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MASTER DISSERTATION

**Study of the susceptibility of *Salmonella*
strains isolated from poultry**

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Dedication

Every challenging work needs self efforts as well as guidance of elders, especially those who very close to our heart.

My humble efforts I dedicated to mu sweet and loving

Father & Mother

Whose affectation, love, encouragement and prays of day and night make me able to get such success and honor.

Along with all hard working and respected Teachers.

Meriem



Dedication

*Thank you God, the most merciful, your love and your
graces have given me the perseverance and the courage
to accomplish this work.*

My dear parents,

*For their efforts and sacrifices throughout my life,
their encouragement and support to persevere until the completion of this work.*

May they find, in this work, the expression of my gratitude.

To my dear friends,

*In memory of our laughter and good times
in memory of all that we have experienced together, I hope with all my heart that will lasts
forever*

Souhila



List of abbreviation.

BPW: Buffered peptone water.

BGA: Brilliant Green Agar.

HE: Hektoen Agar.

H₂S: Hydrogen Sulfide.

MR: Methyl Red.

MIC: Minimal Inhibitory Concentration.

MKTTn: Muller Kauffmann Tetrathionate with novobiocin.

MLVA: Multi Locus Variable Tandam Repeat Analysis.

MLST: Multi Sequence Typing.

PCR: Polymerase Chain Reaction.

qPCR: real time Polymerase Chain Reaction.

PFGE: Pulsed Field Electrophoresis.

RVS: Rappaport Vassiliadis Soy Broth.

SS: *Salmonella*-*Shigella* agar.

TSI: Triple Sugar Iron agar.

VP: Voges – Proskauer.

WHO: World Health Organization.

XLD: Xylose Lysine Desoxycholate.

Table of content

List of Abbreviation

Introduction	1
Bibliographical part	2
1. Generalities.....	2
2. Classification and nomenclature	2
3. Mode of transmission	4
4. Clinical futures.	4
5. Treatment and control	6
6. Salmonella Detection	7
6.1 Pre-enrichment in non-selective liquid medium	7
6.2 Enrichment	7
6.3 Isolation	7
6.4 Biochemical confirmation	8
6.5 Serological confirmation	8
6.6 Molecular methods	8
Conclusion	9
References	10

Introduction

Salmonella is one of the most important foodborne pathogens, recognized by the World Health Organization (WHO) as an important cause of a human illness and death worldwide and had a significant morbidity, mortality (Grimont et al., 2000). Non-typhoidal salmonellosis (NTS) are an important zoonotic ailment caused by the genus *Salmonella* which constitutes a major public health burden, and represent a significant cost in numerous country currently. At a global level, the main source for humans infection are foods of animal origin particularly, meat and poultry product are plays great role to be the primary source of human salmonellosis. The major dissemination rout of the pathogen involve trade in animals and undercooked animal food products, slaughtering process of animal is considered one of the important source of organ and carcass contamination with *Salmonella* (KEDIR ABDI HASSEN, 2020).

Globally, *Salmonella* causes around 93 million cases of gastroenteritis and 155 000 deaths each year. The disease manifestation depends on the serotype involved, virulence factors, infective dose, and host immunity. Young and immunocompromised patients are the most exposed to dangerous complications (Russell, 2012).

In order to prevent from this disease, humans use antibiotics, their discovery has transformed human and animal health. Among *Salmonella* species antimicrobial resistance is a well confirmed phenomenon and antimicrobial resistant, *Salmonella* are increasingly associated with the use of antimicrobial agents. Antibiotics are substance that have significantly contributed to the prevention and treatment of infectious disease in humans, as well in animals as they are used for therapeutic, preventive and growth prompters. However, the overuse and misuse of antimicrobial can generate genomic selective pressures to enable the pathogen to adapt and acquire resistance (KEDIR ABDI HASSEN, 2020).

Antimicrobial-resistant NTS are becoming an important health risk and cause of infection in Algeria. A study made by the Pasteur Institute of Algeria revealed that 11% of food poisoning cases were caused by *Salmonella* spp. in 2011. Overall, 47% of these cases were mainly related to the consumption of chicken meat (Russell, 2012). In many African countries, especially Algeria, meat is a key protein source and poultry production is central to people's livelihoods and it is also a source of contamination. Salmonellosis and the antimicrobial resistant in bacteria in veterinary and human medicine constitute a big global problem. Thus, the objectives of this study were to isolate and determine the prevalence of *Salmonella* from chicken, and estimate the antimicrobial susceptibility of the strains.

