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# Master capstone's project

In pursuit of obtaining a Master's degree in microbial ecology

Under the theme of :

## Prevalence of salmonella in minimally processed vegetables


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# Aknowledgment

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# List of abreviations

**ADN :** Deoxyribonucleic Acid

**C° :** Celsius

**H :** Hour

**H2S :** Hydrogen Sulfide

**RV :** Rappaport vassiliaddis

***S*** **:** *Salmonella*

**Spp :** is an abreviation for species in biology

**XLD :** Xylose Lysine Deoxycholate Agar

**SS :** Salmonella-Shigella

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# Introduction

## Chapter I Introduction:

### 1.1 Background:

Microprocessed vegetables are popular for their convenience and health benefits. However, Salmonella contamination in these products poses a significant food safety risk. Previous studies have identified factors that contribute to the contamination, but further research is needed, especially for minimally processed vegetables.

### 1.2 Research objectives:

This study aimed to determine the prevalence of Salmonella on minimally processed vegetables, identify influencing factors, evaluate containment strategies, and make recommendations for improving food safety practices.

### 1.3 Research questions:

The research questions guiding this study are:

How Prevalent is Salmonella on Minimally Processed Vegetables?

During the production and processing of this vegetable, what factors can lead to Salmonella contamination?

How effective are existing containment strategies at reducing Salmonella contamination?

What are the implications of the research findings for improving food safety practices?

### 1.4 Importance of research:

This study is significant because it addresses the need for a comprehensive understanding of Salmonella contamination on minimally processed vegetables. Findings will help increase food safety and public health knowledge, inform industry stakeholders and policymakers, improve the safety and quality of minimally processed vegetables, and reduce the risk of foodborne illness.

The increasing demand for convenience and healthy food options has led to the popularity of minimally processed vegetables especially in modern days. However, concerns over foodborne pathogens, including Salmonella and many other pathogenic bacterias, have raised questions about the safety of these products. The purpose of this paper is to investigate the prevalence of Salmonella spp. to minimally processed vegetables, with a focus on various factors affecting contamination, detection methods, and potential mitigation strategies. Through a comprehensive review of existing literature, laboratory experiments, and data analysis, this study aims to provide valuable insights into the safety and quality of minimally processed vegetables associated with Salmonella contamination.

# Chapter II : Littérature review

## History of salmonella :

Salmonella is named after American bacteriologist D. E. Salmon who was studiying about cholera outbreaks, he first isolated the bacterium from pig intestines in 1884. Salmonella is a Gram-negative, motile, hydrogen sulfide-producing, acid-labile, facultative intracellular microorganism that commonly causes foodborne diseases such as gastroenteritis and cross-infection between humans and animals worldwide. Many animals are known carriers of Salmonella. One animal vector is chicken, which, when eaten raw, can cause Salmonella Enteritidis, which causes inflammatory diarrhea. Salmonella can also cause severe focal infections in patients with immunocompromised disease. There are more than 2,500 Salmonella serotypes worldwide. (Fatica & Schneider. 2011, Ajmera & Shabbir. 2022).

salmonellosis may be induced in humans through the consumtion of raw vegetables that have been washed only, eggs, meat, poultry and many other products. This bacteria can manifests itself through some symptoms like diarrhea, vomiting, , cramps and fever in individuals ,the illness is usually self-limiting, so there are high-risk populations of old people, young children and the immuno-compromised where the illness may be deadly to them. frequently reported causes of foodborne gastroenteritis is frequently caused by this bacteria and it is estimated to cause around 1.0 million cases of foodborne illness each year in the United States (Fatica & Schneider. 2011).

Poultry and eggs are also associated with Salmonella outbreaks, in fact 33% of infections in 2004–2008 were linked to several other sources including: fruits and vine vegetables, nuts, sprouts, leafy greens like salads, roots, and beans.

through the last 15 years, the incidence of Salmonella infections has not declined in the USA, making the non-typhoidal strains the second most common cause (after Norovirus) of food poisoning, with around 1 million infections per year ( Hernandez-Reyes & Schikora. 2013).

## . II.1 Overview of Minimally Processed Vegetables:

Minimally processed vegetables have exploded in popularity in recent years due to the changing the consumer's preferences and demand for convenience and healthier food options. This section provides a comprehensive overview of minimally processed vegetables, highlighting their characteristics and benefits, minimal processing steps, perishability, and the importance of ensuring quality and safety throughout the production and processing chain ( Carstens et al. 2019).

### II.1.2 Features of Micro Processed Vegetables:

Minimally processed vegetables are characterized by their fresh appearance, texture, and flavor, which closely resemble raw vegetables. These vegetables go through minimal processing steps to preserve their natural properties and minimize nutrient loss. Common processing steps include washing/fluming, shredding and cutting, drying,, all carefully executed to keep the visual appeal and of course the nutritional content of the vegetables

( Carstens et al. 2019).

### II.1.3 Minimum processing steps:

Processing minimally processed vegetables involves several important steps. Cleaning is essential to remove dirt, debris and surface contamination by only 1 to 2 log. Careful cutting techniques such as slicing, dicing or grating are used for added convenience and ease of use. Finally, vegetables are properly packaged to ensure protection from physical damage, microbial contamination, and moisture loss (Sivapalasingam & al. 2004).

## II.1.4 Ephemeral nature:

Vegetables that are minimally processed have a limited shelf life due to their perishability. Over time, their natural enzyme activity, respiration and moisture content promote microbial growth, nutrient degradation and quality loss. Therefore, proper handling, storage, and temperature control are critical to extending shelf life and maintaining the quality of these products in order to reach the consummer in a good state (Ehuwa & al. 2021).

## II.1.5 The importance of choosing MPVs:

Salmonellosis is a growing problem in both developed and developing countries public health .

Salmonella spp. have a variety of animal hosts and transmission routes, and can cause human infection. However, most of them are In developed countries, infections are thought to be caused by Foodborne exposures .Reducing the burden of foodborne salmonellosis is a challenge; it requires Identify major food sources that contribute to disease and prioritize effective intervention strategies ( Pires & al. 2014).

One of the routes that this bacteria can take is through vegetables and it can reach the human by the ones he consumes like fresh uncoocked vegetables that he just washes. Consumption of fresh fruits and vegetables ("fresh produce") has increased significantly since the 1980s due to consumer demand for more nutritious food and a healthier lifestyle, especially in high-income countries throughout the world, a good diet of fresh produce can prevent many chronic diseases like diabetes, cancer, hypertension,obesity and many others. The increase of the consumption of fresh produce will also result in the increase of outbreaks and illness caused by the microbial pathognes , since the fresh produce is often consummed in its raw state which results that the harmful organisms continue to live in it and can cause complications to the consumer's health( Carstens & al. 2019).

