Title: Occurrence and antibiotic resistance of *Arcobacter* spp. isolated from poultry slaughterhouses in Northern Italy.

Author: Chiarini E.[1], Buzzanca D.[1], Chiesa F[2], Botta C.[1], Ferrocino I.[1], Fontanella E.[4], Rantsiou K.[1], Houf K.[3], Alessandria V[1].

[1] Department of Agricultural, Forest and Food Sciences, University of Torino, Italy

[2] Department of Veterinary Sciences, University of Torino, Italy

[3] Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Belgium

[4] Servizio Veterinario – Area B, Igiene alimenti di origine animale, ASL CN2, Italy

Keywords: *Arcobacter butzleri*, microbiota, poultry contamination, 16S rRNA, food safety.

Arcobacter spp. is a bacterial genus belonging to the *Arcobacteraceae* family, identified as a zoonotic pathogen worldwide. The pathogenicity of this microorganism is underestimated due to the lack of knowledge and misdiagnosis of infection, often attributed to *Campylobacter*. The present project aims to detect the presence of *Arcobacter* spp. in the poultry slaughterhouse and to evaluate its antibiotic resistance (AR). Neck skin (BNS) and caecum (BC) from 49 broiler flocks were provided by breeders located in North Italy during slaughtering processes and superficial samples from the slaughterhouse environment (SE) were collected after cleaning procedures. Culture-dependent and independent methods were carried out to identify and characterize *Arcobacter* spp.. Three hundred seventy-one colonies were isolated and 320 *Arcobacter butzleri*, 4 *Arcobacter cryaerophilus*, 3 *Arcobacter cibarius*, 2 *Arcobacter thereius* and 1 *Arcobacter skirrowii* isolates were identified by MALDI-TOF MS (confirmed by species-specific PCR). Bio-typing among isolates was performed considering the presence-absence of three virulence-related genes (*irgA*, *hecA* and *hecB*). Based on the molecular profile, the AR of 126 isolates was examined, showing that 67% of the isolates were resistant to at least one antibiotic. *A. butzleri* isolates from slaughterhouse surfaces were more resistant to antibiotics than those from broilers. Bacterial communities of the broiler neck skin, caecum and environmental swabs were evaluated by metataxonomic analysis based on the 16S rRNA gene Amplicon Sequence Variants. *A. butzleri* was found in environmental swabs (9%) and neck skin samples (4%) resulting the only Campylobacterota member uniquely associated to BNS and SE. *Arcobacter* spp. have been isolated from broilers in all production runs and the microbiota analysis revealed possible cross-contamination between carcasses and slaughterhouse surfaces. The AR detected is of great relevance considering the possible transmission of resistance factors and underlying the importance of slaughtering processes optimization to reduce its presence.

Occurrence and antibiotic resistance of *Arcobacter* spp. isolated from poultry slaughterhouses in Northern Italy

Chiarini E.[1*], Buzzanca D.[1], Chiesa F.[2], Botta C.[1], Ferrocino I.[1], Fontanella E.[4], Rantsiou K.[1], Houf K.[3], Alessandria V.[1]

[1] Department of Agricultural, Forest and Food Sciences, University of Torino, Italy
[2] Department of Veterinary Sciences, University of Torino, Italy
[3] Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, Belgium
[4] Servizio Veterinario – Area B, Igiene alimenti di origine animale, ASL CN2, Italy

*elisabetta.chiarini@unito.it

INTRODUCTION

Arcobacter spp. is a bacterial genus belonging to the *Arcobacteraceae* family, identified as a zoonotic pathogen worldwide.

The pathogenicity of this microorganism is underestimated due to the lack of knowledge and misdiagnosis of infection, often attributed to *Campylobacter* spp..

The genus has mostly been detected in farm animals (chickens, cattle, pigs).

The ingestion of contaminated food or water is the most likely route of transmission of these bacteria to humans.

Poultry meats have a higher percentage of samples detecting *Arcobacter* spp. and its contamination has been linked to food outbreaks. The main symptoms of *Arcobacter butzleri* infection are associated with gastrointestinal disorders.

OBJECTIVES OF THE PROJECT:

A) Isolation of *Arcobacter* spp. from poultry slaughterhouses and characterization of the isolates for their antibiotic resistance.
B) Evaluation of the bacterial communities of broiler and slaughterhouse surfaces by metataxonomic analysis.

