

PhD Thesis Abstract

Title of the Thesis: *The characterization of the bacterial resistome in certain food products sold on the romanian retail market*

Keywords: *Enterobacteriaceae, Escherichia coli, antimicrobial resistance, food products, public health*

The doctoral thesis entitled *The characterization of the bacterial resistome in certain food products sold on the romanian retail market* is structured into two major sections: the first part consists of the bibliographic study, while the second part presents the original research and results obtained during the doctoral studies, organized according to academic methodological standards. The thesis includes the table of contents, introduction, abstract, references, list of abbreviations, list of figures, list of tables, and the list of publications.

Part I - Literature review - This introductory section, structured into three distinct chapters, provides a comprehensive synthesis of the molecular mechanisms and genetic determinants underlying bacterial resistance to antimicrobial agents. Particular emphasis is placed on resistance genes associated with major antibiotic classes, such as aminoglycosides, β -lactams, chloramphenicol, glycopeptides, fluoroquinolones, tetracyclines, and sulfonamides. The final chapter explores the dynamics of antimicrobial resistance within the food sector at global, European, and national levels. This approach highlights the profound implications of the phenomenon for food safety and public health, while also outlining the major challenges inherent to prevention and control strategies targeting this escalating problem.

Part II - Original research and results - This section is composed of three chapters. The first chapter presents the institutional framework in which the investigations were carried out, explicitly defining the general aim and specific objectives of the study, followed by the isolation and identification of bacterial strains from food products. The subsequent chapters focus on the results obtained from antimicrobial susceptibility testing and molecular biology methods, continuing with the analysis of resistance phenotypes and concluding with the detection and characterization of relevant resistance genes.

Chapter 4 details the methodology and results of the investigations concerning the isolation and identification of bacterial microorganisms from various categories of food products. The primary objective of this stage was to evaluate the incidence of bacterial contamination in plant-derived products, based on the analysis of 267 samples of pre-packaged ready-to-eat salads, and in animal-derived products, including 109 poultry meat samples, 90 minced beef samples, and 90 fresh cow cheese samples.

The cultivation and identification protocols for bacterial strains were rigorously implemented according to current international standards. The process included the use of selective and differential culture media, complemented by advanced biochemical and

phenotypic identification techniques, with subsequent confirmation by MALDI-TOF mass spectrometry. To obtain a comprehensive representation of the microbiota present in these products, multiple bacterial strains were isolated and phenotypically characterized from each sample analyzed.

As a result, a total of 246 bacterial strains were identified from plant-derived products, with a significant prevalence of the *Enterobacteriaceae* family (n=116). From dairy products, 205 strains were isolated, of which 126 belonged to *Enterobacteriaceae*.

Poultry meat represented a source of 305 strains, 190 of which were classified as *Enterobacteriaceae*. Finally, 214 strains were isolated from minced beef, with 123 belonging to the same family.

The analysis of plant-derived samples revealed an incidence of contamination with *Escherichia coli* of 2%, while *Enterococcus faecium* and *Enterococcus faecalis* were detected in 2.4% and 4.1% of samples, respectively. Notably, 60% of the salads analyzed presented microbiological contamination, indicating a potential food safety risk. This situation is further exacerbated by the lack of transparency from producers regarding the origin of raw materials, information often being limited to generic indications. Despite the diversity of commercial names and points of purchase, a logistic analysis suggested a possible centralization of salad processing and packaging in common facilities, followed by subsequent differentiation in labeling. This hypothesis is supported by the similarity of microbiological results across different types and batches of salads, suggesting a common origin of raw materials.

In cow cheese products, contamination with *E. coli* was detected in 55% of samples, with 2.4% for *E. faecium* and 4.1% for *E. faecalis*. Poultry meat exhibited the highest rate of *E. coli* contamination of 79.2%, followed by 59% for *E. faecium* and an identical percentage for *E. faecalis*. In beef samples, contamination rates reached 57% for *E. coli*, 50% for *E. faecium*, and 42% for *E. faecalis*. In addition to *E. coli*, other potentially pathogenic species were identified, including *Klebsiella oxytoca*, *Proteus mirabilis*, and *Acinetobacter baumannii*. The presence of these strains, many with the potential to exhibit antimicrobial resistance, amplifies the risk of severe infections, particularly in immunocompromised populations. These results underscore the fact that the investigated food products may represent a significant risk to public health, especially in the context of inadequate processing and storage practices.

