PhD THESIS

Risk evaluations regarding the contamination of poultry meat with germs of the *Salmonella* and *Campylobacter* species

SUMMARY OF THE PhD THESIS

PhD student **Forgaciu Anca Firuța**

Scientific coordinator **Prof.univ. dr. Marian Mihaiu**

INTRODUCTION

The introduction of antimicrobial substances in therapy has revolutionized medicine, but their excessive or erroneous use has led to the development and spread of microbial resistance, a major global public health issue. In modern animal production, antimicrobials are widely used for curative and prophylactic treatments, as well as for growth promotion, frequently exposing animals to antibiotics. This creates conditions conducive to the emergence, persistence, and spread of resistant bacteria, which can cause human infections through food of animal origin. Resistant zoonotic bacteria can also transfer resistance genes to other commensal or pathogenic bacteria.

Salmonella spp. and *Campylobacter* spp. are the main causes of gastroenteritis and bacteraemia worldwide and infections with these bacteria are among the most common foodborne illnesses in Europe. Despite their importance, epidemiological knowledge about *Campylobacter* spp**.** is still developing.

The World Health Organization (WHO) emphasizes the need for an integrated national system for the surveillance of antibiotic consumption and resistance in zoonotic bacteria to monitor and control emerging resistance patterns, thereby protecting public health. This system should include the monitoring of resistance in *Salmonella* and *Escherichia coli* strains from meat, as meat is a major route of human exposure to resistant bacteria. Additionally, the WHO encourages epidemiological research to understand the mechanisms of resistance development and transfer throughout the food chain.

In Romania, there is not yet a national surveillance system for antimicrobial resistance, and data on the prevalence of resistant zoonotic bacteria in food of animal origin are very limited. In this context, the main objective of this research was to evaluate the contamination of poultry meat with *Salmonella* and *Campylobacter* species, test the susceptibility of these bacteria to antimicrobials, and identify microbial resistance genes.

THE STRUCTURE OF THE PhD THESIS

The doctoral thesis entitled "Risk evaluation regarding the contamination of poultry meat with germs from the species *Salmonella* and *Campylobacter*" comprises 94 pages and presents an iconography totalling 19 figures and 7 tables. It is structured into two parts and is prepared in accordance with the drafting standards of IOSUD USAMV-Cluj-Napoca.

The first part of the thesis consists of 24 pages and is divided into 4 chapters.

Chapter 1, entitled *"Poultry meat production: economic impact at global and national levels, international trade, and the Romanian market*", provides information on the economic impact of poultry farming, aspects related to global poultry meat production, and specifics of broiler production in Romanian poultry companies. It also presents statistical data regarding the import and export of poultry meat in various countries.

Chapter 2, "*Foodborne Zoonotic Diseases*," includes general information on the main bacterial zoonoses identified globally, particularly those caused by bacteria of the genus *Salmonella* and *Campylobacter*, as well as data on the human incidence of salmonellosis and campylobacteriosis.

Chapter 3, "*Microbial Resistance*," provides data on the types of microbial resistance, resistance mechanisms, and the global impact of microbial resistance.

Chapter 4, "*Microbial Resistance - Implications for Food Safety*," analyzes the issue of microbial resistance from the perspective of food safety and highlights its significant implications for both animal and human health.

The second part of the PhD thesis is structured into 6 chapters, spanning 50 pages, and includes information on the working hypothesis and pursued objectives, materials and methods used, as well as the results, discussions, and conclusions related to each of the 3 studies presented. The doctoral thesis concludes with chapters on general conclusions and aspects of originality and innovative contributions.

THE OBJECTIVES OF THE PhD THESIS

This doctoral thesis aimed to evaluate the contamination of poultry meat with germs from the species *Salmonella* and *Campylobacter*. To achieve this, three specific objectives were formulated as presented below:

I. **Risk Assessment of Poultry Meat Contamination with Salmonella spp. Strains Isolated from a Large-Capacity Slaughterhouse Unit**

II. **Evaluation of Prevalence and Antibiotic Resistance of Pathogenic Salmonella Strains Isolated from Poultry Meat Products**

III. **Evaluation of Prevalence and Antibiotic Resistance of Campylobacter jejuni Strains Isolated from Cecal Contents of Slaughtered Broilers**

Study 1. Risk Assessment of Poultry Meat Contamination with Salmonella spp. Strains Isolated from a Large-Capacity Slaughterhouse Unit

Introduction

Poultry meat is a popular food both nationally and internationally, ranking second in consumer preferences within the European Union. It is considered a functional food due to its significant contribution of beneficial nutrients to human health. However, bacteria from the genus *Salmonella* are major foodborne pathogens, causing numerous human infections associated with the consumption of contaminated food, including poultry meat.

