**Study of persistence and characterization of the food-born zoonotic pathogen *Arcobacter* spp.**

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This PhD project aims at delineating a sector-specific risk assessment related to the presence of *Arcobacter* spp. in poultry and slaughterhouse. Genes connected to antibiotic resistance and virulence will be determined through physiological tests and genome analysis. Gene regulation and response mechanisms under stress conditions of the bacterium will also be analysed. The results obtained will be useful in expanding knowledge of this microorganism, focusing on its pathogenic potential. Special attention will be paid to antibiotic resistance and the possible transmission of resistance factors to humans, considering its high significance for public health.

**Studio della persistenza e caratterizzazione del patogeno zoonotico di origine alimentare *Arcobacter* spp.**

Questo progetto di dottorato mira a delineare una valutazione del rischio specifico nel settore legato alla presenza di *Arcobacter* spp. nella filiera avicola e nei macelli. I geni legati all’antibiotico resistenza e alla virulenza verranno determinati tramite test fisiologici e analisi del genoma. Verrà inoltre analizzata la regolazione genica e i meccanismi di risposta in condizioni di stress del batterio. I risultati ottenuti saranno utili per ampliare le conoscenze su questo microrganismo, concentrandoci sulla sua potenzialità patogena. Verrà posta particolare attenzione all'antibiotico resistenza e alla possibile trasmissione dei fattori di resistenza all’uomo, considerando l’elevata rilevanza per la salute pubblica.

# **1. State-of-the-Art**

*Arcobacter* spp. is a Gram-negative bacterium originally included in the *Campylobacteraceae* family and following a recent taxonomic revision, the genus was reclassified into the family *Arcobacteraceae* (On *et al.*, 2020)*.* The species of greatest importance are *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii* and *Arcobacter thereius* as they are often associated to clinical conditions (Ramees *et al.*, 2017). They are responsible in humans for diseases such as bacteremia, endocarditis, peritonitis, gastroenteritis, and diarrhoea; numerous problems also occur in animals such as diarrhoea, mastitis, and abortions. The pathogenicity of this microorganism is still underestimated due to the lack of knowledge and misdiagnosis of infection, often attributed to *Campylobacter* spp*.* (Collado and Figueras, 2011; Ramees *et al.*, 2017).

The ingestion of contaminated food or water is considered the most likely route of transmission of these bacteria to humans (Ramees *et al.*, 2017). *Arcobacter* spp. has been isolated from the following food products: chicken meat, red meats (pork, beef and lamb), raw milk, seafood and vegetables (Müller *et al.*, 2020). Among these, poultry meat appears to have the higher percentage of samples in which *Arcobacter* spp. has been detected (Zacharow *et al.*, 2015). The distribution of *A. butzleri* throughout the food chain has been amply demonstrated through investigations on food products from the processing stage to retail and on ready-to-eat products. The unequivocal presence of this bacterium on the surfaces of food processing plants such as slaughterhouses or dairies is favoured by the ability to adhere to different materials and to form biofilm under different conditions. In addition, the adhesion of *A. butzleri* to food surfaces is also a source of cross-contamination (Ferreira *et al.*, 2019).

As for *Campylobacter* spp*.*, closely related to *Arcobacter* spp., normally the infection caused by *Arcobacter* does not require antibiotic treatment. However, the severity or prolongation of symptoms may justify the use of antibiotics (Collado and Figueras, 2011; Ramees *et al.*, 2017). The emergence of antibiotic resistance phenomenon makes the use of antibiotics for clinical treatment less efficient (Collado and Figueras, 2011). Unlike *Campylobacter* antimicrobial susceptibility tests in *Arcobacter* species are not standardized. Many *A. butzleri* strains are resistant to clindamycin, azithromycin, ciprofloxacin, metronidazole, carbenicillin, and cefoperazone (Ramees *et al.*, 2017). Contamination of food by highly antibiotic-resistant bacteria is a public health issue considering the possibility of transmission to humans of genes linked to antibiotic resistance (Gungor *et al.*, 2023).

Furthermore, despite the availability of numerous isolation techniques, there is no recommended standard method for isolation of *Arcobacter* spp.. Due to this limiting factors, many of the important cases may be undetected, resulting in underestimation of the prevalence and epidemiological status of this bacterium (Ramees *et al.*, 2017).

# **2. PhD Thesis Objectives and Milestones**

Taking into consideration the aspects highlighted on *Arcobacter* spp., it is essential to expand the knowledge regarding its pathogenicity considering the high exposure to humans. With this purpose, *Arcobacter* spp. isolates from broiler carcasses (gut and neck skin) during slaughtering and from slaughterhouse surfaces will be analysed.

This PhD thesis project can be divided into the following activities according to the Gantt diagram given in Table 1.

A1) **Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR** to identify the genetic diversity of the isolates.

A2) **Physiological tests of *Arcobacter* spp. isolates for virulence assessment:** tests for antibiotic resistance evaluation using the most used antibiotics in medical field; tests of cell colonization on mucus secreting human cell line (HT29-MTX-E12) and assessment of biofilm formation on microplate; detergent susceptibility analysis of isolates from slaughterhouse surfaces.

A3) **Whole Genome Sequencing:** following Illumina sequencing of *Arcobacter* spp. isolates, bioinformatic analyses will be carried out to annotate genes and obtain pangenome-related information (e.g., core and accessory genes).

A4) ***In vivo* pathogenicity studies**: evaluation of the pathogenic potential of selected *A. butzleri* strains in an *in vivo* murine model. Infection will occur by *gavage* method; the severity of the disease will be assessed for 15 days after infection by stool and blood analysis.

A5) **RNA sequencing analysis:** after the detergent treatment, the RNA sequencing analysis conducted on the samples will allow the highlighting of gene regulation and gene pathways involved in cellular metabolism and bacterial stress response mechanisms. The final goal is to assess the persistence of the pathogen under study.

A6) **Thesis and Paper preparation** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1***Gantt diagram for this PhD thesis project.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***ERIC-PCR*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Physiological tests of Arcobacter spp. isolates*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Antibiotic resistance tests |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Cell colonization and biofilm tests |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Detergent susceptibility tests |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Whole Genome Sequencing*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***In vivo tests*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***RNA sequencing analysis*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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