Bibliographical part

1. Generalities

Salmonella is a rod shaped Gram-negative facultative anaerobe that belongs to the *Enterobacteriaceae* family, within the genus *Salmonella*, around 2600 serotypes are identified, and most of these serotypes have the flexibility to adapt within a range of animal hosts, including humans (Eng et al. 2015).

Salmonella are non-fastidious organism as they can multiply under various environmental conditions outside the living hosts. They do not need sodium chloride for growth, but can grows within the presence of 0.4 to 4% of NaCl. Most *Salmonella* serotypes grows at temperature range of 5 to 47C° with optimum temperature as low as 2 to 4C° or as high as 54C°. They are sensitive to heat and sometimes killed at temperature of 70 °C or above. *Salmonella* grows at a pH range of 4 to 9 with an optimum between 6.5 and 7.5. It require a high water activity (aw) between 0.99 and 0.94, yet can survive at aw<0.2 such as in dried foods (Pui et al., 2011).

While most serotypes don't produce indole, hydrolyze urea, and desaminate phenylalanine or tryptophan, most serotypes readily reduce nitrate to nitrite, ferment a range of carbohydrates with acid production, and are negative for Voges–Proskauer reaction. With the exception of *S. enterica subsp. arizonae* and *S. subsp. diarizonae*, most serotypes utilize arginine, ornithine, decarboxylate lysine and hydrogen sulfide. Similarly, most serotypes utilize citrate with the exception of some few serovars of *S. Choleraesuis*, *S. Typhi*, and *S. Paratyphi A*. While most serovars don't utilize lactose, dulcitol is mostly utilized by all serovars with the exceptions of *S. enterica subsp. Arizonae* (Jajere, 2019).

2. Classification and nomenclature

Salmonella was first discovered and isolated from the intestines of pigs infected with classical swine fever, by Theobald Smith in 1855. The bacterial strain was named after Dr. Daniel Elmer Salmon, an American pathologist who worked with Smith. The nomenclature of *Salmonella* is controversial and still evolving. Currently, the Centers for Disease Control and Prevention (CDC) uses the nomenclatural system of *Salmonella* recommended by the World Health Organization (WHO) Collaborating Centre (Eng et al., 2015).

A phylogenetic tree can be derived from the comparison with 16S rRNA or other gene sequences. The genus *Salmonella* is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis.

The type species, *S. enterica*, can be further classified into six subspecies based on their genomic relatedness and biochemical properties. The subspecies are assigned with roman numerals: *S. enterica* subsp. *Enterica* I; *S. enterica* subsp, *S. enterica* subsp. *Arizonae* IIIa, *S. enterica* subsp. *Diarizonae* IIIb, *S. enterica* subsp. *Houtenae* IV, and *S. enterica* subsp. *Indica* VI. Among all the subspecies of *Salmonella*, *S. enterica* subsp. *enterica* (I) is found predominantly in mammals and contributes approximately to 99% of *Salmonella* infections in humans and warm-blooded animals. In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Eng et al., 2015).

In the past, *Salmonella* has been named according to the original location of isolation, such as *Salmonella* London and Indiana. This system was replaced by the classification based on the susceptibility of isolates to different bacteriophages which is also known as phage typing. Phage typing is generally employed when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype. It is very reproduce and more than 200 definitive phage type (DT) have been reported so far (Pui et al., 2011).

Epidemiologic classification of *Salmonella* is predicated on the host preferences. The first group includes host-restricted serotypes that infect only humans like *S. Typhi*. The second group includes host-adapted serotypes which are related to one host species but can cause disease in other hosts serotypes like *S. Pullorum* in avian. The third group includes the remaining serotypes. Typically, *S. Enteritidis*, *S. Typhimurium* and *S. Heidelberg* are the three most frequent serotype recovered from humans annually (Pui et al., 2011).

Since the primary Kauffman-White serotype scheme based on surface molecular antigen variation published in 1934, serotyping has become the foremost important tool for identifying and classifying the *Salmonella* strains for quite 80 years. The Kauffmann-White scheme classifies *Salmonella* according to three major antigenic determinants composed of flagellar H antigens, somatic O antigens, and capsular K antigens. This was adopted by the International Association of Microbiologists in 1934. Over 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* subspecies *enterica*. Although not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* do occur (Pui et al., 2011)

In brief, O antigens are lipopolysaccharide (LPS) of the outer bacterial membrane. They are heat stable, insoluble in alcohol and dilute acids. H antigens are heat-labile proteins related to the peritrichous flagella and may be expressed in one among two phases. The phase 1 H antigens are specific and related to the immunological identity of the actual serovars,

whereas phase 2 antigens are non-specific antigens containing different antigenic subunit proteins which may be shared by many serovars. K antigens which are heat-sensitive carbohydrates are produced by *Salmonella* serovars that express a surface-bound polysaccharide capsular antigen (Pui et al., 2011).

