if they have not been exposed to the antimicrobial agent in question.

The aim of the present study was to investigate the distribution, association and the transferability of various resistance genes in resistant E. coli isolated from meat and meat products of Norwegian origin. The isolates investigated have been included in the NORM-VET programme during the years 2000–2003.

Materials and methods

Bacterial strains and sampling procedures

The NORM-VET programme investigated 944 E. coli isolates from meat and meat products of poultry (359), pork (295), cattle (190) and sheep (100) during the years 2000, 2001, 2002 and 2003. These 944 E. coli isolates from meat and meat products have been subjected to susceptibility testing and the MICs of various antimicrobial agents have been determined. The methods and the quality control system used by NORM-VET are described in the annual reports. All bacterial strains included in the surveillance programme are stored at -80°C after susceptibility testing. The break-points, testing ranges and substances have varied somewhat between the years. In the present study we decided to use the break-points used in NORM-VET 200414 for classification of the strains as resistant or susceptible and further to include only the isolates resistant to at least one of the antimicrobial agents that has been included in the test panels every year. The following break-points were used: streptomycin, >8 mg/L; sulphonamides, >256 mg/L; tetracycline, >8 mg/L; ampicillin, >8 mg/L; trimethoprim, >4 mg/L; nalidixic acid, >16 mg/L; enrofloxacin, >0.25 mg/L; chloramphenicol, >16 mg/L; gentamicin, >4 mg/L; florfenicol, >16 mg/L; cefotiofur, >2 mg/L; and neomycin, >4 mg/L. The isolates included in year 2000, n = 362, were tested using a disc diffusion method; break-point, ≥20 mm. Most commonly observed was resistance to streptomycin (15.1%), followed by resistance to sulphonamides (14.5%), tetracyclines (6.6%), ampicillin (6.5%) and trimethoprim (4.5%). Resistance to quinolones, cephalosporins, neomycin and gentamicin was rare (~1% or less). A total of 241 isolates were classified as resistant to at least one of the tested antimicrobial agents. These isolates were subjected to further investigations as described below.

In a previous study, the 241 resistant strains have been screened for the presence of integrons and the inserted gene cassettes have been characterized. Most of the streptomycin-resistant isolates have also been investigated for the strA-strB and aada genes encoding resistance to streptomycin.

PCR

The isolates were screened for resistance genes using PCR. Tetracycline-resistant isolates (n = 62) were screened for the presence of tet(A), tet(B) and tet(C). Ampicillin-resistant isolates (n = 61) were screened for the presence of the blaTEM and blaSHV genes. All strains resistant to chloramphenicol (n = 8) were investigated for the cat gene. All strains resistant to sulphonamides (n = 135) were screened for the presence of the sul1 and the sul2 genes. Sulphonamide-resistant strains that were negative for sul1 and sul2 were in addition investigated for the sul3 gene. Strains resistant to streptomycin were investigated for the strA-strB and aada genes (seven strains not investigated previously).

All primers used to detect resistance genes are described in previous publications as shown in Table 1, which also shows primer sequences and the annealing temperatures used. The template was prepared by boiling a bacterial pellet in sterile distilled water and 5 μL of suspension was added to 45 μL of a mixture of PCR reagents containing 1× PCR buffer (Qiagen PCR Buffer, Qiagen GmbH, Hilden, Germany) with 1 U of Taq DNA polymerase (Qiagen), 10 pmols of each primer and 200 μM (each) dNTP. The PCR products were analysed by agarose gel electrophoresis. Positive and negative controls were used in each run. The following positive control strains were used: E. coli with plasmid RSF1010 (sul2, strA-strB), E. coli Se 131 (accession no. AJ238350 (sul1, aada1)), E. coli Rt 17 (tet(A)), E. coli He 146 (tet(B)), Aeromonas salmonicida subsp. salmonicida with plasmid pRAS3 (tet(C)), E. coli with plasmid pBR325 (cat), E. coli U39 (sul3), E. coli Dak2 (blaTEM) (provided by F. Aarestrup, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark) and E. coli 76-33094-7 (blaTREMA) (provided by F. Aarestrup).

