



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



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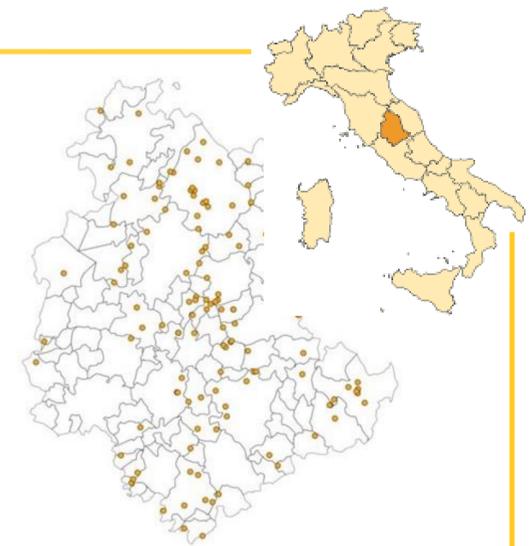


## BACKGROUND

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 (EUVSEC) 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-value≤0.05.



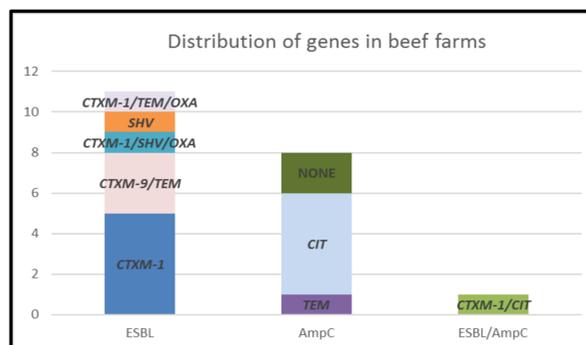
## RESULTS

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.

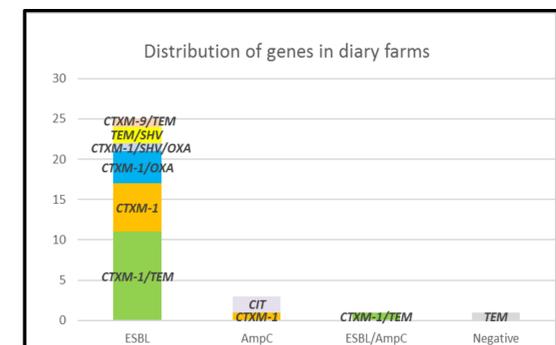
Out of 51 isolates (21 from beef farms and 30 from dairy ones) from MCctx, 36 (71%) were ESBL-producers, 10 (19%) were AmpC-producers, 3 (6%) were both, and 2 (4%) were negative. These isolates belonged to phylotype A (63%), C (20%), D (6%), E (6%), and F (4%). The 36 ESBL-producers presented mainly 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 CIT-group, in particular they were all characterized as *cmv-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

	MC <sup>nal</sup>			MC <sup>ctx</sup>		
	Positive (%)	Negative (%)	total	Positive (%)	Negative (%)	total
dairy	195 (90%)	21 (10%)	216 (100%)	88 (40%)	128 (60%)	216 (100%)
beef	183 (66%)	93 (34%)	276 (100%)	40 (14%)	236 (86%)	276 (100%)
total	378	114	492	128	364	492



Graph 1: Gene content in beef farms



Graph 2: Gene content in dairy farms



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## 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.

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