## II.2 Microbial safety of Minimally processed vegetables :

This part will examine the microbiological safety of the minimally processed vegetables, with a particular focus on the prevalence of Salmonella. as a foodborne pathogen. The risks associated with microbial contamination, including bacterial, viral, and fungal contamination, are discussed. In addition, the consequences of eating minimally processed vegetables contaminated with Salmonella were analyzed, including the severity and dangers of foodborne illness and what it can come with impacts on public health.

### II.2.1Microbial Contamination Risks:

Minimally processed vegetables are susceptible to microbial contamination at various stages of production and processing and it depends on various factors. The primary sources of contamination include soil, irrigation water, equipment, and human handling like cutting chopping while processing the fresh produce. The use of irrigation water containing human pathogens will automatically lead to the direct contamination of crops and cause outbreaks of human illness (Markland & al. 2017)

The most commonly implicated etiological agents in fresh produce borne illnesses include bacteria such as Salmonella, Escherichia coli (E. coli), Shigella spp,Clostridium spp, Listeria and Campylobacter. Viral contaminants such as norovirus,Sapovirus,Rotavirus and hepatitis A virus can also be a problem. In addition, fungal pathogens such as Aspergillus sp. and Fusarium spp. lead to food safety problems (Alegbeleye & al. 2018).

## II.3 Salmonella as foodborne pathogens:

Salmonella is a major foodborne pathogen often associated with fresh produce, including minimally processed vegetables. It can cause gastrointestinal infections such as salmonellosis, with symptoms ranging from mild gastroenteritis to severe illness. Salmonella infection can be life-threatening in the elderly, infants, and vulnerable people with compromised immune systems. The ability of Salmonella to survive and grow on vegetables underscores the importance of implementing proper safety measures. Salmonella infections are more common in areas of intensive livestock farming, especially poultry, cattle, and swine due the different transmission routes. These bacteria can contaminate a large variety of animal and plant such as fruits and vegetables. The main carriers of nontyphoidal Salmonella are farm animals such as cattle, pigs, poultry, game birds and pets, which shed the bacteria in their feces. This fecal contamination can then spread to its turn into food sources and additionally to the environment. Many infected livestock are asymptomatic, but they may carry and shed high levels of Salmonella in their faeces, often it's very hard to detect or undetectable at all by routine testing. This highlights the risks of transmission from asymptomatic animals to humans and emphasizes the need for effective control measures to prevent contamination and subsequent infection (Nair & al. 2015).

## II.3.1Genralities about salmonella :

Salmonella belongs to a group of bacteria called gram negative bacteria, shaped like rod and can have fllagellas helping it to move around it devides to two species : *Salmonella bongori* and *Salmonella enterica.*

The S. enterica is divided into seven subspecies denoted by Roman numerals, most human pathogenic salmonella belongs to the isolated subspecies, while other subspecies are mainly associated with cold-blooded vertebrates. There are more than 2,600 serotypes of Salmonella enterica. Serotypes vary widely in their ability to infect different hosts such as mammals and birds and can be divided into three groups based on host range: broad host range serotypes, or broad, host-adapted, and host-restricted serotypes . Host-restricted serotypes are only associated with one host species. Certain serotypes, namely typhoid, paratyphoid A, paratyphoid C, and sendai typhoid, are pathogenic in humans only. Likewise, Salmonella abortus mainly affects goats and sheep, whereas Gallinarum and Pullorum are restricted to poultry. Pigs are susceptible to S. typhi serotypes, while horses can be infected with S. abortus serotypes. However, some serotypes adapted to specific hosts can still cause disease in alternate hosts. In their natural hosts, both host-restricted and host-adapted serotypes lead to systemic infection and often establish themselves in intracellular niches such as macrophages (Silva & al. 2014).

Biochemical tests for somatic (O) and flagellar (H) antigens were used to characterize bacteria in different serotypes. Ingestion of nontyphoidal Salmonella serotypes may be the cause of salmonellosis in humans. Salmonellosis typically causes vomiting, diarrhea, convulsions, and fever in an individual. Although the disease is usually self-limited, high-risk groups exist in the young, the elderly, and the immunocompromised, where the disease can be fatal. Salmonellosis is one of the most commonly reported causes of foodborne gastroenteritis with an estimated 1 million cases of foodborne illness in the United States each year (Fatica & Schneider. 2011).

Salmonella serotypes are broad host range and can infect a wide variety of animals, including

Mammals, birds, reptiles, amphibians, fish and insects. Exist Additionally, Salmonella can grow and survive in plants Protozoa, soil and water and many different and various conditions

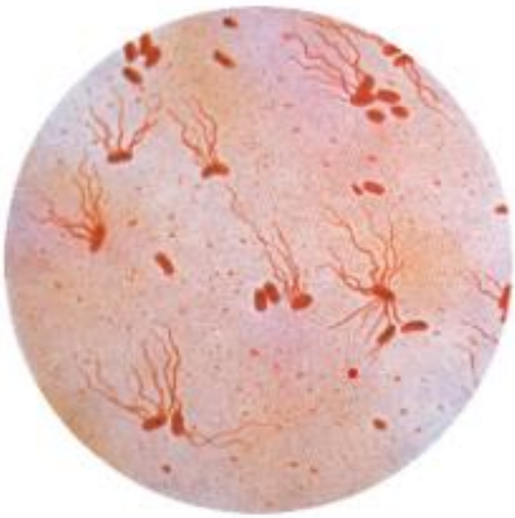
(Silva & al.2014).

Therefore Salmonella has a broad host range Can be transmitted directly to humans through feces of wild animals, domestic animals, etc. Pets, or indirectly by eating a variety of common foods: Poultry, beef, pork, eggs, milk, fruits, vegetables, spices, and nuts or by contaminated water ,other food , contaminated utensils, or the hands of the one who handles food

( Hernandez-Reyes & Schikora. 2013).

