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MASTER DISSERTATION

Theme

Prevalence and antibiotic susceptibility of *Salmonella* strains isolated from chicken carcasses, chicken offal and chicken intestines

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Table of content

Table of Contents

Abstract

List of abreviation

List of abbreviations

ATM : aztreonam

AMP :ampicilline

AMC :*amoxicilline*

CIP : Ciprofloxacine

CEZ:cefazoline

CTX :ceftazidim

ERT : ertapenem

FOX :cefoxitine

GEN : Gentamicine

GMT : gelose touati modified

H2S :hydrogene sulfure

ISO : International organization for standarization

LEM : laboratory of ecologymicrobial

MDR : Multiple Drug Resistance

MIC : Minimum inhibitory Concentration

NTS : non-typhoidal *Salmonella*

RV: Rappaport Vassiliadis

TDA: Tryptophan desaminase

TSI: Triple sugar Iron Agar

WHO: World Health Organization

XLD: Gelose xylose lysine desoxycholate

List of figure

List of figures

List of table

List of tables

INTRODUCTION

Salmonella is a Gram-negative zoonotic pathogen (Yang *and al.*, 2018)it is one of the major causes of foodborne infectious diseases, posing a serious threat to public health. It can enter the food supply chain at different stages of production, processing, distribution, and marketing (Shen *and al.*, 2021). Gastroenteritis is the most common manifestation of *Salmonella* worldwide, followed by bacteremia and enteric fever (Heredia and García, 2018) *Salmonella* is mainly found in poultry, eggs and dairy products Fresh fruits and vegetables are other food sources implicated in the transmission of *Salmonella* (Eng *and al.*, 2015)

In general, food animals such as pigs, poultry and cattle are the main sources of *Salmonella* infection. The main routes of dissemination of pathogens are trade-in animals and uncooked animal food products. The slaughter process of food animals in abattoirs is considered to be one of the main sources of contamination of organs and carcasses with *Salmonella*. The emergence of antibiotic-resistant foodborne pathogens has raised public concern as these pathogens are more virulent and lead to the increased mortality rate of infected patients(Eng *and al.*, 2015)

Although different serotypes have been associated with salmonellosis, a limited number of them are responsible for most human infections; S. enterica Enteritidis being the most common in the EU (39.5% in 2013) and the USA (14.5% in 2012), followed by *S. enterica Typhimurium* (including its monophasic variant) (28, 8% in EU, 2013; 11.6% in the USA) *Salmonella* Enteritidis is commonly associated with poultry and its products, while *S. Typhimurium* has a wider range of species, including pigs and poultry. Cattle as well as poultry. Therefore, foods of animal origin, especially contaminated poultry products (eggs and poultry meat), have been considered the main vectors of *Salmonella* infection and associated with the global outbreak of S. Enteritidis In Furthermore, various epidemiological studies have confirmed the great contribution of poultry-based foods to the burden of salmonellosis (Antunes *and al.*, 2016)Increasing human population and urbanization, per capita income, globalization, and changes in consumption trends (more protein in the diet) have increased the consumption of animal products. The consumption of these products is estimated to reach 376 million tonnes in 2030 (Dhama and al. 2013).

This high demand for animal products results in intensive animal production and processing of products, with an increased circulation of food on a global level. This situation could lead to faulty processing practices and an increased risk of contamination with

foodborne pathogens at any point in the farm-to-table chain. Animal origin is a serious concern because it is difficult to control. Many factors can cause contamination, including those from the environment (associated wildlife, water from different sources, disposal of animal waste, etc.) and human handling of animals (slaughtering and processing practices, storage procedures, etc. Microbial pathogens can cause disease through the consumption of animal products contaminated with microorganisms or their toxins (Heredia and García, 2018)

These microorganisms can proliferate via the food chain and a pool of resistance genes can be transmitted to human pathogens, reducing the availability of effective drugs to treat infectious diseases caused by these microorganisms The increasing isolation of *Salmonella* antibiotic-resistant in humans and other animals is a public health concern *Salmonella* that is resistant to antibiotics can be found in multiple surveys using pulsed gel electrophoresis. Strains of *Salmonella* are reported to possess the bla_{CMY} gene, which results in plasmid-level resistance to ceftiofur and ceftriaxone, and are multidrug-resistant (Medeiros *and al.*, 2011) Thus, the main objectives of our survey were to report the prevalence of *Salmonella* present in the carcass and intestine of chicken and chicken offal and dairy products (cheese, cream, milk) in Bejaia. Furthermore, the antibiotic resistance profiles of these isolates.

