Resistance to apramycin of *Salmonella* and *E.coli* isolated from swine.

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**Summary:** The aim of this study was to determine the prevalence of aminoglycosides antibiotic resistance in *Salmonella* spp. and *E. coli* strains. 32 *E. coli*, and 47 *Salmonella* spp., isolated from cases of enteritis in growers and fatteners from 1998 to 2002 in Umbria and Marche regions, were tested. Susceptibility to gentamicin, tobramycin and streptomycin was determined by Kirby-Bauer method, apramycin by microdilution method. 92.4 % of the strains tested were susceptible to apramycin, 77.2 % to gentamicin, 67.1 % to tobramycin and 35.4 % to streptomycin. A positive statistical association between gentamicin and apramycin (RR = 7.63; p = 0.014), tobramycin and apramycin (RR = 9.22; p = 0.027) was demonstrated. There is no difference between the association apramycin-streptomycin, suggesting a mechanism of resistance related to the presence of the aminoglycoside acetyltranspherase IV enzyme. The trend based on estimated OR from the resistance of the strains for every year considered was significant (p = 0.00049), showing a progressive decrease from 1998 (OR = 1) to 2002 (OR = 0.3).

**Keywords:** aminoglycosides, enteritis, gentamicin, tobramycin, streptomycin.

**Introduction:** Apramycin, an aminoglycoside antibiotic has been used in veterinary medicine since 1980, in oral treatment of Gram negative bacterial enteritis of swine (Johnson et al., 1994). The main objective of this study was to determine the prevalence of antibiotic resistance among strains of *Salmonella* spp. and *E.coli* isolates from cases of enteritis in growers and fatteners in Umbria and Marche regions. Furthermore, the relationship between susceptibility to apramycin and related aminoglycosides was evaluated. Finally, trend of resistance to these aminoglycosides from 1998 to 2002 was investigated.

**Materials and methods:** *Salmonella* spp. and *E.coli* isolates were identified as described elsewhere (Quinn et al., 1999); *Salmonella* spp. strains were serotyped according to Popoff (Popoff et al., 1997). MIC were performed using the microdilution method according to the National Committee for Clinical Laboratory Standards document for veterinary antimicrobial susceptibility tests (NCCLS, 1998). The dilutions ranged from 0.25 mg / ml to 128 mg / ml. Apramycin was kindly provided by EliLilly. The interpretative criteria used for apramycin were based on previous report (Prescott et al., 2000): strains with MIC ≤ 16 mg / ml were regarded as susceptible. Susceptibility to gentamicin, tobramycin and streptomycin was determined by the Kirby-Bauer test using antimicrobial containing disks (Oxoid) according to the NCCLS document described above. The hypothesis of association between the resistance to apramycin and others aminoglycosides was tested using the Fisher's exact test. Values of p less than 0.05 were considered significant. The measure of this association was expressed by the relative risk (RR). The chi square value for the trend and the odds ratio were used to verify the resistance of the strain to aminoglycosides for every year considered.

**Results:** 79 strains, 32 belonging to *E. coli* species, and 47 to *Salmonella* spp., were used in this study. Among *Salmonella* spp., the predominant serovar was Typhimurium (69 %), followed by Panama, (7 %), Seftemberg (4 %), Anatum (4 %) and Bredney (4 %), while other serovars did not exceed 2 %. In figure 1 frequency (percentage) of strains related to MIC values are shown. The interpretative criteria used for apramycin were based on previous report (Prescott et al., 2000): strains with MIC ≤ 16 mg / ml were regarded as susceptible: so, 92.4 % of the strains tested were classified as susceptible. A
High percentage of strains tested were susceptible to gentamicin (77.2%), tobramycin (67.1%), but not to streptomycin (35.4%). The relationship between the resistance of isolates to apramycin and others antibiotics is shown in Table 1. The trend based on odds ratio (OR) estimated from the resistance to all the aminoglycosides for every year considered is shown in figure 2.

**Table 1** Results of univariate analysis

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>7.63</td>
<td>1.53</td>
<td>37.98</td>
<td>0.014</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.122</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>9.22</td>
<td>1.09</td>
<td>78</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Discussion and Conclusions: 92.4% of the strains tested were classified as susceptible to apramycin. These results are consistent with previous reports, as resistance to apramycin is rare among Gram-negative bacteria. Strains were either clearly susceptible (< 8 mg/ml) or resistant (> 128 mg/ml) none of the strains tested showed values between 16 to 64 mg/ml. Such distribution, rather than a normal, seems the bimodal distribution typical for most antibiotics and for most pathogens (Prescott et al., 2000). A positive statistical association between gentamicin and apramycin (RR = 7.63; p = 0.014), tobramycin and apramycin (RR = 9.22; p = 0.027) was demonstrated. Since aminoglycoside acetyltranspherase IV degrade apramycin, gentamicin and tobramycin, but not streptomycin, these results may suggest a mechanism of resistance related to this enzyme. The trend of the resistance of strains to aminoglycosides for every year considered is significant (p = 0.00049) showing a progressive decrease from 1998 to 2002. These data need further investigations but these results could also be explained by a decreased employment of aminoglycosides in feed.
**Survival of Salmonella and Escherichia coli in pig slurry:** simulation of decay

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**Summary:** Spreading of slurry infected with multi-resistant Salmonella Typhimurium DT104 (MRDT104) on arable land might constitute a risk of transmission to wildlife. To estimate survival time on farmland, we modeled the bacterial decay based on Escherichia coli data from a plot study carried out in spring 2002 in Denmark. Time until undetectable levels were modeled under different scenarios: 1) E. coli in swine slurry, 2) Salmonella in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds. A log-linear model extended with time^2 and time^3 was used to describe bacterial decay. For scenarios 2 and 3, we assumed that the level of bacteria in the slurry would be log 4.0 cfu/g and log 3.4 cfu/g, respectively, and a similar effect of spreading and decimation to that of E. coli. Hereby, it was estimated that Salmonella counts fell below detectable levels after 10 and 5 days, respectively.

**Keywords:** microbial ecology, transmission, multi-resistant Salmonella Typhimurium DT104, environmental persistence, decimation

**Introduction:** Spreading of slurry infected with multi-resistant Salmonella Typhimurium DT104 (MRDT104) on arable land has been considered a potential hazard for transmission to wildlife. Therefore, spreading has been restricted for herds positive to MRDT104. Our aim was to model decay of Escherichia coli and Salmonella after spreading of contaminated slurry on farmland, and to estimate the survival time.

**Materials and Methods:** The modeling was based on Escherichia coli data from a plot study carried out in spring 2002 in Denmark. Here, E. coli was measured quantitatively on day 0, 7, 14, 21, and 28 after application on soil using 4 different methods (see Boes & Alban, in this issue). Salmonella was not detected when slurry was ploughed in, and hence, these data were not used for the modeling. Data from the three remaining application methods (harrowed only, slurry injection, and hose application) were used. We were interested in estimating the time from disposal until undetectable levels under different scenarios: 1) E. coli in swine slurry, 2) Salmonella in slurry from clinically infected swineherds, and 3) MRDT104 in slurry from sub-clinically infected swineherds.