Figure 1 :A photomicrograph of *Salmonella typhosus* bacteria using a flagellar sain technique.Also know as *Eberthella typhi and Bacillus typhosus,* is the cause of Typhoid fever.

https://phil.cdc.gov/PHIL_Images/09132002/00032/PHIL_2115_lores.jpg



## II.3.2 Caracteristics of Salmonella :

*Salmonella* is a Gram-negative, non-sporulating facultatively anaerobic organism, rod-shaped bacteria with flagella that belongs to the Enterobacteriaceae family. It is a facultatively anaerobic organism that does not form spores. Pathogenic strains of Salmonella can grow in a wide and very different range of conditions due to its capacity of adaptation, including temperatures between 8°C and 45°C, pH values from 4.0 to 9.5, and low water activity . S. enterica is commonly found in animal fecal materials, which can contaminate water and crops. The majority of human pathogenic serotypes belong to the subspecies enterica (I) of S. enterica. Most Salmonella infections in humans are primarily foodborne, with contaminated food or water being the main sources of transmission that can be from various factors. Symptoms of salmonellosis resemble mostly gastroenteritis, with an incubation period ranging from six to 48 hours. While the disease is generally self-limiting, invasive salmonellosis can occur in vulnerable populations. Antibiotic treatment is typically needed for severe cases or individuals at risk of health complications. Non-typhoidal S. enterica infections result in a significant number of cases in the United States each year, with a considerable proportion leading to hospitalizations. Salmonella spp. is responsible for a substantial portion of foodborne outbreaks, hospitalizations, and deaths, as well as significant economic costs ( Carstens & al. 2019).

## II.3.3 Identification of salmonella :

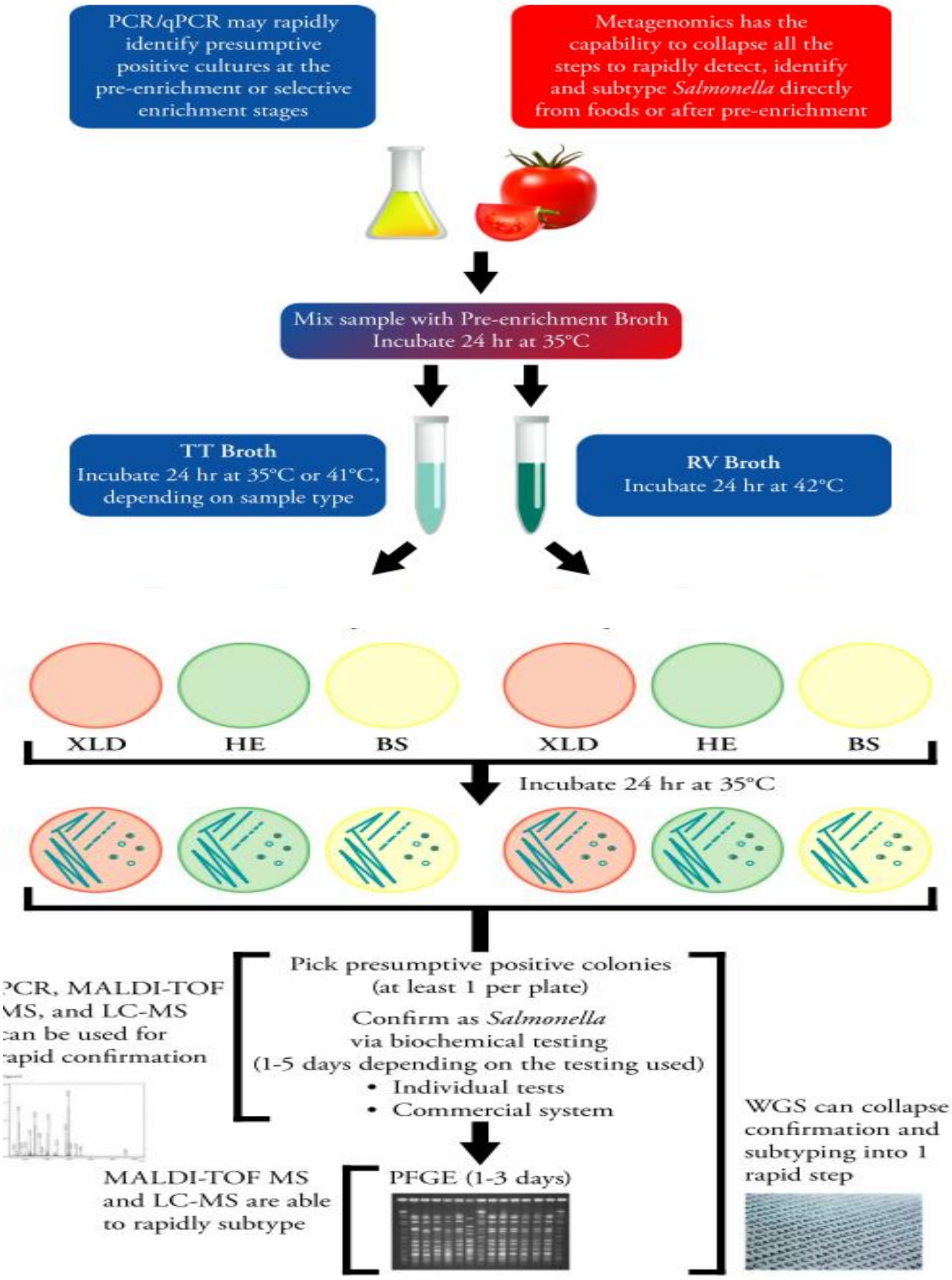
Detection and identification of this pathogen in food and environmental sources is critical to ensuring food safety. Traditional cultivation techniques are the standard for regulatory agencies, but they are time-consuming and therefore impractical for perishable food inspections. However, advances in microbiology and molecular biology have led to faster detection methods. Analysis of food and environmental samples remains challenging, and accurate identification depends on the isolation of pure strains. However, the development of DNA sequencing technologies has revolutionized the detection, identification, and subtyping of Salmonella, resulting in a culture-independent diagnostic framework. This review focuses on current methods and next-generation state-of-the-art methods for the detection,identification and subtyping of Salmonella in food and environmental samples ( Bell & al. 2016).

Salmonella infections pose significant economic and public health concerns, with an estimated annual cost of 3.7 billion dollars . In the United States, the Salmonella genus causes around 1.35 million infections each year, resulting in approximately 26,500 hospitalizations and 420 deaths, according to the Centers for Disease Control and Prevention (CDC) [38]. Salmonella can manifest in different disease syndromes, including Salmonella gastroenteritis, characterized by symptoms such as stomach cramps, diarrhea, fever, and occasionally vomiting (Aljahdali & al. 2020).

The plasticity of Salmonella's bacterial genomes is influenced by horizontal and vertical gene transfer, driven by the presence of mobile genetic elements (MGEs) like plasmids. These plasmids play a crucial role in Salmonella's ability to survive in various food animal sources and cause infections in humans. They carry genes that provide selective advantages, such as antibiotic resistance and virulence factors. This genomic plasticity allows Salmonella to adapt and thrive in different environments, contributing to its pathogenic potential in humans (Aljahdali & al. 2020).

