Antibiotic resistance in calves in Umbria, Italy: prevalence, genetic characterization and possible risk factors

BACKGROUND

Antimicrobial resistance (AMR) has been recognised as a One-Health problem, with livestock being a reservoir of AMR genes.

AIM OF THE STUDY

The aim of this study was to assess dairy and beef calves in Umbria as a reservoir of AMR and ESBL- and AmpC-producing *E. coli*.

MATERIALS AND METHODS

Sampling: 123 farms with more than 50 units were recruited in the study. On each farm, four samples were collected from the pens of calves <60 days of age.

Laboratory analysis: each sample was pre-enriched and then cultured on McConkey agar (MC), MC+ nalidixic acid (MCnal) and MC+ cefotaxime (MCctx). For each herd, one isolate from MCctx was tested using a MIC method (EUVCSEC) and then characterized for the phylogenetic group (Clermont, 2013).

Molecular analysis: the isolates were tested with a blaTEM/blaSHV/blaOXA-1-like multiplex PCR, a blaCTX-M multiplex PCR including phylogenetic groups 1, 2 and 9 and a blaCTX-M-8/-25 simplex PCR (Dallenne, C., 2010). Moreover, the presence of genes encoding the pAmpC enzymes were investigated by multiplex PCR (Pérez-Pérez, 2002). PCR products were analyzed by agarose gel electrophoresis (2%) and visualized under UV light.

Statistical analysis: the differences in prevalence of AMR between dairy and beef farms were investigated using chi-squared test and the odds ratios (OR) were assessed, using beef calves as the baseline value. The threshold of significance was set at p-values<0.05.

RESULTS

The prevalence of MCctx and MCnal positive samples is shown in table 1. The odds of isolating an *E. coli* resistant to nalidixic acid or cefotaxime was 4.72 (95%IC: 2.82-7.89) and 4.06 (IC95%: 2.64-6.24) higher in dairy farms than beef ones, respectively.

Out of 51 isolates (21 from beef farms and 30 from dairy ones) from MCctx, 36 (71%) were ESBL-producers, 10 (19%) were AmpC-producers, 3 (6%) were both, and 2 (4%) were negative. These isolates belonged to phylotype A (63%), C (20%), D (6%), E (6%), and F (4%). The 36 ESBL-producers presented mainly blaCTX-M-1, alone (21%) and in association with blaTEM (25%). Interestingly, all blaCTX-M belonging to group 1 were blaCTX-M-15, that is reported worldwide as associated with uropathogenic strains in man. The AmpC-producers presented mainly genes from CTX-group, in particular they were all characterized as cmy-2. The differences in gene content beef and dairy farms are shown in graph 1 and 2.

### Table 1. number and proportion (%) of samples positive for culture on McConkey agar supplemented with antibiotics, according to the production type

<table>
<thead>
<tr>
<th></th>
<th>MCnal (%)</th>
<th>MCnal (%)</th>
<th>MCctx (%)</th>
<th>MCctx (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>total</td>
<td>total</td>
<td></td>
</tr>
<tr>
<td>dairy</td>
<td>195 (90%)</td>
<td>21 (10%)</td>
<td>216 (88%)</td>
<td>128 (60%)</td>
</tr>
<tr>
<td></td>
<td>21 (10%)</td>
<td>total</td>
<td>total</td>
<td></td>
</tr>
<tr>
<td>beef</td>
<td>183 (69%)</td>
<td>93 (34%)</td>
<td>276 (40%)</td>
<td>236 (80%)</td>
</tr>
<tr>
<td></td>
<td>3 (1%)</td>
<td>total</td>
<td>total</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>378</td>
<td>114</td>
<td>492</td>
<td>364</td>
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</tbody>
</table>

Graph 1: Gene content in beef farms

Graph 2: Gene content in dairy farms

### CONCLUSIONS

This work highlights the role of calves as a reservoir of AMR in Umbria and describes an association between the production type and the risk of AMR.

### BIBLIOGRAPHY