Bibliographic synthesis

Taxonomy and nomenclature

Salmonella is an enterobacteria of the Enterobacteriaceae family. The genus *Salmonella* has 2 species: *Salmonella enterica*, and *Salmonella bongori* (Judicial Commission Of The International Committee On Systematics Of Prokaryotes 2005). *Salmonella* received its name from Daniel E. Salmon, the veterinarian who was the first to isolate (what was then called) "*Bacillus choleraesuis*» from pig intestines in 1884. This name was changed in 1900 to "*Salmonella* choleraesuis" by Lignieres.

Today, the genus *Salmonella* is divided into only two species*: Salmonella* enterica and *Salmonella bongori*, with *S. enterica* being divided into 6 additional subspecies. In the past, the subspecies of *S. enterica* were considered as subgenera and the serovars/serotypes of *Salmonella* as distinct species, which, according to what is done today, would give more than 2,600 species of *Salmonella.* The terms "serovars" and "serotypes" are generally considered synonymous. The World Health Organization (WHO)/Institut Pasteur use the term "serovar", while the Centers for Disease Control (CDC) and the American Society for Microbiology (ASM) originally used the term "serotype", but gradually replaced it with "sérovar" to maintain international consistency (Oludairo *and al.*, 2022).

The nomenclature of *Salmonella* is complex, and scientists use different systems to refer to and communicate about this genus. However, consistency in *Salmonella* is necessary for communication between scientists, health officials, and the public. Unfortunately, current usage often combines multiple nomenclature systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serovars (serovars), which is confusing(Brenner and al. 2000) . The nomenclature of the genus *Salmonella* has evolved from the initial concept of a serotype and a species proposed by Kauffmann based on the serological identification of O (somatic) and H (flagellar) antigens. Each serotype was considered a separate species (eg, *S. Paratyphi* A, *S.* Newport, and *S. Enteritidis*); this concept,if used today, would result in 2,463 species of *Salmonella.* Other taxonomic proposals have been based on the clinical role of a strain, on the biochemical characteristics that divide serotypes into subgenera, and finally, on genomic relatedness. Proposals for nomenclature changes within the genus have been summarized previously (Brenner *and al.*, 2000).

Salmonella enterica is divided into six subspecies, enterica (I), salamae (II), arizonae (IIIa), diarizonae (IIIb), indica (IV), houtenae (VI) (Wang et al., 2020) More 60% of all

Salmonella and 99% of serovars causing disease in warm-blooded animals are members of subspecies I. Other *Salmonella*, particularly subspecies IIIa (Arizona) and S. bongori, are associated with disease in cold-blooded organisms with Arizona and are occasionally responsible for systemic disease in humans. What is particularly intriguing about subspecies I serovars is that their ability to cause disease in animals encompasses a spectrum of host specificity and disease severity (Chan *and al.*, 2003).

Bacteriology

Salmonella is a genus belonging to the *Enterobacteriaceae* family. It is a Gram-negative, facultatively anaerobic, oxidase-negative, catalase-positive, non-spore-forming bacillus. The size varies between 2.0 and 5.0 μm in length by 0.7 to 1.5 μm in width. Most *Salmonella* species are motile by peritrichous flagella, except for *S.* Gallinarum, *S.* Pullorum and some mutants. The optimum growth temperature for *Salmonella* is 37°C. However, minimal growth was recorded between 2 and 4°C and at a maximum temperature of 54°C (Boubendir ;2019).

Transmission

Transmission of nontyphoidal *Salmonella* to humans can occur through ingestion of contaminated food or water, consumption of infected animals, or direct or indirect contact with *Salmonella*-infected animals in homes, veterinary clinics, zoos, or other public or private places (Eng *and al.*, 2015). The consumption of raw or undercooked meat remains a risk factor for salmonellosis.