Serveral techniques can be employed to identify  this bacteria, including cultural, biochimical,and molecular techniques. Limited studies exist on the isolation, identification, and distribution of Salmonella serotypes from diverse sources. Thus, this study aimed to assess the prevalence and distribution patterns of significant Salmonella serotypes in diarrheagenic human infants, young animals, sewage waste, and fresh vegetables. The goal was to gain insights into the occurrence and spread of Salmonella serotypes in these specific sources, aiding in the development of preventive strategies for controlling Salmonella infections ( Nair & al. 2015).

**Fig.2.** Overview of the U.S. Food and Drug Administration Bacterial Analytical Manual (FDA-BAM) workflow for the detection, isolation and subtyping of Salmonella. Detection and isolation of Salmonella requires 5 days. Subsequent confirmation and subtyping may take up to a week longer. Various, newer molecular methods such as PCR/qPCR, MS, WGS and metagenomics, may shorten the time to result and may be incorporated into the workflow at the indicated steps ( Bell & al. 2016).



Salmonella isolation was conducted following the guidelines recommended by the FDA [11]. In summary, a 1 ml sample from the transport swab was mixed with 9 ml of buffered peptone water and incubated at 37°C for 18 hours for pre-enrichment. After pre-enrichment, 0.1 ml of the inoculum was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated at 42°C for 24 hours for selective enrichment.

Following the enrichment step, a loopful (10 µl) of the enriched culture was streaked onto xylose lysine desoxycholate (XLD) agar and incubated at 37°C for 24 hours. Presumptive Salmonella colonies, which appeared as slightly transparent red halos with a black center surrounded by a pink-red zone on the XLD agar, were selected for further screening.

These selected colonies (4-5 colonies per plate) were subjected to biochemical characterization to confirm their identity as Salmonella ( Nair et al. 2015).

## II.3.3.a Taxonomy and Classification :

**Kingdom :** Bacteria

**Phylum :** Proteobacteria

**Class :** Gammaproteobacteria

**Order :** Enterobacteriales

**Family :** Enterobacteriaceae

**Genus :** Salmonella

**Species :** Salmonella Enterica

**Subspecies** : S. enterica subsp. enterica (I), S. enterica subsp. salamae (II), S. enterica subsp. arizonae (IIIa), S. enterica subsp. diarizonae (IIIb), S. enterica subsp. houtenae (IV), S. enterica subsp. indica (VI) Serotypes: ~2600

(Knodler and Elfenbein. 2019).

### II.3.3.b Pathogenicity of salmonella :

Salmonella spp. is a bacterium that can infect humans through contact with animals carrying the bacteria or by consuming contaminated aliment or water. Approximately 94% of non-typhoidal S. enterica infections are caused by foodborne transmission in the United States of America. Salmonellosis , the disease caused by the bacteria Salmonella, typically manifests as gastroenteritis with symptoms such as abdominal cramps ,diarrhea, and fever. The incubation duration can vary from 6 to 48 hours after exposure to it.

The exact infectious dose of Salmonella has not been determined, but the disease is generally self-limiting, it means that it resolves on its own without specific treatment. However, in certain individuals at higher risk, such as young children which have yet an immature immune system, the immunocompromised, and the elderly, invasive salmonellosis can occur. In such cases, the infection can spread beyond the gastrointestinal tract like reaching different organs of the body and may require antibiotic treatment.

It is important to note that antibiotics are typically reserved for severe cases of salmonellosis or cases that are more susceptible to complications. In general, supportive care, such as maintaining hydration and electrolyte balance, is sufficient for managing most cases of salmonellosis ( carstens and al. 2019).

## II.4 Factors Influencing Salmonella Contamination on Minimally Processed Vegetables:

This section focuses on factors affecting the prevalence of Salmonella contamination in minimally processed vegetables. Pre-harvest and post-harvest factors, including potential sources of contamination such as contaminated irrigation water, soil, animal and human handling. In addition, we investigated the effects of environmental conditions such as temperature, pH, humidity, and organic matter on the survival and growth of Salmonella.

### II.4.1 Pre-harvest factors :

Water is an essential source for all living beings and it's used in all domaines and one of them is agricultural practices, a good source of fresh water will help in the production of good fresh produce that will bring a healthy balance in the human food regime, as is it is known agricultural water is very precious and some partof the world are lacking from it due to its limited quantities, Other water types and sources for irrigation are being investigated in the U.S. and around the world, Treated water is being explored as an alternative source for irrigation to increase water availability. However, studies have shown the presence of harmful bacteria in surface water used for irrigation, leading to outbreaks of foodborne illness. Strict water standards, emission reduction technologies, and expanded use of treated water are gaining attention in agriculture (Markland & al. 2017).

Soil Contamination is caused by a various of bacteria, Salmonella can survive in soil and contaminate vegetables when soil is contaminated with animal or human feces or other sources of contamination. Pathogenic and zoonotic microorganisms can be released by animals carrying it into the soil, which in its turn contaminates water sources in the area (Park & al.2012).

Animal contact also leads to contamination,Wild animals, livestock, and livestock can spread Salmonella and contaminate fields or crops through grazing or their droppings.

It can spread through direct or indirect contact.Pathogens with an animal reservoir can contaminate fields when animals or their droppings are present.

Fecal pathogens such as E. coli can survive in cow manure for up to 70 days.

. Produce grown or harvested in the ground or in contact with improperly composted manure may become contaminated (Sivapalasingam & al. 2004).

### II.4.2 Post harvest factors

Human handling is one of the causes that leads to the contamination of fresh produce becase of poor sanitation and bad harvesting, grading, and packing from the workers, this can allow salmonella to enter vegetables and cause foodborne illnesses (Prendergast & al. 2009).

Contaminated Equipment such as knifes and Surfaces. Improper handling ,cleaning and sanitization of processing equipment and surfaces can lead to cross-contamination of vegetables. The spread of salmonella among food can be caused by the lack of good handling and sanitizing according to the USFDA (Ehuwa and al. 2021).

Storage and transportation conditions are also an important step to take in consideration when it comes to good mesures, Inadequate conditions like bad transportation or bad storage can promote the growth of Salmonella on fresh produce .Poor hygiene practices along the food chain from slaughtering, cutting or harvesting, processing, storage, distribution, transportation to preparation can expose the consumer to foodborne infections that may be fatal especially for young people, elders and immuno-compromised

(Kroupitski & al.2021).