Human salmonellosis is one of the most frequently reported zoonoses at the European level, with most infections being foodborne. Infections with *Salmonella* can vary in severity and are influenced by numerous factors, including the serovar involved and the immune status of patients. Statistical data indicate a significant prevalence of infections with *Salmonella* strains associated with poultry meat consumption.

Contamination of carcasses with *Salmonella* spp**.** can occur at various stages of meat processing. Monitoring antimicrobial resistance in zoonotic bacteria is essential for managing public health risks and developing appropriate control and prevention measures.

Aim of the study

The aim of this study was to isolate and identify germs from the genus *Salmonella* in samples from poultry meat collected in a large-capacity slaughterhouse. This was achieved through antemortem inspection of broiler chickens and postmortem inspection of carcasses, followed by bacteriological analysis.

Materials and Methods

The study included a total of 403 samples collected from a large-capacity slaughterhouse unit with two slaughter lines. The antemortem inspection of broiler chickens and the postmortem inspection of chicken carcasses were conducted in two batches, in accordance with current regulations. Isolation and identification of *Salmonella* strains were performed using the automated VIDAS® bioMérieux analyzer, following the manufacturer's protocol.

Results and discussions

All requirements for the antemortem inspection were met, with negative results for salmonellosis testing.

Regarding the postmortem examination, a number of 505 carcasses from the first batch showed various lesions, including bruises and fractures (14.25%), contaminated viscera (11.48%), ascites and edema (10.49%), cellulitis (20.30%), tumors (2.57%), peritonitis and perihepatitis (4.75%), pericarditis (3.36%), overexertion (0.19%), hepatitis (10.69%), dermatitis (1.38%), poor defeathering (0.79%), death before slaughter (0.39%), death on arrival (2.57%), contamination (2.97%), and fever (4.75%).

For the second batch, 503 carcasses exhibited lesions, including bruises and fractures (6.75%), contaminated viscera (28.23%), ascites and edema (10.93%), cellulitis (14.91%), tumors (1.39%), peritonitis and perihepatitis (4.57%), pericarditis (0.39%), overexertion (2.98%), hepatitis (3.57%), dermatitis (0.79%), poor defeathering (1.98%), death before slaughter (0.99%), death on arrival (3.77%), contamination (10.93%), and fever (7.75%).

Out of the 403 samples analyzed for the evaluation of poultry meat contamination with germs from the genus *Salmonella*, 397 were compliant and 6 samples were non-compliant due to the identification of *Salmonella infantis*, indicating a contamination rate of 1.49%.

The contamination level of poultry meat with germs from the genus *Salmonella* can be influenced by various factors, such as the transport of animals from the farm to the slaughterhouse, including the duration of transport, as well as slaughter processes. Adherence to regulations regarding washing, immersion, or cooling procedures is crucial in this context, as it can significantly reduce contamination.

The presence of *Salmonella* spp**.** on the carcasses identified in this study was not associated with specific lesions found during the postmortem examination. Therefore, a direct correlation between them could not be established.

Conclusions

This study aimed to conduct antemortem inspections of broiler chickens and postmortem inspections of carcasses, followed by bacteriological analysis. The antemortem inspection focused on document control and negative certification in the bacteriological examination for salmonellosis, as well as evaluating the health and welfare conditions of the birds prior to slaughter. The results obtained in this respect were in compliance with specific legislation.

The postmortem examination of both batches included in the study revealed the following types of lesions: bruises/fractures, contaminated viscera, ascites/edema, cellulitis, tumors, peritonitis/perihepatitis, pericarditis, overexertion, hepatitis,

dermatitis, poor defeathering, death before slaughter, death on arrival, contamination, and feverish state.

Surveillance of poultry meat for contamination with germs from the genus *Salmonella* through the collection of representative samples from the neck skin in batch 1 revealed 6 non-compliant samples out of a total of 403 samples (contamination rate of 1.49%).

A better collaboration between the authorities involved in the prevention and control of zoonotic diseases and the economic operators within the food chain is especially recommended.