Conjugation experiments

Conjugation experiments were carried out with the resistant isolates from meat as donor strains (n = 229, nalidixic acid-resistant isolates excluded n = 12) and nalidixic acid-resistant E. coli DH5α (MIC ≥20 mg/L) as the recipient strain. Conjugation was carried out in nutrient broth for 12 h, and the matings were prepared as described previously. After incubation the matings were diluted 10-fold in 0.9% saline. Samples of 0.1 mL were subsequently spread onto Mueller–Hinton agar plates containing 20 mg/L nalidixic acid (Sigma Chemical Co., St Louis, MO, USA), selecting recipients from the donors. Discs (Neo-Sensitabs®, Rosco, Taastrup, Denmark) containing relevant antimicrobial agents (corresponding to the resistance profile of the donor) were placed onto the agar surface. The plates were incubated overnight at 37°C, and suspected transconjugants (colonies that were able to grow within the inhibition
**Antibiotic resistance genes in Escherichia coli**

**Table 1.** Primers and annealing temperatures used in the PCR experiments

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5'→3')</th>
<th>Target gene(s) or region</th>
<th>PCR product size (bp)</th>
<th>Annealing temp. (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>strAstrB-F</td>
<td>TCTCCTGGAATGGAGCCCTCTG</td>
<td>strA-strB</td>
<td>538</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>strAstrB-R</td>
<td>CATGCTCATTATTTGATCCGCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aadA-F</td>
<td>GAGACATAGGCGGTCCTGTTGG</td>
<td>aadA1</td>
<td>198</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>aadA-R</td>
<td>TCGGATCGGATGAGGTACGCAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sul1-F</td>
<td>CCGGATCATGTCGGAATGTTACC</td>
<td>sul1</td>
<td>433</td>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>sul1-R</td>
<td>GCCGATCGGATGAGGTACGCAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sul2-F</td>
<td>GCGCTAAGAGCGATGCGATTT</td>
<td>sul2</td>
<td>293</td>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>sul2-R</td>
<td>GCCGATCGGATGAGGTACGCAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sul3-F</td>
<td>GAGCAAGATTTTGGAATCG</td>
<td>sul3</td>
<td>789</td>
<td>51</td>
<td>32</td>
</tr>
<tr>
<td>sul3-R</td>
<td>CATCGAGCTAACATAGGCCGTGGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetA-F</td>
<td>TTGTTCTGGAAGTGCCAGTAA</td>
<td>tet(A)</td>
<td>370</td>
<td>43</td>
<td>19</td>
</tr>
<tr>
<td>tetA-R</td>
<td>GACGGTCGTCAGGAGGCAAGCA</td>
<td>tet(B)</td>
<td>435</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>tetB-F</td>
<td>CTGACTATCCATCTTCTTGTG</td>
<td>tet(C)</td>
<td>588</td>
<td>53</td>
<td>34</td>
</tr>
<tr>
<td>tetB-R</td>
<td>GCTTCAAGAGCGGTTACATGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetC-F</td>
<td>CTTCAAGAGCGGTTACATGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetC-R</td>
<td>GCGCTAAGAGCGGTTACATGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tem-F</td>
<td>TTCTTGAGAAGCGAAGGCG</td>
<td>tem(B)</td>
<td>1150</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>tem-R</td>
<td>ACCTCAGTGGAAAGGACACAC</td>
<td>tem(C)</td>
<td>885</td>
<td>52</td>
<td>27</td>
</tr>
<tr>
<td>shv-F</td>
<td>CACCTCAAGGAGTAGATTGTTG</td>
<td>shv(B)</td>
<td>209</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>shv-R</td>
<td>TTAGGGTTGGCAATGCTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cat-F</td>
<td>GGTGAGCTGGTGATATGG</td>
<td>cat</td>
<td>209</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>cat-R</td>
<td>GGGATTGCGTGGAGACGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

zones of the discs) were subsequently selected. Transconjugants were tested for resistance to relevant antimicrobial agents (corresponding to the resistance profile of the donor) in a standard disc diffusion test using commercial drug discs (Neo-Sensitabs®), with semi-confluent growth on Mueller–Hinton agar. The inhibition zones used to classify the strains as resistant or susceptible were those recommended by the Norwegian AFA Group, described in ‘User’s guide Neo-Sensitabs®’ (http://www.rosco.dk). The presence of resistance genes in the transconjugants was confirmed by performing PCR as described earlier with DNA from the transconjugants as template. The transconjugants, in parallel with the donors, were plated out on lactose–saccharose–bromothymol blue agar plates and cultured at 37°C overnight. The colonies were inspected in order to confirm transconjugants as E. coli DH5α and not donor strains with mutations leading to a resistant phenotype (E. coli DH5α colonies are significantly smaller than wild-type E. coli colonies).

**Statistical methods**

All statistical analyses were performed with the SAS-PC-System version 9.1 for Windows (SAS Institute Inc., 1996). Comparisons of the associations between resistance genes were performed using the Pearson’s χ² exact test. The statistical significance was set at a P value of <0.05. An association between two genes can be positive, indicating that the genes are found together, or negative, indicating that the genes are not found together.

