# ESCHERICHIA COLI RESISTANCES TO 3RD AND 4TH GENERATION CEPHALOSPORIN FROM FARM TO FORK: THE RISK OF TRANSMISSION TO CONSUMER.

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## INTRODUCTION

The food animal industry contributes to the spread of antimicrobial resistant (AMR) microorganisms through the food chain and the environment.

Food processing techniques are used to extend the shelf life of food products, but some bacteria can survive or be only sub-lethally damaged. At the same time, cell death and lysis can release DNA and resistant genes into the environment, allowing other bacteria to receive or incorporate and express parts of free DNA. The increasing demand for raw or minimally processed food can also contribute to the spread of AMR due to the bacterial replication. Pigs are exposed to different antibiotic treatments during the breeding cycle, which results in the development of different pools of resistant genes. A significant risk factor is the use of 3rd and 4th generation cephalosporins. The microorganisms isolated are cephalosporin resistant Escherichia coli (E. coli), chosen because of their increasing involvement in human infections. The ability of E. coli to produce β-lactamases leads to the deactivation of the antibiotic molecule. This study focuses on the pork food chain in order to evaluate the risk of E. coli AMR transmission from animal to consumer.

## METHODOLOGY

Sample collection was carried out in northern Italy during 2019/2020 (Period A) and repeated in 2020/2021 (Period B).

Eight fattening pig farms were selected, and faecal swabs were collected. All the sampled pigs were identified and followed to slaughterhouses. Carcasses were sampled with sterile sponges. Meat products, derived from pigs that harboured resistant E. coli in their faeces, were directly sampled at slaughter. After processing, seasoned (coppa or pancetta) and fermented products (salami) were sampled.

E. coli was isolated from all samples and tested for the ability to produce ESβL and AmpC using the disk diffusion method.

DNA from phenotypically confirmed and intermediate ESβL and AmpC E. coli isolates was extracted. A Real-time PCR was applied to verify the presence of the ESβL associated genes

bla-CTX-M1, bla-CTX-M2, bla-TEM and bla-SHV. The AmpC genes bla-MOX, bla-CIT, bla-DHA, bla-ACC, bla-EBC and bla-FOX were verified using a multiplex PCR protocol.

Molecular typing (Enterobacterial Repetitive Intergenic Consensus sequences) was used to identify potential phylogenesis between AMR and non-AMR bacterial strains belonging to the same food chain (faeces-carcasses-fresh meat-meat product).

RESULTS

The prevalence of phenotypically ESβL E. coli isolated from faeces was 7 %, 9.9 % was isolated from carcasses. In fresh and fermented meat, 12.65 % and 10 %, respectively, of E. coli harboured ESβL. Approximately 29 % of the E. coli isolated from seasoned meat products in period A were phenotypically ESβL, while in period B, no E. coli was found. Phenotypic AmpC E. coli prevalence reached almost 4 % in faecal samples and carcasses. In pig meat and meat products, no E. coli with an AmpC profile was found. In period A, genotypic analysis confirmed the presence of ESβL genes in 66.7 % of the E. coli that showed phenotypic resistance or an intermediate profile, and in Period B, this rate reached 93 %. Overall, no AmpC genes have been found but 25.8 % of the phenotypic AmpC bacteria showed ESβL genes. BlaTEM was the most frequently found ESβL gene. Only one phenotypic resistance profile was found to be similar in strains isolated from the faeces, carcass, fresh meat and fermented products of the same pig, and their genotypic profile similarity was supported by typing analysis (except for fermented products). Phylogenetic similarities of strains isolated both from faeces-carcass and carcass-meat product were highlighted by molecular typing.

DISCUSSION

In Europe, sales of 3rd and 4th generation cephalosporins decreased by 24 % between 2011 and 2018 (ESVAC, 2018). Despite this, the data collected here showed an increase of ESβL resistance from 2019 to 2021, often supported by the presence of resistance genes. At the same time, phenotypic AmpC resistances also increased. No AmpC genes have been found in our study, possibly suggesting an aspecific mechanism of AMR.

The major strength of this study was the traceability of the samples that allowed us to analyse the farm to fork system and to better understand the spread of resistant bacteria along the food chain.

Molecular typing showed, in only one case, that the same E. coli phylogenetic group is transmitted from the animal, through the carcass, to the fresh meat. Results showed that similar strains have been found in faeces-carcass or in carcass-meat product, highlighting that the transmission directly from farm to fork is possible but not common. However, the food producing environment and cross-contamination can play an important role in the dissemination of AMR. Further sequencing analyses are scheduled in order to consolidate the data found.