Kitchen practices such as frequency of cleaning surfaces or not using a cutting board for raw meat are specific risk factors for *Salmonella* infection (Mughini-Gras and al., 2017) . Contact with animals is responsible for several cases of human salmonellosis each year, and the risk to public health varies between animal species, age group, husbandry practice and state of health. Certain human subpopulations are considered more at risk due to biological or behavioral risk factors.Many human infections caused by direct contact with animals such as cattle, horses, cats, dogs etc. are attributed to occupational causes, the agricultural environment and contamination at the family level (Hoelzer and al., 2011).

Reservoir and Host

The host specificity of certain *Salmonella* depends on the ability of the serovar to adapt to the environment of its hosts. This specific ability to adapt to the host environment is regulated by several microbial characteristics, which are responsible for the expression of clinical manifestations in specific hosts. Other important determinants are the infectious dose of a particular serovar, the animal species infected, the age of the host, and the immune response. It has been shown that a particular mechanism making a serovar virulent for a particular animal species could make the same serovar less or even avirulent for another animal species. This phenomenon is called "serovar host specificity" or "serovar host adaptation" (Jajere; 2019)

*Salmonella*is an intestinal pathogen (D'Aoust; 1994) present in the intestines of humans and animals -their main reservoir- they can, following fecal contamination, survive in the environment (water and soil) for several months (Korsak; 2004) Their ubiquity results in a large spectrum of reservoirs: humans and animals, mammals, birds. Their ability to survive also allows them to persist in secondary reservoirs such as foods of animal origin, fruits and vegetables (Todd, Greig and al. 2008).

Antibiotic resistance

In developed countries, antimicrobial drug resistance in non-typhoidal *Salmonella* organisms is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals. Such drugs may be used either therapeutically or prophylactically, or for growth promotion (feed additives). Despite legislation targeted at controlling the overall usage of antimicrobials in food-producing animals, in recent years there have been significant increases in developed countries in the occurrence of resistance in non-typhoidal *Salmonella* spp. Such increases have been observed in many countries, not only in Europe but also in North America. Of particular concern in such organisms is the development of resistance to key antimicrobials such as fluoroquinolones and more recently extended-spectrum β-lactamases.

In developed countries, it is increasingly accepted that most of these strains are of zoonotic origin and acquire their resistance in the animal host before being transmitted to humans through the food chain (Threlfall ;2002)

In the early 1960s, the first incidence of resistance of *Salmonella* to a single antibiotic, namely chloramphenicol, was reported. Since then, the frequency of isolation of *Salmonella* strains with resistance to one or more antimicrobial agents has increased in many countries, including the United States, United Kingdom and Saudi Arabia. Antimicrobial agents such as ampicillin, chloramphenicol, trimethoprim and sulfamethoxazole are used as traditional first-line treatments for *Salmonella* infections. *Salmonella* spp. resistant to these agents are called multidrug-resistant (MDR) (Eng and al; 2015)

Of particular importance in the 1990s was a multidrug-resistant strain of *Salmonella* typhimurium of definitive phage type (DT) 104, showing resistance to six commonly used antimicrobials, with approximately 15% of isolates also showing reduced susceptibility to ciprofloxacin. Mutations in the *gyrA* gene of these isolates were characterized by a LightCycler PCR-based *gyrA* mutation assay, and at least four different mutations were identified. Multiple resistances (to four or more antimicrobials) are also common in poultry-associated pathogens (Eng and al; 2015).

Discussion

Salmonella is a zoonotic pathogen with significant economic and health effects on animals and people worldwide, The most common method of transmission of *Salmonella* to humans is through the consumption of contaminated food
(Boubendir;2019)