**Results**

**Occurrence of resistance genes**

The prevalences of the various resistance elements among the 241 resistant isolates included in the study are shown in Table 2. The investigated resistance genes were responsible for resistance in at least two-third of the isolates.

Resistance to streptomycin was mediated by the strA-strB genes and/or the aadA1 gene cassette (within class 1 and class 2 integrons) in the majority of streptomycin-resistant isolates. The presence of both strA-strB and an aadA1 cassette within the same strain was detected in nine strains. All these strains were resistant to three or more antimicrobial agents.

Resistance to sulphonamides was encoded by sul2 in the majority of the strains, whereas sul1 had a more limited distribution. The presence of multiple sulphonamide resistance genes, both sul1 and sul2, occurred in five isolates. All these strains were resistant to six or more antimicrobial agents. The recently described sul3 gene was detected in three strains. It is possible that more isolates may carry sul3 as only the 14 isolates resistant to sulphonamides that were negative for sul1 and sul2 were investigated for sul3.

Resistance to tetracycline was mediated by tet(A) or tet(B) in the majority of the tetracycline-resistant isolates. Only one strain carried tet(C). The presence of multiple tetracycline resistance determinants was detected in one isolate which carried both tet(A) and tet(B). This isolate (from pork) was the most multiresistant isolate (resistant to eight antimicrobial agents) found among all the 944 isolates investigated.

Resistance to ampicillin was encoded by the βl TMP genes in the majority of the ampicillin-resistant strains. Gene cassettes within integrons played a minor role as only one gene cassette (the oxa-30) in the 61 isolates investigated was responsible for ampicillin resistance. 15

Resistance to trimethoprim was mediated by gene cassette within integrons (dfrA1, dfr2a, dfrA12) in the majority of the trimethoprim-resistant strains. 15
Table 2. Distribution of genetic elements in relation to phenotypically expressed resistance

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Resistance marker</th>
<th>No. (%) of positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin (n = 143)</td>
<td>strA-strB</td>
<td>92 (64)</td>
</tr>
<tr>
<td></td>
<td>aadA</td>
<td>29 (20)</td>
</tr>
<tr>
<td></td>
<td>at least one</td>
<td>112 (78)</td>
</tr>
<tr>
<td>Sulphonamides (n = 135)</td>
<td>sul1</td>
<td>22 (16)</td>
</tr>
<tr>
<td></td>
<td>sul2</td>
<td>101 (75)</td>
</tr>
<tr>
<td></td>
<td>sul3</td>
<td>3b</td>
</tr>
<tr>
<td></td>
<td>at least one</td>
<td>121 (90)</td>
</tr>
<tr>
<td>Tetracycline (n = 62)</td>
<td>tet(A)</td>
<td>34 (55)</td>
</tr>
<tr>
<td></td>
<td>tet(B)</td>
<td>25 (40)</td>
</tr>
<tr>
<td></td>
<td>tet(C)</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>at least one</td>
<td>59 (95)</td>
</tr>
<tr>
<td>Ampicillin (n = 61)</td>
<td>bltTEM</td>
<td>40 (66)</td>
</tr>
<tr>
<td></td>
<td>oxa-30a</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>at least one</td>
<td>41 (67)</td>
</tr>
<tr>
<td>Trimethoprim (n = 41)</td>
<td>dfrA1a</td>
<td>24 (59)</td>
</tr>
<tr>
<td></td>
<td>dfr2aA</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>dfr12Aa</td>
<td>3 (7)</td>
</tr>
<tr>
<td></td>
<td>at least one</td>
<td>28 (68)</td>
</tr>
<tr>
<td>Chloramphenicol (n = 8)</td>
<td>cat</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Integronsa</td>
<td>class 1 integron</td>
<td>29 (12)</td>
</tr>
<tr>
<td></td>
<td>class 2 integron</td>
<td>14 (6)</td>
</tr>
</tbody>
</table>

Table 3. Association between antimicrobial resistance genes and class 1 and class 2 integronsa

<table>
<thead>
<tr>
<th>tet(A)</th>
<th>tet(B)</th>
<th>dfrA1</th>
<th>sul1</th>
<th>sul2</th>
<th>strA-strB</th>
<th>aadA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>tet(A)</td>
<td>(+++)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tet(B)</td>
<td>++</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dfrA1</td>
<td>++</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sul1</td>
<td>+++</td>
<td>(++)</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sul2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>(++)</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>strA-strB</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(++)</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>aadA1</td>
<td>++</td>
<td>(+)</td>
<td>+++</td>
<td>+++</td>
<td>(++)</td>
<td>(++)</td>
</tr>
<tr>
<td>cat</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>bltTEM</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>int1</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
<td>+++</td>
<td>(++)</td>
<td>(+)</td>
</tr>
<tr>
<td>int2</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>(++)</td>
</tr>
<tr>
<td>dfrA12a</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

a Only antimicrobial resistance genes that exhibited an association with another gene at the P < 0.05 level are shown. The levels of significance of the association (as assessed by the χ² exact test) were as follows: –, P > 0.05; +, 0.05 ≥ P > 0.01; ++, 0.01 ≥ P > 0.001; ++++, P ≤ 0.001. Parentheses indicate negative associations.

