Tetracycline, sulphonamide and trimethoprim resistance genes and integrons in *E. coli*

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Aim of the study

Commensal *Escherichia coli* are present in large numbers in nearly all animal species and are regarded as general indicators for resistance among Gram-negative bacteria. The aim of this study was to investigate the antimicrobial resistance in *E. coli* against three antimicrobials that are frequently administered orally to pigs through supplemented feed.

Materials and methods

1. Bacterial isolates and antimicrobial susceptibility

A total of 100 commensal *E. coli* isolates from pigs and chickens were selected on the basis of different antimicrobial resistances (tetracycline, sulphonamides, trimethoprim, ampicillin and streptomycin) from a collection obtained for the resistance monitoring programme of the Belgian Federal Agency for the Safety of the Food Chain. All strains were tested for susceptibility by a micro broth dilution method (Sensititre, Trek Diagnostic Systems) using Eucast breakpoints.

2. Resistance genes

The *E. coli* isolates were investigated by multiplex and simplex PCR for the presence of common resistance genes against tetracycline (Ng et al., 2001), sulphonamides (Kerm et al., 2002; Perreten et al., 2003) and trimethoprim (Lee et al., 2001).

3. Integron detection and characterization

a. Screening for presence of integron class 1, 2 and 3: triplex PCR targeting intI1, intI2 and intI3 respectively (Su et al., 2006).

b. Amplification of variable cassette regions (VCR’s) of integrons class 1 and 2 (Figure 1 and 2) by PCR using previously described protocols (White et al., 2001; Su et al., 2006).

c. RFLP analysis of amplified VCR’s.

d. Sequencing of VCR’s with unique RFLP patterns (Macrogen Amsterdam).

Results

1. Resistance genes

The total results for *sul*, *tet* or *dfrA* genes are shown in Figure 3. Different gene combinations were found for each gene type (Figure 4). Of the sulphonamide resistant isolates, 19% were negative for the *sul* genes. Of the tetracycline and trimethoprim resistant strains, 18% and 28% appeared negative for respectively all *tet* and *dfrA* genes tested. Other *tet* and *dfr* resistance genes will be tested. In the cases of the sulphonamide resistant isolates that were negative for all known *sul* genes, the resistance mechanism will be further investigated.

2. Integron characterization

Table 1 shows the results of the screening on integron presence.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genes/Region</th>
<th><em>E. coli</em> from pigs</th>
<th><em>E. coli</em> from poultry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrase genes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IntI1</td>
<td></td>
<td>43%</td>
<td>71%</td>
</tr>
<tr>
<td>IntI2</td>
<td></td>
<td>10%</td>
<td>2%</td>
</tr>
<tr>
<td>IntI1 + IntI2</td>
<td></td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>IntI3</td>
<td></td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Variable cassette region</td>
<td></td>
<td>42%</td>
<td>71%</td>
</tr>
<tr>
<td>Integron class 1 VCR</td>
<td></td>
<td>80%</td>
<td>80%</td>
</tr>
</tbody>
</table>

*Percentage calculated on the basis of the intI or intI2 positive strains.

RFLP analysis resulted in 9 different VCR patterns for integrons class 1 and 7 different VCR patterns for integrons class 2.

So far, 6 different types of integron class 1 VCR’s and 1 type of integron class 2 VCR were sequenced. The different gene cassettes found in these VCR’s are shown in Table 2.

<table>
<thead>
<tr>
<th>Integron type</th>
<th>Gene cassettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>class 1</td>
<td><em>dfrA1</em> ; <em>aadA1</em></td>
</tr>
<tr>
<td>class 1</td>
<td><em>aadA1</em></td>
</tr>
<tr>
<td>class 1</td>
<td><em>dfrA7</em></td>
</tr>
<tr>
<td>class 1</td>
<td><em>bla-OXA-30</em> ; <em>aadA1</em></td>
</tr>
<tr>
<td>class 1</td>
<td><em>aadB</em> ; <em>aadA2</em></td>
</tr>
<tr>
<td>class 2</td>
<td><em>dfrA17</em> ; <em>aadA5</em></td>
</tr>
<tr>
<td>class 2</td>
<td><em>aadA1</em></td>
</tr>
</tbody>
</table>

Conclusions

This study shows a high prevalence of *tetA* and *sul2* in respectively tetracycline and sulphonamide resistant *E. coli* isolates. *dfrA1* was the predominant trimethoprim resistance gene, followed by *dfrA12* and *dfrA7*. In general, a large variety of *dfrA* genes was found. In many strains, combinations of different genes encoding for one type of resistance were found. As expected with the selected resistance phenotypes of the *E. coli* isolates (sulphonamide, ampicillin and streptomycin resistant), a high integron prevalence was found with integron class 1 being predominant. Different gene cassettes were found in these integrons, with *aadA1* being most prevalent.

References


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