Chicken consumption is increasing considerably in Algeria. This increase is mainly due to its reasonable price, its appreciable taste and its nutritional value (Lounis and al; 2020). This study aims to study the prevalence and resistance to antibiotics in the chicken carcass, chicken offal and chicken intestine sold in certain butcher shops in Bejaia province, concerning its consumption leading to food poisoning. It is therefore important to identify the contamination of the product by *Salmonella* and to develop new control methods adapted to the different poultry farms, slaughterhouses and butcheries. The analysis of 199 samples including 124 chicken carcasses, 33 chicken intestines, and 42 chicken offal revealed that 0.50% of the samples contain *Salmonella* which represents *Salmonella* from chicken offal that does not exceed the threshold of $5.10^{5\ germs/}$grams, while the prevalence found on the chicken carcass and chicken intestine is 0%, *Salmonella* is a pathogenic gene, despite its presence at a low load sufficient to cause fatal damage, The presence of this pathogen is interpreted either by a lack of hygiene during handling or poor storage conditions or by contamination during slaughter, transport, the environment and personnel (BOUDOUIKA and al ;2017)

The prevalence of *Salmonella* contamination in bejaia butcher shops from chicken offal is 0.50%, while for the chicken carcass and chicken intestine is 0%, the latter result represents a low rate. of contamination, but in disagreement with the prevalence found a few years ago from the Algiers province revealed a prevalence of 6.67% at the level of trade (Hamitouche et al; 2012), in comparison to the result of the year 2020 *Salmonella* was found in 16% (16/34) of poultry samples collected during this study, thus a prevalence of 4% from chicken organs and intestines (Addalou, Barach, and Touati 2021). In other times the prevalence was estimated at 0% in Draa ben kheda in the city of tizi ouzou in 2019 (AFFADJEN Imane and al; 2019) which is in accordance with the results obtained in Algiers in 2020 shows a prevalence of 0% (Lounis and al; 2020). In other countries such as Meknes in MOROCCO from September 2005 to October 2007, 25 broiler farms sampled revealed 25% of farms infected with *Salmonella* (Abdellah et al). In other cases in Egypt, the incidence of *Salmonella* in the raw chicken liver was 40% (El-Aziz 2013). We first note that the prevalence at the level of

breeding and slaughterhouses studied in previous years is more important contrary to recent studies carried out on samples collected from different butcheries represents a low rate of this pathogenic agent this explains on the one hand, the environmental conditions of cleaning and disinfection. On the other hand, can be explained by the stress that the samples undergo during their transport and then their conservation under the cold regime. In our study we ended up with a total absence of *Salmonella* in all of the 124 chicken carcasses and 33 chicken intestines is a low prevalence of chicken bats, which can explain on the one hand the norm of hygiene implemented in the industry in farms and on the other hand, the detection threshold of the environment which is low. After having tested different antibiotics (aztreonam, cefotaxime, cefoxitin, ertapenem, amoxicillin) on the strain of *Salmonella* is found to be sensitive to all antibiotics in this case there is a high probability of therapeutic success. in disagreement with the previous study, their results showed high rates of antimicrobial resistance were reported against tetracycline (56.25%) and ciprofloxacin (18.75%) (Addalou, Barache, and Touati 2021).

MIC test of *Salmonella* was sensitive to gentamicin (MIC=0.5), ertapenem, and cefotaxime and resistant to cefazolin (MIC>8), ampicillin (MIC>16) and ciprofloxacin (MIC=4), in another CMI test study shows student resistance to ciprofloxacin superior at 8 µg / l, gentamicin showed a low level of resistance (Addalou, Barache, et Touati 2021).

Following our study, the following proposals are essential to minimize the risks associated with the appearance of foodborne *Salmonella*. These proposals are based on the implementation of control measures at all stages of the food chain.

For industries:

- Screening animals before slaughter to limit the number of pathogens at the slaughter site, and the application of good hygienic practices at slaughter reduces the contamination of carcasses by feces.
- Regular decontamination of preparation tools
- Training for staff in food hygiene rules and the HACCP system is an essential measure.

For people who handle food:

- Must be vigilant in the preparation of these foods and respect the rules of hygiene that apply to this preparation.

- Professional food handlers who experience fever, diarrhea, vomiting, or visibly infected skin lesions should report it to their employer immediately.

For the public:

- Make sure that food is properly cooked and still hot when served
- Get into the habit of cleanliness, and wash your hands thoroughly and frequently with soap, especially after contact with animals.
- Breeding or companionship or after going to the toilet.
- Separate raw foods from cooked foods.
- Keep food at the right temperature, especially respecting the cold chain.