Association between resistance genes

Statistically significant (P < 0.05) associations between the occurrences of individual resistance genes among all the isolates were detected (Table 3). Several of the discovered associations found were obvious, such as the associations between integron components (sul1, int1, int2) and gene cassettes (dfrA1, aadA1). The positive association found between the strA-strB genes and sul2 was expected since these resistance genes are known to co-reside on small broad-host-range plasmids (like RSF1010 and pBPI) with a wide distribution among coliform bacteria.23

Unexpected positive association between tet(A) and the genetic elements sul1 (r = 0.64), dfrA1 (r = 0.65), aadA1 (r = 0.44) and int1 (r = 0.31) were found. This indicates an association between tet(A) and class 1 integrons. A positive association was also found between tet(B) and the sul2 gene. In addition, positive associations were found between the cat gene and both the sul2

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**Figure 1.** Proportion (%) of positive conjugation in all isolates versus with and without the presence of class 1 integron (bars: 95% confidence interval), and the strA-strB genes. Further conclusions should be made with caution due to a limited number of isolates positive for the cat gene.

**Conjugal transfer**

The conjugation experiments showed that 38% (n = 87) (95% CI: 31.7–44.6) of the resistant strains were able to transfer resistance genes to the susceptible recipient strain. In most cases, multi-resistant strains were able to transfer all their resistance features to the recipient strain. Only 10 multiresistant isolates did not transfer all their resistant features (four isolates not to tetracycline, two isolates not to ampicillin, two isolates not to trimethoprim, one isolate not to gentamicin and one isolate not to tetracycline and sulphonamides).

Transfer of resistance features varied significantly (P < 0.001, χ² test) between isolates harbouring class 1 integrons and those without as shown in Figure 1. In total, 77% of the strains
Antibiotic resistance genes in *Escherichia coli*

![Figure 2. Proportions (%) of positive conjugation in relation to the number of antimicrobial agents the strains were resistant to (bars: 95% confidence interval).](image)

harbouring class 1 integrons transferred their resistance features to *E. coli* DH5α whereas 33% of the resistant strains not carrying class 1 integrons were able to transfer their resistance features to the recipient strain.

The proportion of isolates with positive conjugation (at least one resistant feature transferred) in relation to increased multi-resistance is presented in Figure 2. The presence of class 1 integrons did also increase gradually by increased multiresistance. This suggests that the possibility of transferring resistance increases both by increased resistance (increased number of resistance elements present) and by the presence of class 1 integrons.

**Discussion**

Most of the investigated resistance genes were detected at high rates, indicating that these genes have major roles in conferring resistance among the isolates investigated. To our knowledge there are very few reports describing the occurrence of resistance genes in *E. coli* being part of the contamination flora of meat. However, there are several reports describing the presence of resistance genes in food-borne pathogens such as *Salmonella* spp. and *E. coli*12,24 and in intestinal *E. coli* from domestic animals.10,18,19,25–27 Comparisons between these findings and our findings showed that most of the resistance genes we investigated were also present at high rates in resistant coliform bacteria from other sources.

The presence of different genes within the same strain, encoding resistance to the same antimicrobial agent, was detected in 15 strains. We have shown that such strains are more frequently associated with multiresistance than expected by chance. Different resistance elements are often clustered within limited areas on plasmids or on the chromosome.9–13,28 Selection of one resistance marker may therefore lead to co-selection of the other resistance genes not involved in direct selection force. The clustering of resistance genes also ensures the inheritance of all resistance elements. A linkage between resistance genes and other genes contributing to increased bacterial survival and fitness may also lead to the maintenance of multiple resistance genes in the same strain. However, when analysing the whole dataset we detected a negative association between genes encoding resistance to the same antimicrobial agent such as tet(A) and tet(B) (this was also found in other investigations),24,26 between sul1 and sul2, as well as between strA-strB andaadA1. In most cases it is therefore probably not cost-beneficial for the bacteria to carry or acquire more than one resistance gene encoding resistance to a specific antimicrobial agent. Incompatibility between plasmids carrying resistance determinants can also explain why the presence of different genes encoding resistance to the same antimicrobial agent is relatively uncommon.