Study 2 – Study on the Prevalence and Antibiotic Resistance of Pathogenic Salmonella Strains Isolated from Poultry Meat Products

Introduction

Bacteria of the genus *Salmonella* are among the most important foodborne pathogens, frequently causing zoonoses globally and representing a significant public health problem. Infection with *Salmonella* spp. often manifests as gastroenteritis caused by numerous non-typhoidal serovars. These infections are predominantly associated with the consumption of contaminated foods such as meat, eggs, milk, and dairy products. Poultry meat is a food product frequently implicated in the contamination with *Salmonella* strains, justifying the implementation of a surveillance system in slaughtering facilities. Antimicrobial resistance is an emerging problem, limiting therapeutic options for human salmonellosis. The excessive use of antimicrobial agents in animal production and human therapy has promoted the emergence and spread of resistant bacteria, with numerous *Salmonella* strains showing resistance to multiple classes of antimicrobial agents, leading to more severe infections, therapeutic failures, and significant societal costs. Monitoring bacterial resistance is essential for tracking this phenomenon and developing appropriate control strategies.

Aim of the study

The objective of this study was to identify the prevalence of *Salmonella* strains in poultry meat products, to test their susceptibility to antimicrobials, as well as to identify and evaluate the presence of resistance genes among the isolated strains.

Materials and Methods

The study included a number of 112 *Salmonella* strains isolated from chicken meat and chicken meat products collected between 2011-2021 in Romania. The

isolation of *Salmonella* spp. from meat samples was performed according to the standardized protocol SR EN ISO 6579/2003/AC/2017, while the determination of the susceptibility profiles to tested antimicrobial agents was carried out using the diffusion method, according to CLSI guidelines, using biodiscs impregnated with different amounts of active substance. The confirmation of the identified *Salmonella* spp. strains and the identification of specific resistance genes was performed using the molecular PCR method (polymerase chain reaction). The results obtained were subsequently statistically interpreted using specific tests.

Results and discussions

Of the total *Salmonella* strains included in the study (n=112), isolated between 2011-2021, 56% were found to be positive for *Salmonella* Enteritidis (*S*. Enteritidis) (n=63), while 25% were positive for *Salmonella* Typhimurium (*S*. Typhimurium) (n=28). Based on the results of antimicrobial susceptibility testing of the pathogenic *Salmonella* strains included in the study (n=91), an increase in resistance levels was observed over the years 2011-2021. Of the 91 *Salmonella* strains tested, 78 were found to be MDR. High levels of resistance to antibiotics such as tetracycline (74%), sulfonamides (54%), or ampicillin (32%) were also observed. The vast majority of the isolates were susceptible to cephalosporins and quinolones, with only 8% and 4% respectively showing resistance to these two classes of antimicrobial agents. The prevalence of pathogenic MDR *Salmonella* strains showed an increasing trend during the period 2015-2021 (n=34), compared to the period 2011-2014 (n=28). The prevalence of resistance genes was closely correlated with the resistance patterns. The most prevalent resistance genes identified in the study were those encoding tetracycline resistance, specifically *tetA*, *tetB, and tetC* (n=79%), as well as genes encoding resistance to sulfonamides (*sul1, sul2*) (n=85%).

According to the results of the present study, *Salmonella* strains were isolated from meat and poultry meat products every year during the 10-year period included in the evaluation. The most prevalent serovar identified in the study was *S*. Enteritidis (n=56%), a result similar to data reported in other previous studies. The present study also identified high levels of resistance to commonly used antimicrobial agents in poultry production, such as tetracycline (84%), nalidixic acid (78%), or ampicillin (78%). These results are consistent with those reported in other similar studies. Regarding the presence of resistance genes among the analysed strains, the *sul1* gene (93.4%) was the most frequently isolated, followed by *tet* genes (86.8%) and *blaTEM* (64%). Four strains carried the *cat1* gene, responsible for chloramphenicol resistance, a type of resistance less frequently reported in studies conducted in the field. Overall, the prevalence of resistance genes was correlated with the phenotypic characteristics of the isolates. The resistance patterns identified differed in the two time intervals analyzed (2011-2015 and 2016-2021), which could be influenced by a change in the therapeutic protocol over time.

Conclusions

This study is one of the most comprehensive studies on the prevalence and antibiotic resistance of pathogenic *Salmonella* strains isolated from meat and poultry meat products conducted in our country. The results indicated high levels of resistance to tetracycline, nalidixic acid, ampicillin, and streptomycin. Therefore, more judicious administration of these compounds in the therapy of *Salmonella* spp. infections is recommended.

Study 3. Study on the prevalence and antibiotic resistance of Campylobacter jejuni strains isolated from cecal content of slaughtered chickens

Introduction

Infection with *Campylobacter* spp. is one of the most common bacterial causes of human gastroenteritis worldwide, but epidemiological studies are still ongoing. Most infections occur sporadically, without an obvious source, and can lead to severe complications, such as Guillain-Barre syndrome. Poultry and their products are the main vehicles of infection. Industrial slaughter of poultry presents considerable risks of bacterial contamination, but reducing the bacterial load on carcasses could significantly reduce cases of human campylobacteriosis. With the necessary monitoring of *Salmonella* spp. and the planned introduction of *Campylobacter jejuni* for process hygiene criteria for slaughtered broilers in the European Union (EU), microbiological data are needed to implement appropriate measures in the slaughtering process.