National surveillance systems are important means to know and monitor the quality of these foods, and therefore to detect and react to their onset to prevent them from spreading.

Conclusion

This project has made it possible to highlight new knowledge regarding the prevalence and distribution of *Salmonella* on chicken carcasses, chicken offal and chicken intestines in Bejaia. Thus their resistance to antimicrobials has been determined, and one strain of *Salmonella* detected.

The low rate of *Salmonella* contamination reported in this study shows that despite the hygiene practices carried out, the bacterium has not been eradicated. Consequently, measures would make it possible to reduce contamination throughout production. It is indeed fundamental to respect certain hygiene practices. Additionally, no results show the widespread presence of antimicrobial-susceptible *Salmonella* strain, which is why it may be a good alternative for treatment.

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Résumé

Salmonella est l'un des agents pathogènes les plus souvent associés aux maladies d'origine alimentaire dans le monde. Les produits animaux, notamment la viande de poulet, sont considérés comme les principales sources de contamination. L'objectif de cette étude était d'isoler, d'identifier à fin de générer des nouvelles connaissances quant à la prévalence et la distribution de *Salmonella* sur les carcasses de poulets, abats de poulets et intestins de poulets recueillis à partir de différentes boucheries de Bejaia et tester leur sensibilité à certains antibiotiques. Au cours des 2 mois de la phase terrain de cette étude. Un total de 199 d'échantillons (carcasses de poulet, abats de poulets et intestins de poulets) ont été récupérés de différentes communes de Bejaia. Après isolement, les isolats suspectés ont été identifié à l'aide des tests biochimiques. Après cela, les souches ont été soumis à des tests de sensibilité aux antimicrobiens avec 5 antibiotiques pour la méthode de diffusion par disque, tandis que pour la méthode de CMI nous avons testé 4 antibiotiques. 0.50% d'échantillon était contaminée par *Salmonella* provenant d'un abat de poulet .ainsi, cette isolatest révélé sensible à tous les antibiotique testé : aztreonam, cefotaxime, cefoxitin, ertapenem et amoxicillin. Tandis que pour les CMI La souche de *Salmonella* était sensible à la gentamicine (MIC=0,5), à l'ertapeneme et au céfotaxime et résistante à la céfazoline (MIC>8),à l'ampicilline (MIC>16) et à la ciprofloxacine (MIC=4). Bien que les pratique d'hygiène effectué par l'ensemble des producteur et vendeur ont aboutis à un faible taux de germe menacent le consommateur.

Mots clés : Prévalence, *Salmonella,* carcasse de poulet, abats de poulet,intestin de poulet, sensibilité aux antibiotiques.

Abstract

Salmonella is one of the most common pathogens associated with foodborne illness worldwide. Animal products, especially chicken meat, are considered the main sources of contamination. The objective of this study was to isolate, identify and generate new knowledge about the prevalence and distribution of *Salmonella* on chicken carcasses, chicken offal and chicken intestines collected from different butcheries in Bejaia and test their sensitivity to certain antibiotics. During the 2 months of the field phase of this study. A total of 199 samples (chicken carcasses, chicken offal and chicken intestines) were collected from different municipalities in Bejaia. After isolation, suspected isolates were identified using biochemical tests. After that, the strains were tested for antimicrobial susceptibility with 5 antibiotics for the disk diffusion method, while for the MIC method we tested 4 antibiotics, and an average of 0.50% of the sample was contaminated with *Salmonella* from chicken offal. Thus, this isolate was found to be sensitive to all the antibiotics tested: aztreonam, cefotaxime, cefoxitin, ertapenem and amoxicillin. While for MICs the *Salmonella* was sensitive to gentamicin (MIC=0.5), ertapeneme and cefotaxime and resistant to cefazolin (MIC>8), ampicillin (MIC>16) and ciprofloxacin (MIC=4).
Although the hygiene practices carried out by all the producers and sellers result in a low germ rate, they threaten the consumption of the Product.

Keywords: Prevalence, *Salmonella*, chicken carcass, chicken offal, chicken intestine, antibiotical susceptibility.