To contextualize and validate the scientific relevance of these findings, this chapter also includes a comparative analysis of similar studies conducted across different geographical regions. This approach enabled the evaluation of correlations and discrepancies between the bacterial contamination profiles identified in our samples and those reported internationally, offering additional perspectives on the epidemiology of microorganisms of concern in food safety.

Chapter 5 is dedicated to the phenotypic characterization of antimicrobial resistance profiles in the bacterial strains isolated. Susceptibility testing was carried out using the agar disk diffusion method, employing standardized antibiotic concentrations:

Gentamicin (10 µg), Amikacin (30 µg), Ampicillin (10 µg), Piperacillin/Tazobactam (36 µg), Ciprofloxacin (5 µg), Amoxicillin/Clavulanic acid (30 µg), Aztreonam (30 µg), Chloramphenicol (30 µg), Meropenem (10 µg), Moxifloxacin (5 µg), Tigecycline (15 µg), Cefoxitin (30 µg), Cefotaxime (5 µg), and Trimethoprim/Sulfamethoxazole (25 µg). The interpretation of inhibition zone diameters was conducted according to the standards established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), with automated reading using the ADAGIO system (Bio-Rad).

The results obtained for bacterial isolates from ready-to-eat salads, fresh cow cheese, poultry, and beef revealed heterogeneous yet concerning resistance phenotypes, with high prevalence of resistance to β -lactams, fluoroquinolones, and trimethoprim-sulfamethoxazole, as well as the emergence of isolates resistant to critically important antibiotics such as carbapenems and tigecycline. Enterococcal isolates further demonstrated variable resistance rates to vancomycin, with notable differences across species and food categories.

Overall, the findings emphasize that the investigated food products represent a potential reservoir for multidrug-resistant bacteria, with direct implications for food safety and public health. The identification of multidrug resistance phenotypes and the presence of strains resistant to last-resort antibiotics highlight the urgent need for continuous monitoring and implementation of stricter control measures across the food production and distribution chain.

In Chapter 6, the research focuses on the molecular characterization of antimicrobial resistance mechanisms for a batch of 24 bacterial isolates, primarily *Escherichia coli* and *Klebsiella pneumoniae*, sourced from various food products of animal and plant origin.

Precise taxonomic identification was achieved using MALDI-TOF MS technology, yielding high correlation scores (>2.0) that ensured the reliability of the species determination prior to molecular screening. The results, consolidated in the form of a hierarchical clustering heatmap, revealed a remarkable prevalence of resistance determinants, genotypically confirming the previously observed phenotypic multi-drug resistance profiles.

The analysis identified a predominant group of samples, dominated by *E. coli* isolates from meat, characterized by the universal (100%) presence of the *bla*TEM and *sul1* genes, frequently associated with *bla*CTX-M, *aac-6-Ib*, and *qnrB*. This suggests the dissemination of multidrug-resistant plasmids through the food chain.

In contrast, *K. pneumoniae* isolates from dairy products displayed adaptive specialization through the possession of *bla*SHV and *oqx*B genes, while an *Enterobacter ludwigii* strain from salad demonstrated a critical risk due to the presence of the *qnrS* gene in a product consumed without thermal treatment.

Although a concerning accumulation of PMQR and ESBL-type genes was observed, the complete absence of the carbapenemases KPC, OXA-48, and MBL

confirms the continued viability of these antibiotics as reserve options, consistent with trends reported by EFSA.

The discussions emphasize the robust phenomenon of co-selection, where the selective pressure exerted by sulfonamides indirectly sustains resistance genes to cephalosporins, thereby transforming food products into vectors for the dissemination of resistance within the "One Health" framework.