The prevalence of chloramphenicol resistance among the isolates included in the NORM-VET programme during the period 2000–2003 was low, 0.8%. A reason for this might be that use of chloramphenicol for domestic animals has been prohibited in Norway since 1990. The prevalence of chloramphenicol resistance in commensal *E. coli* from animals has been found to be considerably higher in other countries. This is reported by several antibiotic resistance monitoring programmes such as DANMAP (http://www.dsvf.dk/Default.asp?ID=9604) and MARAN (http://www.cidc-lelystad.wur.nl/UK/publications/other+publications/maran/). The presence of chloramphenicol resistance among the isolates investigated in the study might be due to contamination of the meat by *E. coli* of non-animal origin. There is reason to believe that most *E. coli* occurring in meat originates from the faecal flora of the domestic animals. However, even if the *E. coli* isolates most probably originate from the faecal flora of the animals in question, it cannot be excluded that the meat may be contaminated from the environment of the slaughteringhouses or the cutting plants or eventually from people working there. The occurrence of chloramphenicol resistance in *E. coli* of human origin in Norway is unknown. The drug is approved for systemic use in Norway, but the usage is very limited. Since 1998 the sale of chloramphenicol has been ≤0.005 DDD/1000 inhabitants/day.14 Another explanation for the detected chloramphenicol resistance might be that the resistance genes (the *cat* gene in six of the eight resistant isolates) could have a close genetic linkage to other resistance genes leading to a co-selection of the *cat* gene when antimicrobial agents are used. The *cat*-positive strains included in our study were all multiresistant; the conjugation experiments indicated that four of the strains contained transferable DNA elements carrying the *cat* gene and other resistance genes (encoding resistance to at least five different antimicrobial agents).

Several of the associations between resistance elements found were obvious. However, the associations between tet(A) and the genetic elements sul1, dfrA1, aadA1 and intI indicate an association between tet(A) and class 1 integrons. The same associations (in *E. coli*) have also recently been found by others.24,26 The conjugation experiments carried out in the present study showed that the resistance elements were located on the same transferable element, probably conjugative plasmids. This suggests a widespread distribution of conjugative plasmids carrying tet(A) and class 1 integrons. Possible explanations could be that a specific plasmid carrying tet(A) and a class 1 integron is successful and has a wide distribution or it could be that tet(A) and the class 1 integron constitute a part of an ‘antibiotic resistance island’ and that this resistance island has been integrated into various conjugative plasmids. Further investigations should be carried out in order to characterize the plasmids and the genetic organization of tet(A) and class 1 integrons carried by them. On a recently sequenced incF plasmid (pAPEC-O2-R, 101 kb) from an avian *E. coli*, a class 1 integron co-resides with tet(A).28 However, on plasmid pRAS1 from the fish pathogen *Aeromonas salmonicida* a class 1 integron is also
localized adjacent to tet(A), but the organization of the resistance elements is very different from that on pAPEC-O2-R. Our results suggest further that the possibility of transferring resistance increases both by increased multiresistance (increased number of resistance genes present) and by the presence of class 1 integrons. The presence of class 1 integrons is therefore unfavourable as such genetic elements are associated with transferable genetic elements, enabling the resistance genes to spread in the bacterial populations. It is therefore desirable if the prevalence of class 1 integrons is low. Only 2 of the 14 isolates carrying class 2 integrons were able to transfer their resistant features. A preferred target-site for Tn7 exists within the chromosome of E. coli; this might influence the transfer frequency of resistance associated with class 2 integrons.

The collection of isolates investigated in the present study was performed in such a way that it constituted a representative sample of E. coli in meat of Norwegian origin. Such collections are valuable as they can form a basis to perform risk analysis of the spread and development of antimicrobial resistance in a ‘farm to fork perspective’. In the future such studies may be more common as more countries establish surveillance programmes for antimicrobial resistance. The strain collections obtained in such surveillance programmes will be of great value (as long as they are representative of the microorganism investigated) for further analysis of the distribution of resistance genes and other characteristics that may allow for comparison between the different origins of the products. The development of methods based on DNA microarray analysis as a tool for screening of resistance genes, pathogenic factors and plasmid characteristics will contribute to a more efficient and rapid analysis of huge strain collections. Thereby, the amount of data generated that may be used both to follow the epidemiological situation of antimicrobial resistance as well as for risk analysis will increase dramatically.

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Transparency declarations

None to declare.

References

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