Aim of the study

The purpose of this study was to identify the prevalence of *Campylobacter jejuni* strains in caecal content samples from birds and to evaluate their susceptibility to antimicrobials.

Materials and Methods

The study was conducted on a number of 147 caecal content samples collected from slaughtered chickens from poultry slaughterhouses in the counties of Cluj, Maramureș, and Satu-Mare in 2022. After isolating *Campylobacter jejuni* according to the

standard protocol, antimicrobial susceptibility was tested using the microdilution method in microtiter plates.

Results and discussions

Of the total samples included in the study (n=147), 61 (41.49%) were found to be positive for *Campylobacter jejuni*. Based on the results of the antimicrobial susceptibility testing of the isolated *Campylobacter jejuni* strains, it was found that 42 strains (68.85%) were resistant to ciprofloxacin, while 24 strains (39.34%) showed resistance to tetracycline. Also, a significant number of strains were resistant to nalidixic acid (40; 65.57%). In the present study, a lower prevalence of resistance to gentamicin and streptomycin was identified. All strains were susceptible to erythromycin.

Foodborne infection caused by *Campylobacter jejuni*, frequently reported worldwide, can also be due to errors occurring in the technological process of birds intended for human consumption. In the present study, 41.49% of the collected samples were found to be positive for *Campylobacter jejuni*, with results similar to those reported in other countries. Regarding antimicrobial susceptibility, the results indicated a high level of resistance to ciprofloxacin (68.75%) and nalidixic acid (65.57%), but also to tetracycline (39.34%). None of the isolated strains showed resistance to erythromycin, and only a small percentage of isolates were resistant to streptomycin (8.2%) and gentamicin (1.64%).

Conclusions

The high prevalence of *Campylobacter jejun*i strains resistant to antimicrobials is recognized as a public health problem both in human and veterinary medicine. The veterinarian must ensure the health of animals and contribute to reducing or even avoiding errors in the slaughtering flows of animals intended for human consumption, so as not to negatively affect the health of the population. In the context of the results obtained in the present study, it is recommended to improve laboratory examinations regarding *Campylobacter jejuni* before poultry meat products are released for consumption, as well as to improve consumer knowledge regarding the preparation of poultry meat.

GENERAL CONCLUSIONS

- The evaluation and monitoring of the contamination with *Salmonella* spp. are crucial elements in the prevention of salmonellosis. A better collaboration between the authorities involved in the prevention and control of zoonotic

diseases and the economic operators operating within the food chain is especially recommended.

- The results obtained in the second study indicated high levels of resistance of the isolated *Salmonella* strains to tetracycline, nalidixic acid, ampicillin, and streptomycin, as well as the presence of associated resistance genes. Therefore, a more judicious administration of the aforementioned compounds in the therapy of *Salmonella* spp. infections is recommended.
- The results indicated in the third study showed a high prevalence of *Campylobacter jejuni* in cecal content samples and a high level of resistance to ciprofloxacin, nalidixic acid, and tetracycline.
- Based on the results and conclusions presented, closer collaboration between regulatory authorities and food industry operators is recommended to improve the control of salmonellosis and campylobacteriosis within the food chain. Additionally, a more rational use of antibiotics, especially those for which high levels of resistance have been identified in the studies conducted, is necessary to reduce the selection pressure on pathogenic bacteria.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

At an international level, this thesis contributes to the enrichment of knowledge in the field through publications (both ISI and BDI) on the prevalence and antibiotic resistance of *Salmonella* and *Campylobacter* species in fecal content, meat, and poultry meat products. It also addresses the mechanisms of resistance development and the potential role of animal-origin foods in the dissemination of multi-resistant bacterial strains.

The studies included in this doctoral thesis provide new and up-to-date data on the presence of resistant bacteria in animal-origin foods, the associated resistance patterns, and the presence and dissemination of resistance genes within the food chain. Furthermore, the thesis encompasses one of the most comprehensive studies on the prevalence and antibiotic resistance of pathogenic *Salmonella* strains isolated from poultry meat and meat products conducted in our country. The studies conducted during the doctoral research period contribute to the understanding of resistance mechanisms and the dynamics of its transmissibility within the food chain. The resulting publications increase the international visibility of the research conducted, with the potential to transfer the obtained results into medical and socio-economic practice, aiming to protect public health and improve the quality of life.