3.Mode of transmission

Salmonella infection appears to be one among the most common samples of an enteric disease that's transmitted from animals to humans. The transmission occurs both through food products, like meat, dairy products, eggs, and by direct contact between animals like reptiles, insects and humans through the fecal-oral route (Olsvik et al., 1985). In developed countries the principal common source of infection is food. The identification of food source causing disease is usually difficult, but it's the most important measure to stop the spread of the infection. Water could represent a source of contamination. Eggs and meat (chicken, pork) remain the major sources, but vegetables and fruits shouldn't be neglected. Chicken and other birds can carry the microorganism and exposure to those birds has been related to the acquisition of the infection. Cases of infection have also been reported after contact with pets. Often, the animal is asymptomatic. Person to person transmission can also happen (Popa and Popa, 2021).

4.Clinical futures.

Infectious microbial diseases constitute a major cause of death in many parts of the world, Particularly in developing countries. *Salmonella* has been identified as an important food and Water-borne pathogen that can infect human and animals resulting in significant morbidity and mortality (EL Hussein et al., 2012).

Infections caused by *Salmonella* should be divided into minor and major disease. Minor salmonellosis caused by NTS strains is characterized by self-limiting diarrhea, rarely resulting in bacteremia or meningitis. Major salmonellosis is represented by enteric fever. The clinical picture of enteric fever includes fever, headache, and malaise and sometimes cough (Popa and Popa, 2021).

NT serovars of *Salmonella enterica* are among the most prevalent zoonotic pathogens affecting animals and humans worldwide. These bacteria are mainly transmitted to humans through the ingestion of contaminated food of animal origin particularly poultry, pork, beef meat, milk, eggs, and derived products. However, many other foods including fruits and

vegetables contaminated with animal or human feces can also be involved (Fernández et al., 2018).

In general, *Salmonella* infection can be involved in bacteremia and focal infections such as osteomyelitis, meningitis, gastroenteritis, and urinary tract infections. NTS infection most commonly presents as gastroenteritis with diarrhea, fever, and abdominal cramps. Commonly in history, For *Salmonella enteritidis*, eating undercooked chickens may cause inflammatory diarrhea. Other raw or undercooked products such as eggs, meat, and dairy products can also cause *Salmonella* infection and may be found in the history of the patient. An immunocompromised state is also common for *Salmonella* infection. Infection is common in children less than one year of age due to underdeveloped immune systems. In adults, *Salmonella* infection is present in chronic steroid use, malignancy, immunodeficiency from organ transplantation, and acquired immunodeficiency syndrome. *S. enterica subspecies arizonae* is a common cause of infection in patients with immunodeficiency states (Ajmera and Shabbir, 2021).

Salmonella have a large sort of domestic and wild animal hosts. The infection may or may not be clinically apparent. Within the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it's going to be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently. In livestock, there is several well-known clinical enteritis because of species-adapted serotypes, such as *S. Pullorum* or *S. Abortus equi*. Other clinically apparent or in apparent infections are caused by serotypes with multiple host (Acha and Szyfres, 2001).

The principal causes of clinical salmonellosis in cattle are serotype *S. Dublin* and *S. Typhimurium*. Other serotypes can sometimes be isolated from sick animals. Salmonellosis in adult cattle occurs sporadically, but in calves it usually acquires epizootic proportions. The disease generally occurs when stress factors are involved. Serotype *S. Dublin*, adapted to cattle, has a focal geographic distribution. In adult cattle, the disease begins with high fever and therefore the appearance of blood clots with the feces, followed by profuse diarrhea, then a drop by blood heat to normal. Signs of abdominal pain are very pronounced. The disease could also be fatal during a couple of days or the animal may recover, during which case it often becomes a carrier and new cases appear. Calves are more susceptible than adults, and in them the infection gives rise to true epidemic outbreaks, often with high mortality. Septicemia and death are frequent in newborns. The carrier state is a smaller amount frequent among young animals and occurs primarily in adult cattle. The infection is nearly always spread by the feces of a cow that's shedding the agent, but it's going to also originate from milk (Acha and Szyfres, 2001).