### II.4.3 Environemental conditions :

Salmonella is known for its capacity of survival in different environemental conditions even when it's unfavorable to it :

- Temperature: Salmonella can grow rapidly at temperatures around 37° and it is optimum for it but the range temperature of Salmonella growth is between 5°C and 47°C. Insuitable temperature is a major favorable factor for foodborne infections for a large range of pathogenic bacteria, Higher temperatures in the range of 25-37°C promote the growth of Salmonella, while cooler temperatures and pH values below 3.94 are effective in inhibiting bacterial growth. Temperature control is important to ensure safety and stability of fresh produce (madi & al. 2012).
- pH: Salmonella tolerates a wide pH range as it tolerates temperature aswell , but favors growth in slightly acidic to neutral conditions (pH 6-7). Australian New South Wales government food safety guidelines recommend maintaining a pH of 4.2 or below to effectively control the growth of Salmonella in raw egg products. However, the effectiveness of pH control is affected by factors such as the type of acid used, water

activity temperature and the presence of various ingredients such as garlic, ginger and pepper. When exposed to environmental stressors such as carbon starvation, Salmonella can develop cross-tolerance and resistance to various stressors. Studies have shown that a weak acid (pH 5.8) can acclimate Salmonella and increase their tolerance to lower pH, heat, high concentrations of sodium chloride, and certain antimicrobials. Exposure of Salmonella to acidic conditions before inoculation with mix of previous ingredients at a pH of 4.2-4.5 improved their survival and persistence at low temperatures (4°C).

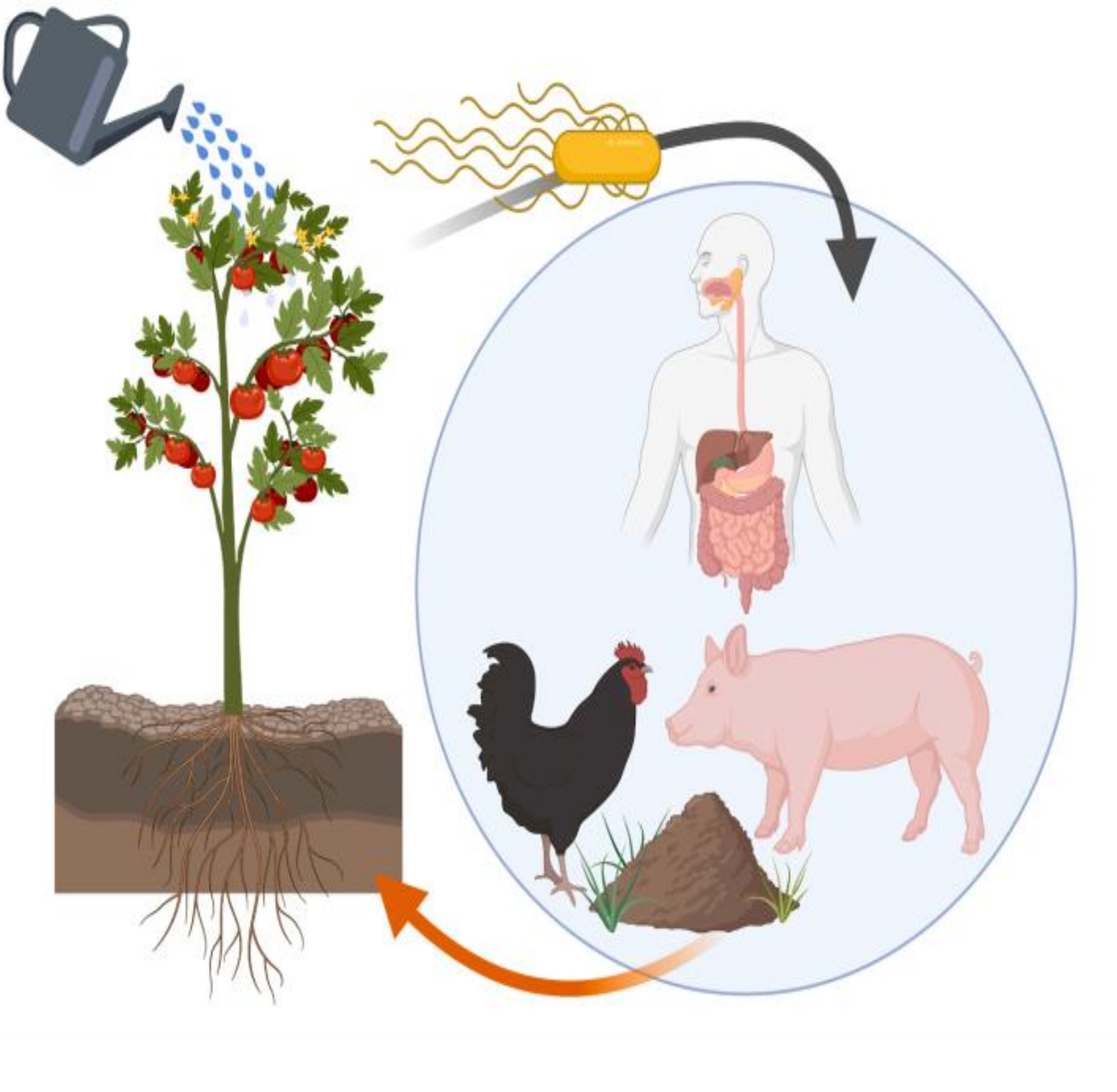
The sensitivity of Salmonella typhimurium for exemple to acid stress appears to be influenced by growth temperature. Salmonella typhimurium has been found to grow in a temperature range of 25-37°C and a pH of 4.5. Lowering the pH of homemade mayonnaise for exemple in salad reduced the survival of Salmonella regardless of temperature. A study was conducted and it was found that the minimum pH value that allowed the growth of Salmonella typhimurium was 3.94 in the temperature range of 25–35 °C (Keerthirathne & al.2016).

- High moisture : high moisture levels is a favorable factor for bacteria growth and survival in environement such as salmonella, this bacteria can also survive in intermediate and low moisture levels for extended periods of time
  (Ehuwa and al. 2021).
  vegetable moisture is affected by a lot of factors, including environmental conditions, irrigation methods, and the presence of pathogens in irrigation water. Monitoring and treatment of water sources and proper irrigation practices play a key role in maintaining the microbial quality of irrigation water and reducing the risk of pathogen transmission to leafy greens (Mogren & al. 2018).
- Organic matter can also play part in the growth of salmonella as it can be a source of nutrition for this pathogenical bacteria and help it survive longer and enable it to multiply and colonize it surroundings,for exemple decaying plants or organic-rich loam that are playing a very important part of its growth ,supporting the survival of this bacteria and contributing to water contamintion also
  (Alegbeleye & Sant'Ana. 2023).

