

Food-associated bacteria, including foodborne pathogens such as *Listeria monocytogenes*, are now thought to be one of the main sources and drivers of the spread of antibiotic-resistance genes. Resistance genes acquired by bacteria cause phenotypic resistance. There are cases where bacteria carrying antibiotic resistance genes show sensitivity to antibiotics. Currently, susceptibility tests ignore sensitive strains, focusing only on resistant isolates. Differences between the genotype (set of genes) and phenotype (observed characteristics) of antibiotic resistance result due to the presence of so-called 'silent' antibiotic resistance genes. However, it is possible to reverse the silencing of resistance genes through food preservation methods such as pascalisation. Pascalisation is one of the leading methods of non-thermal food fixation, which uses high pressures as a fixing agent. Although this technology is considered to be effective and to produce safe, high-quality food, there are still many issues related to the potential safety risks of its use. These include the impact on the antibiotic susceptibility profile among food pathogens such as *L. monocytogenes*.

The project therefore involves a comprehensive analysis of antibiotic-susceptible *L. monocytogenes* strains isolated from food and containing 'silent' antibiotic resistance genes, investigating the impact of pascalisation on altering the antibiotic-susceptibility profile of these pathogens and assessing the potential of strains containing 'silent' antibiotic resistance genes to induce phenotypic antibiotic resistance. These objectives will be achieved by performing analyses to determine the frequency of 'silent' antibiotic resistance genes among isolates using Next Generation Sequencing. Subsequently, the potential to alter the antibiotic susceptibility profile by investigating the ability to induce phenotypic resistance will be assessed, and the impact of pascalisation on the degree of change in antibiotic susceptibility of *L. monocytogenes* strains will be investigated, both in terms of changes in antibiotic resistance and expression of 'silent' antibiotic resistance genes (Real-Time PCR method) and induction of phenotypic resistance. The project also involves the selection and characterisation of strains that exhibit the phenomenon of silencing of antibiotic resistance genes through mutations, by determining the frequency of reversion and the molecular basis of this phenomenon (genetic changes in the region encoding resistance genes or regulatory elements of these genes) and the impact of pascalisation on this phenomenon.

The proposed project is an innovative and novel approach to the topic, as it will fill the knowledge gap on the antibiotic resistance potential of susceptibility strains carrying antibiotic resistance genes in the genome. The study will determine whether the phenomenon of 'silencing' of antibiotic resistance among these pathogens is reversible, as well as the effect of specific parameters of the pascalisation process on the potential associated with changes in the antibiotic-susceptibility profile of antibiotic-susceptible *L. monocytogenes* strains (increase in antibiotic-resistance and expression of 'silent' antibiotic-resistance genes). Secondly, the study will use state-of-the-art methods, including whole genome sequencing and bioinformatics methods. The project will also provide information on the mechanism of 'silencing' of antibiotic resistance genes. There is a lack of research in the literature on this topic in the context of food-derived *L. monocytogenes* strains. Studies to date have only focused on the survival of antibiotic-resistant strains or strains. Given that *L. monocytogenes* is one of the most dangerous pathogens found in food, and that the problem of antibiotic resistance of strains isolated from food is still being dynamically researched, this problem undoubtedly requires constant monitoring, especially in the context of emerging new food preservation technologies.