Two serotypes, *S. Pullorum* and *S. Gallinarum*, are adapted to poultry. They're not very pathogenic for man, although cases of salmonellosis caused by these serotypes are described in children. A wide variety of other serotypes are frequently isolated from domestic poultry; for that reason, these animals are considered one among the principal reservoirs of *Salmonella*. Carrier birds lay infected eggs that contaminate incubators and hatcheries. Fowl typhoid occurs mainly in adult birds and it is transmitted by the faecal matter of carrier fowl. On an affected poultry farm, recuperating birds and apparently healthy birds are reservoirs of infection. *Salmonella* un-adapted to fowl also infect them frequently. The infection in adult birds is usually asymptomatic, but during the primary few weeks of life, its clinical picture is analogous to Pullorum disease (loss of appetite, nervous symptoms, and blockage of the cloacae with diarrheal fecal matter). The highest mortality occurs during the primary two weeks of life. Most losses occur between six and ten days after hatching (Acha and Szyfres, 2001).

5. Treatment and control

Salmonellosis is a self-limiting illness that ceases in a week (Ehuwa et al., 2021). In most cases, treatment focus should be on the correction of dehydration and electrolyte disturbances. Supportive care is necessary for acute diarrhea and dehydration symptoms. Antipyretic therapy may also be provided if needed. Uncomplicated NTS localized to gastroenteritis without sepsis symptoms is not treated with antibiotics except patients less than 3 months of age and patients with immunocompromised states. Initial therapy should be with third-generation cephalosporin like ceftriaxone for at least 7 to 10 days. Once bacterial susceptibilities are known, antibiotic treatment can be transitioned to azithromycin or a fluoroquinolone (Ajmera and Shabbir, 2021).

Given current conditions under which cattle and poultry are raised, transported, marketed, and slaughtered, as well as existing food processing practices, it is impossible to obtain *Salmonellae*-free foods of animal origin. Control is currently based on protecting man from infection and reducing its prevalence in animals. Veterinary meat and poultry inspection and supervision of milk pasteurization and egg production are important for consumer protection, another important control measure is the education of food handlers, both in commercial establishments and in the home, about correct cooking and refrigeration practices for foods of animal origin, and about personal and environmental hygiene. Epidemiological surveillance by health authorities is necessary to evaluate the magnitude of the problem in each country, locate the origins of outbreaks, and adopt methods designed to reduce risks (Acha and Szyfres, 2001).

In animals, salmonellosis control consists of elimination of carriers, which is currently possible for Pullorum disease and fowl typhoid by means of serologic tests bacteriologic control of foods, mainly of such ingredients as fish, meat, proper management of herds and poultry farms (Acha and Szyfres, 2001).

6. *Salmonella* Detection

The routine method for detecting *Salmonella*. spp includes the use of a non-selective Pre-enrichment, selective enrichment, and subsequent culturing on selective media. The identification is based on biochemical tests followed by serotyping (Rodríguez-Lázaro and Hernández, 2021).

6.1 Pre-enrichment in non-selective liquid medium

The enrichment of portion of the food sample is to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock, in a non-selective pre-enrichment media, such as Buffered Peptone Water (BPW), and to increase the number of target cells as these are generally not uniformly distributed in foods, typically occur in low numbers, and may be present in a mixed microbial population (Joseph A. Odumeru, 2012).

6.2 Enrichment

The recovery of *Salmonella* from food is often complicated by the presence of high numbers of indigenous micro flora in food that can interfere with the isolation of pathogens, Success in isolating *Salmonella* is usually enhanced by the inoculation of incubated pre-enrichment broth into selective enrichment media cultures such as Selenite Cystine broth (SC), Rappaport Vassiliadis Soy broth (RVS), Tetrathionate Broth (TT), or Muller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn) and incubated at temperature of 37°C or 42°C for 18-24 hours (Joseph A. Odumeru, 2012).

6.3 Isolation

The incubated selective enrichment broth are streaked on selective media such as *Salmonella*- Shigella agar (SS), Brilliant Green agar (BGA), bismuth-sulfite agar, Hektoen Enteric agar (HE), and Xylose Lysine Desoxycholate agar(XLD). These selective media allow the growth of *Salmonella* organism, while at the same time suppressing the propagation of other bacteria. The colors with the coliforms formed on these media are used for differentiating colonies of *Salmonella* serotypes. For instance, *S. Typhi* on SS appears as

colorless colonies with a black center. Typically, *Salmonella* colonies on XLD appear as red colonies with or without black centers (Jajere, 2019).