MATERIALS & METHODS

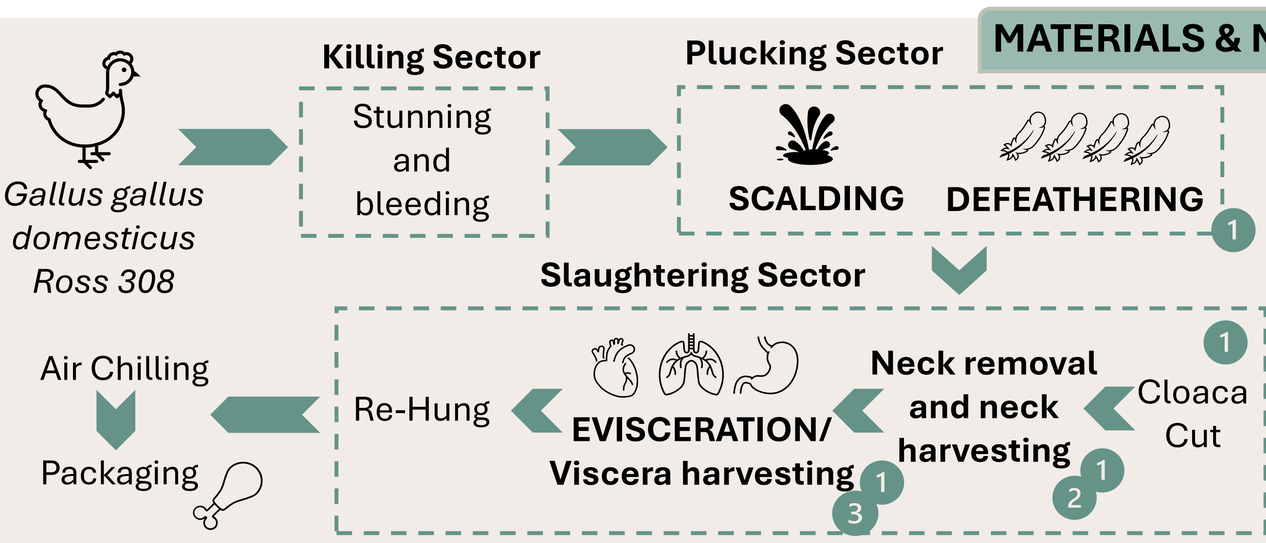


Figure 1. Schematic representation of poultry slaughtering process.

Samples collected during the process:

1 56 swabs from the slaughterhouse environment (SE) 2 49 broiler neck skin (BNS) 3 49 broiler caecum (BC)

Analysis of the samples:

A) Isolation of *Arcobacter* spp. from the samples and identification using the MALDI-TOF MS. Evaluation of the presence-absence of virulence-related genes: *irgA*, *hecA* and *hecB*.
Evaluation of antibiotic resistance to seven antimicrobials.

B) DNA extraction of BC, BNS and SE.
Metataxonomic analysis based on the 16S rRNA gene Amplicon Sequence Variants.

A) RESULTS-CULTURE DEPENDENT ANALYSIS

Isolates identified:

322 isolates of *Arcobacter butzleri*
1 isolate of *Arcobacter cryaerophilus*
1 isolate *Arcobacter thereius*

107 isolates were selected for antimicrobial resistance evaluation. The selection was made considering (i) the vegetable or supplemented feed fed to the animals, (ii) the administration or non-administration of antibiotics and (iii) the profile of the putative virulence genes of each isolate (presence/absence of the *hecA*, *hecB*, *irgA* genes).

The majority of isolates are highly resistant to antibiotics.