## II.4.4 Salmonella controls :

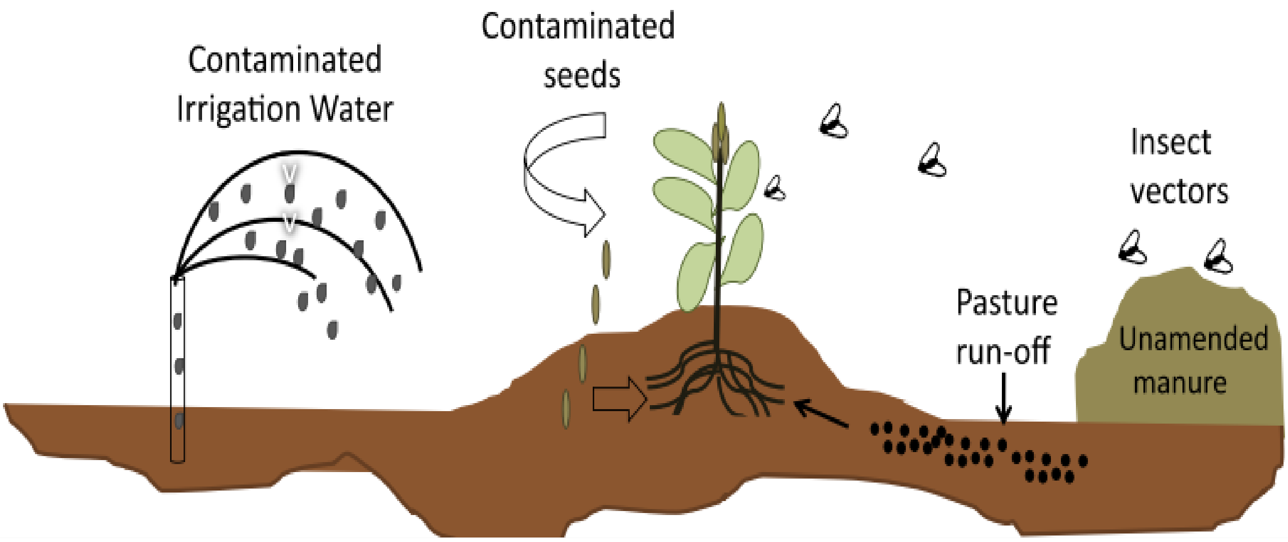
*Salmonella* was reported that it can persist in agricultural soil for a long period in various conditions and is able to move to the non-infected parts of the plants to colonize the entire plant and therefore becoming a risk for the public health, Contamination of crops can occur at any step of the food production chain, during both pre-harvest and post-harvest processing steps. contaminated irrigation water is one of the causes that leads to The pre-harvest contamination , workers, insufficient of the execution of the plan of work, bad sanitation, or fecal contamination . While post-harvest contamination can occur during , processing, trnasportation of the produce and its packaging . Additionally, there could be mechanical damages too during the transportation and it can increase the survival of human pathogens on the surface of diverse plants (Zarkani and Schikori. 2021).

**Fig. 3.** An overview on *Salmonella* in agriculture. The figure illustrates the ability of *Salmonella* to contaminate crop plants, such as tomato, rising the potential risk of its transmission back to humans. The contamination of crop plants could be due to the use of soil or irrigation water that are contaminated with this bacterium. This figure was created with Biorender.com (Zarkani and Schikori. 2021).



The scientific community keeps advancing in research and has now a deeper understanding about foodborne bacteria contamination in processing environements and argriculture in past few years. Studies found about bacterial survival in soils,plants,manure. Cutting and harvesting tools and the use of some chemicals,irrigation water can contaminate or harm the produce (Martinz-Vaz and al. 2014).

**Fig. 4.** Enteric pathogens in leafy greens: routes of contamination. Representation of the conditions that can cause the contamination of leafy greens with *Enterobacteria* in the pre-harvest environment
(Martinz-Vaz and al. 2014).




Studies conducted that good agricultural practices encourage the growth of some competing bacteria that may reduce incidence of produce contamination, because usually oraganically managed soils have a much higher microbial activity and diversity and biomass to conventional soils (Franz and Van Brugggen. 2004).

One of the uselful ways to eliminate pthogenic mecrobial from fresh produce like lettuce is the use of Ozone due to its effeciency,but it depends on several factors like the residual ozone in the medium and quantity of ozone used. A study showed that an application of 2ppm ozonized water treatement on lettuce is found effective in a period of 2 minutes , it actually desinfects it, it work actually on decreasing microbial load leaving a nice sensory quality during cold storage. This method is also more useful than chlorine and organic acid treatements because it help maintain a good quality of the fresh produce ( balali and al. 2020)

Storage temperature of food can also play a big role in the safety of the consummer for example :

- Food business operators must make sure that their work meets the criteria of shelf-life
- Nordic countries applied variying recommended storage temperature that goes from 3C° to 8C°

Temperature abuse would compromise the food quality which of course would affect the consumer health (Söderqvist. 2017).

Nowdays finding sources of good water (potable water) is challenging and it is essential for the production of fresh produce, although, accessibility to safe water is progressively becoming a challenge worldwide resulting in increased food safety risks, especially in dry season ,irrigation water is used to maximize crop yield. And with time some sources of fresh water get contaminated.

Water requirement depends on the country and its capacities and developpement, status and climate conditions, as aresults it differs from a place to another (Iwu and Okoh. 2019).

## II.4.5 Salmonella prevention :

Many methods of precessing food can make it safer for the consumer from the contamination of many microbial pathogens but complete eradication of salmonella can be quite a challenge because of its capacity to adapt and survive in many environement .but it's always possible to try to reduce the present of unpleasant organism by following some measures in order to get to our goal.

Many contamination occur while harvesting or distributed to retail food establishments or it can even be while handling it like cutting,touching, we have also ready to eat salads RTE that are exposed to a wide range of risk and condition from growth, harvest,preparation and distribution, this can increase the cahnces of getting contaminated with pathogenic bacteria that has genetic elements which help it to survive to antimicrobial pressure. This is one of hte main reasons that good hygiene practices from the beginning are essential for a good healthy produce, from farm to fork to avoid any sort of contamination with pathogens . The prudent use of antibiotics in farming is a good way to reduce the number of potential pathogens that can exist in farm foods, these kind of bacteria like salmonella showed that it can gain resistance to these treatements, so it’s necessary to investigate more on their prevalence in these foods in order to keep fresh produce/foods out of reach of contamination ( Taban and al. 2013).