6.4 Biochemical confirmation

The resulting presumptive colonies isolated on plating media are then incubated in both Triple Sugar Iron test (TSI) and Lysine Iron agar (LIA), followed by tests including urease test and other additional tests for urease negative cultures such as Voges Proskauer (VP), methyl red (MR), and Indole tests. Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in some cases) formation of hydrogen sulfide (blackening of the agar) (Jajere, 2019).

6.5 Serological confirmation

Utilize polyvalent antisera for flagellar (H) and somatic (O) antigens. Isolates with a typical biochemical profile, which agglutinate with both H and O antisera are identified as *Salmonella* species. Several rapid latex agglutination assay Enzyme-linked immunosorbent assay (ELISA) tests are widely used for the rapid detection of *Salmonella* (Joseph A. Odumeru, 2012).

6.6 Molecular methods

Molecular techniques such as real-time polymerase chain reaction (qPCR), Polymerase chain reaction (PCR), Multiplex PCR, Reverse transcriptase PCR (RT-PCR), Nucleic acid hybridization, have become a very effective alternative in food microbiology diagnostic for rapid and specific detection of foodborne pathogens such as *Salmonella* in foods and food-related environments (Joseph A. Odumeru, 2012).

Traditional methods of *Salmonella* detection are available and affordable for laboratory, however they are time consuming and labor intensive requiring well trained experienced technicians, additionally it can be imprecise and may result in false positive identification may originate from multiple contamination sources (Tang et al., 2019). To overcome the limitation, molecular methods are becoming an alternative choice for identification. As they are faster, does not require the same technical expertise, can generate a wealth of objective information in a short time, allow for the detection and characterization of isolates at the genetic level. Although it remains more expensive and not accessible for all laboratory (Yoshida et al., 2016).

Conclusion

During this study the prevalence of *Salmonella* in different municipalities in Bejaia as well as their antimicrobial resistant pattern was determined, 16 *Salmonella* strains were recovered.

The high level of contamination with *Salmonella* reported in this study highlights the poor hygiene condition all along the poultry sale procedure, from breeder to the market in Algiers. Thus to establish the safety of human from contamination, it should be focused to implement protective measures of hygiene all along the production process additionally the isolation of *Salmonella* in chicken for humans consumption focus on the importance of consumer education on proper handling and preparation of food in order to reduce the risk of contamination.

Furthermore, our results show the widespread presence of *Salmonella* strains resistant to antimicrobial and multi drug resistant strains. These results are related to the use, overuse and misuse of antimicrobial agents in production animals which act as a potential risk factor for the occurrence and the emergence of antibiotics resistance in public health. For that reason, random antibiotics usage should be restricted to minimize public health hazards of spreading multi-drug resistant pathogens, also in order to monitor the *Salmonella* resistance in poultry and limit the risks of transfer resistant pathogens to humans.

Therefore, basis on the conclusion above, the veterinarian should be given awareness for the farmers owners to not use antibiotics without regulation in food product of animals origin. The consumer should avoid cross-contamination of food of animal origin with good hygienic practice. A surveillance programs of using of antibiotic and strict biosecurity measures have to be implemented in order to limit the spread of these health-threatening bacteria in the local and national poultry industries.

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Abstract

Aims: *Salmonella* is one of the major food borne pathogen responsible for outbreaks of foodborne illnesses in humans and animals worldwide. The aims of this study was to determine the prevalence of *Salmonella* in poultry products in Bejaia and to estimate resistance rates towards some antibiotics.

Methods: A total of 134 samples of giblets, intestine, minced chicken meat and chicken merguez were collected in different municipalities of Bejaia. After isolation, the strains were identified using biochemical tests. After that, the strains were tested for their antibiotic susceptibility using disc diffusion method.

Results: *Salmonella* was detected in 12% of the total samples. High antimicrobial rates were observed against tetracycline (56.25%), ciprofloxacin and levofloxacin (18.75%) and gentamicin (6.25%). All the strains were found susceptible to β -lactam antibiotics, tobramycine and chloramphenicol.

Conclusion: Importance of consumer education on proper handling of food and restriction on irrational use of antibiotics in humans and animals are suggested for reduction of *Salmonella* contamination risk and resistant strains.

Key words: *Salmonella*, Chicken meat, Giblets, Identification, Antimicrobial susceptibility