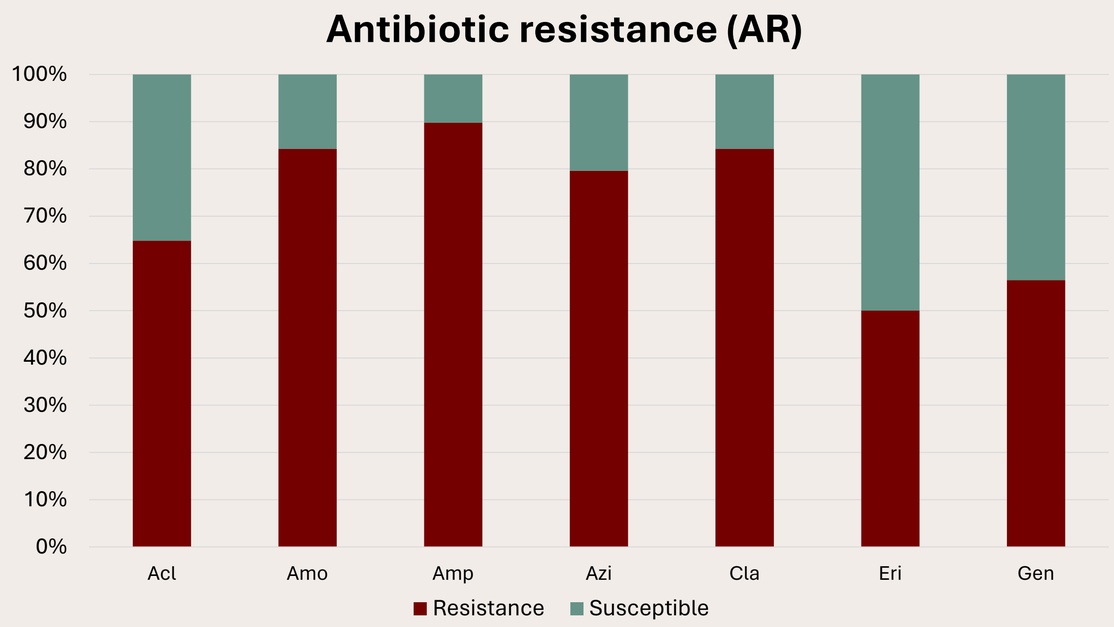
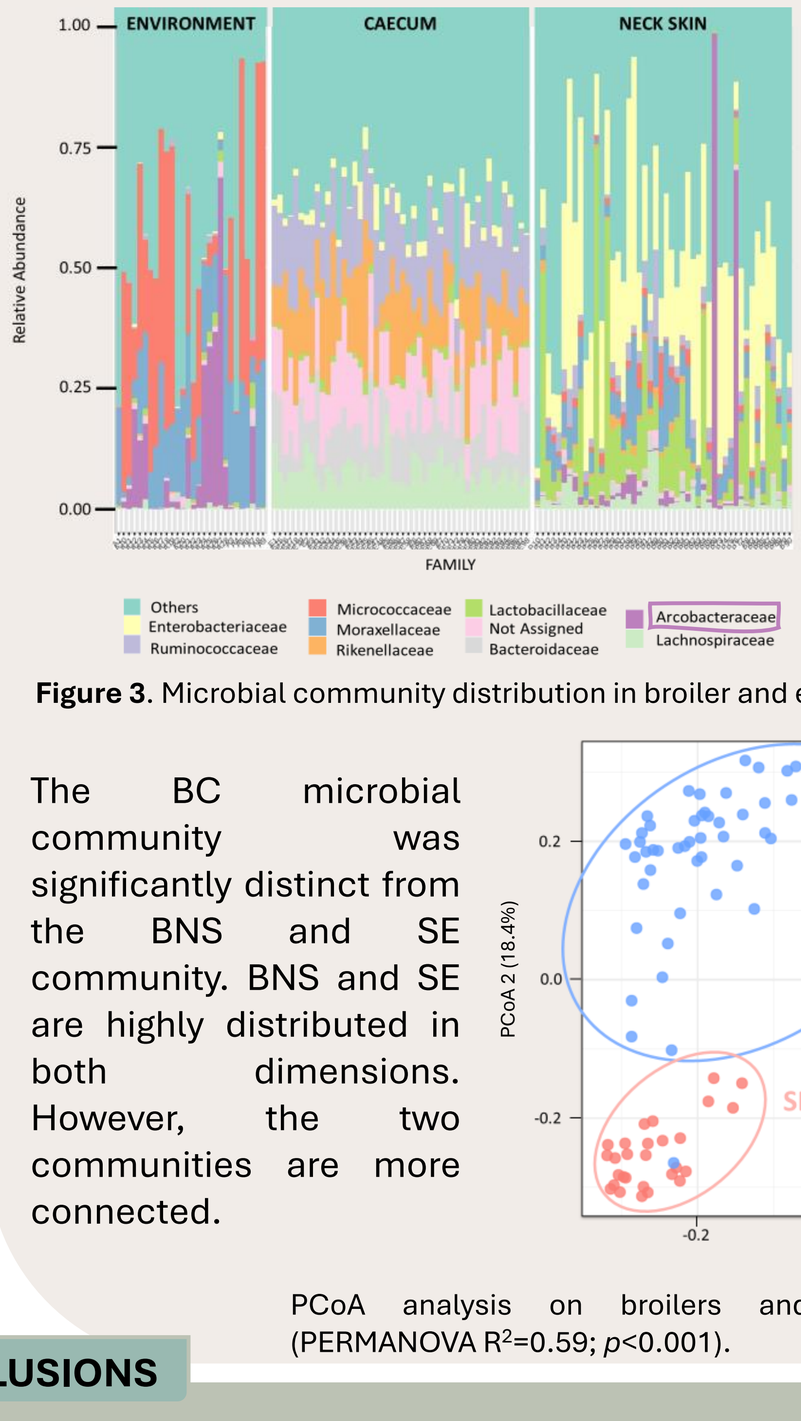