Temperature may play an important role in the conservation of fresh produce to keep it healthy for the consummer, because the failure of maintaining proper conservation temperature can result in giving favorable conditions to several pathoghen growth, and leading to causing illnesses for the consummer. Sanitizing any tool used for cutting or choping of these fresh produce including for example knives and cutting boards. Fresh produce has to be also seperated of meat due to the possibilities of carrying bacteria to it . a knife can cross-contaminte salad(lettuce) with salmonella enterica if it has been used to cut raw meat,chicken just before. Proper cutting, wrapping with plastic and storing in retails, proper refrigeration temperature, all these maintained and the reduce of bacterial pathogen contamination is applied ( Carstens and al. 2019).

Consumers and food handlers should educate, train themselves about food and practices that prevent not only about salmonella but about how to prevent foodborne diseases , that where the importance of the national and regional surveillance netwroks in giving instructions, identifying and monitoring, to let people know about its risks and how to behave while processing food or consuming it in order to halt its propagation among population. The contact between people and domestic animals especially among young people encountering reptiles,cats, dogs, should be under supervision. Laboratories around the world work on tackling salmonella with effective methods (Ehuwa and al. 2021).

## II.4.6 Control measures recommended for salmonella contamination prevention :

**a.Prevention methods :**

- Preventive measures should be applied at all stages of food Chain: from primary production to processing, distribution, sale and consumption, foor an effective work.
- Salmonella prevention steps recommended in the food handling manually should be followed precisely.
- Contact between children and pets requires supervision.
- The public is advised to follow the National and Regional Foodborne Illness Surveillance System in order to Detect salmonellosis outbreaks early and respond quickly and prevent the spread among population

**a.1 Recommendations for the public and travellers :**

- Food must always be properly cooked at a good cooking temperature wether if its boiling or frying and served hot.
- The consumption of only pasteurized milk and its products is safer than consumming raw milk.
- Fruits and vegetables should be washed well in good conditions before consumption
- Washing hands well with appropriate hand sanitizing products after touching animals or using the toilet.
- Edible ice must be made from drinking water for a safer consumption.

**a.2 Recommendations for food handlers :**

- - Grocers should pay attention and follow ingredients Rules for the hygienic preparation of food.
- - Provision of five fundamental elements to lay the groundwork for safer food Training for Professionals and Food Safety consumer. The focus is:

1- keep clean
2- separate ingredients Cook food
3- cook it correctly
4- store it at the correct temperature

5- use drinking water.

**a.3 Recommendations for producers of fruits and vegetables :**

- - Practice good personal hygiene..
- - Fecal contamination should be avoided.
- - Only treated fecal waste is allowed to use in agriculture as farm composting or land fertilizers
- Irrigation water should be treated and managed properly to avoid contamination of crops.

**a.4 Recommendations for producers of aquaculture products :**

- Wash your hands regularly with soap and water and practice good personal hygiene, especially before handling or handling fish.
- Regularly remove debris, excess vegetation and debris that may degrade water quality and negatively impact fish health to ensure a clean pond environment.
- Monitor water quality parameters such as temperature, pH, dissolved oxygen and ammonia levels to ensure optimal conditions for fish growth and health.
- Regularly clean and sanitize harvesting equipment and keep it sanitary to prevent the spread of disease or contamination.
- Prioritize fish health by regularly assessing fish condition, looking for signs of disease or stress and taking appropriate action to correct any problems promptly. Consult with a veterinarian or fisheries specialist regularly to ensure the overall health and welfare of your fish (Pires & al. 2014 , Ehuwa & al. 2021)

# Materials and Method

**III.1 Objectives :**

The aim of this study is to identify and isolate in different medias of Salmonella strains from parsley and coriander took from various markets across Bejaia city .

**III.2 Sampling :**

The study was carried out between April 28 and may 3, on a total of 20 samples (10 of coriander/ 10 of parsley), we brought each one of them from different markets across the city of Bejaia,we processed to this operation by covering our hands with medical gloves to not contaminate them with hand germs, each sample was put in a sterile plastic bag alone and was labelled and closed well, then took directly to the laboratory of the university for the experiment.

**III.2.1 Pre-enrichement :**

Once we got to the laboratory we prepared enough peptone water for all the samples that we'll prepare , we put 225 ml of this prepared solution in 20 bags, after that we take 25g of each sample from their bags and put inside the bags containing the 225 ml of peptone water and close it fermely and shake it , than we incubate them inside the incubater for about 18 to 24 hours at 37° C.

**III.2.2 Enrichement :**

after the pre-enrichement process is finished, we take out the plastic bags out of the incubater and we began by opening them by order and take with the pipette pasteur 1 ml of the solution inside each one of the bags than we proceed to the enrichement in 20 tubes that contains 10 ml of RV (rappaport vassiliadis), after that we incubate all the 20 tubes in the agitating bath water at 42°C for about 18 to 24 hours.

**III.2.3 Isolation :**

After the pre-enrichement and enrichement process is done we recuperate the tubes from the incubator, we prepare two kind of medias for each sample one is XLD (Xylose lysine-desoxycholate) and SS (Salmonella,Shigella) we innoculate from the 20 tubes for each XLD and SS gelose in petri boxes then we incubate all thes petri boxes at 37°C.

**III.2.4 Purification :**

After the incubation process , the petri boxes are examined for bacteria characteristics of salmonella (a black center and a slightly red colored translucent zone on XLD gelose and smooth and opaque or colorless on SS gelose). They were subcultured on XLD and SS gelose using a deplatinum loop, in order to obtain pure cultures.

**III.2.5 Identification :**

Gram stain is the most commonly used staining technique in bacteriology. It is a differential staining method that divides bacteria into two groups: Gram-positive bacteria and Gram-negative bacteria. Gram-positive bacteria retain their purple color (gentian violet), while Gram-negative bacteria (including Salmonella) can be decolorized with alcohol and then stained pink with safranin.

**2.5.1 Study on the biochemical characteristics of Enterobacteriaceae strains:**

Microscopic and macroscopic characteristics of colonies are not sufficient to accurately identify bacteria. Other characteristics, especially biochemical or metabolic ones, need to be studied.

# RESULTS & Discussion

### IV.1.Results of the isolation and identification :

Salmonella was isolated on xylose-lysine-deoxycholic acid (XLD) and Salmonella-Shigella (SS) media to elucidate the presence and characteristics of this pathogenic bacteria. The purpose of this study was to gain insight into methods for the detection and isolation of Salmonella in the 20 samples ( 10 parsley samples,10 coriander samples), which is critical for public health and food safety.

**Isolation on XLD media:**

The use of XLD agar results in the growth of a variety of colonies, characterized by red to pink colors with or without black centers. These colonies corresponded to potential Salmonella isolates.