Figure 2. Evaluation of resistance of *Arcobacter* spp. to amoxicillin-clavulanic acid (Acl), amoxicillin (Amo), ampicillin (Amp), azithromycin (Azi), clarithromycin (Cla), erythromycin (Eri) and gentamicin (Gen).

B) RESULTS-CULTURE INDEPENDENT ANALYSIS



A. butzleri was found in environmental swabs (9%) and neck skin samples (4%) resulting the only Campylobacterota member uniquely associated with BNS and SE.

Figure 3. Microbial community distribution in broiler and environmental samples.

The BC microbial community was significantly distinct from the BNS and SE community. BNS and SE are highly distributed in both dimensions. However, the two communities are more connected.

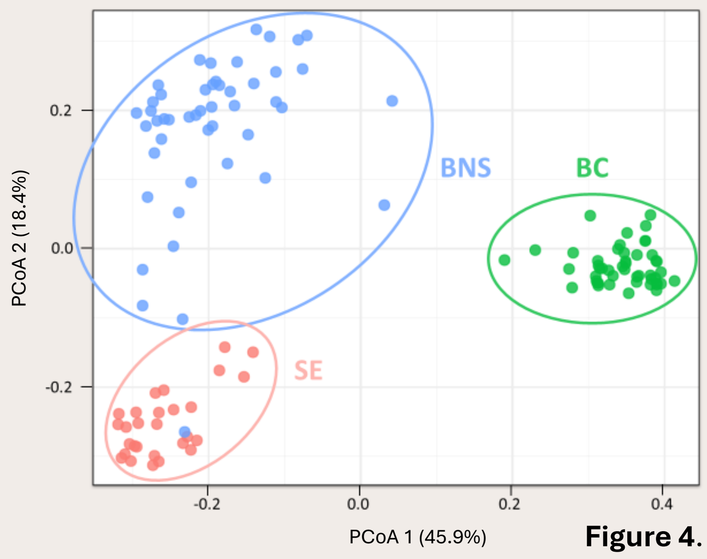


Figure 4. PCoA analysis on broilers and environmental samples (PERMANOVA R^2=0.59; p<0.001).

CONCLUSIONS

Arcobacter spp. have been isolated from broilers in all production runs and the microbiota analysis revealed possible cross-contamination between carcasses and slaughterhouse surfaces. The AR detected is of great relevance considering the possible transmission of resistance factors and underlying the importance of slaughtering processes optimization to reduce its presence.

Research funded by the CRT Foundation.

References

Ferreira *et al*. (2019) 'A meta-analytic perspective on *Arcobacter* spp. antibiotic resistance', Journal of Global Antimicrobial Resistance.
Ramees *et al*. (2017) '*Arcobacter*: An emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - A comprehensive review', *Veterinary Quarterly*.
Isidro *et al*. (2020) 'Virulence and antibiotic resistance plasticity of *Arcobacter butzleri*: Insights on the genomic diversity of an emerging human pathogen', *Infect Genet Evol*.