The selective nature of XLD agar, which contains bile salts and xylose as key components, successfully inhibits the growth of many non-Salmonella species, thereby increasing the specificity of the isolation.

As we can see in these figures below taken in the laboratory the results of (5th,6th,7th samples of coriander,on XLD)

**Fig.5:** growth of salmonella colonies on the Fifth sample of coriander on XLD

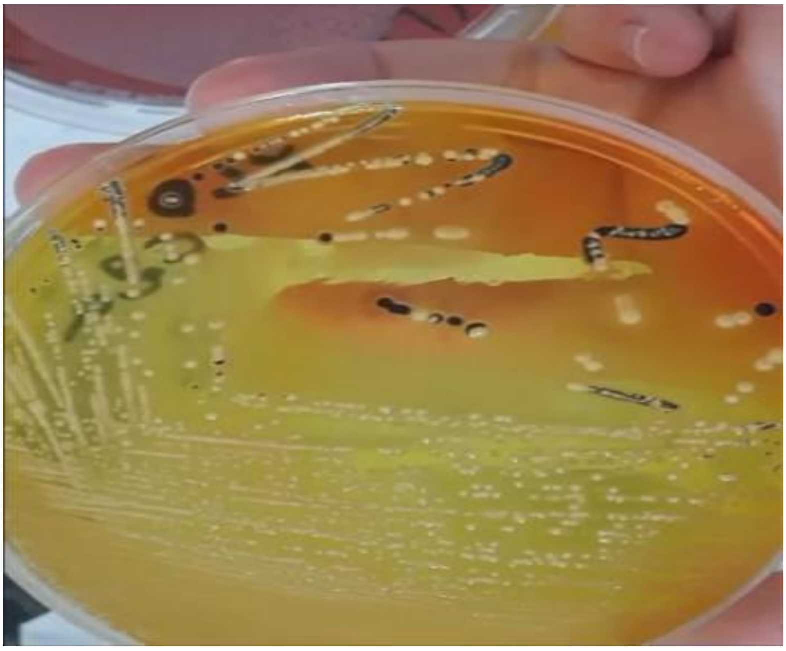


**Fig 6:** growth of salmonella colonies on the Sixth sample of coriander on XLD

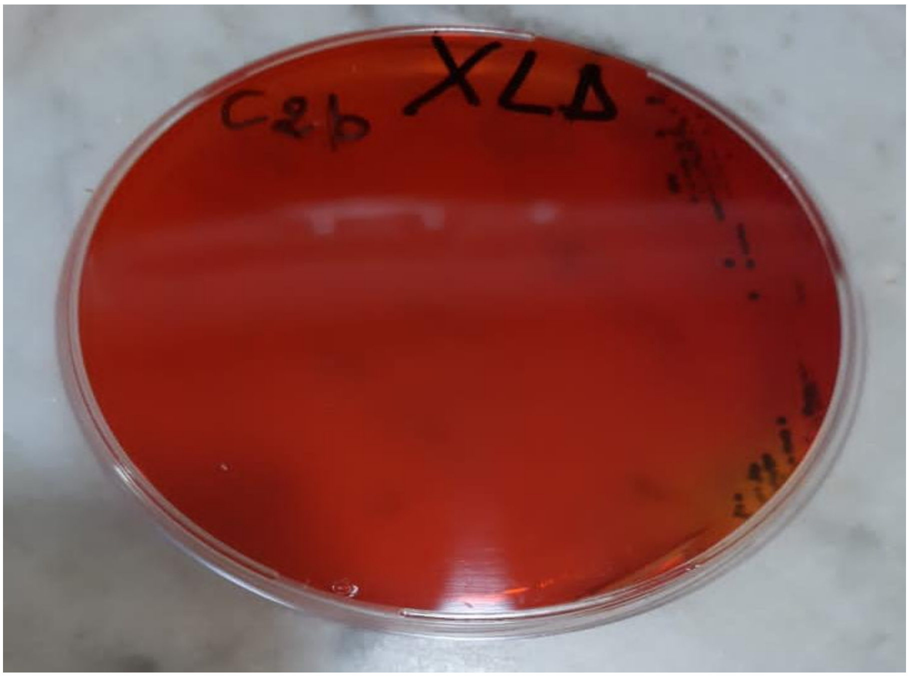
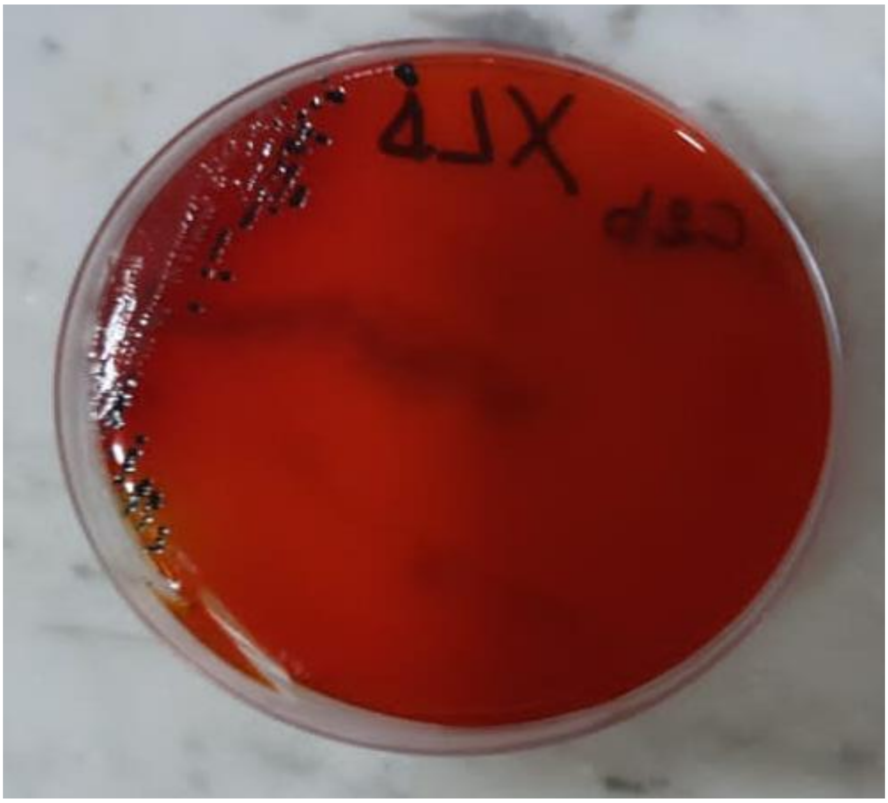



**Fig7:** growth of salmonella colonies on the Seventh sample of coriander on XLD

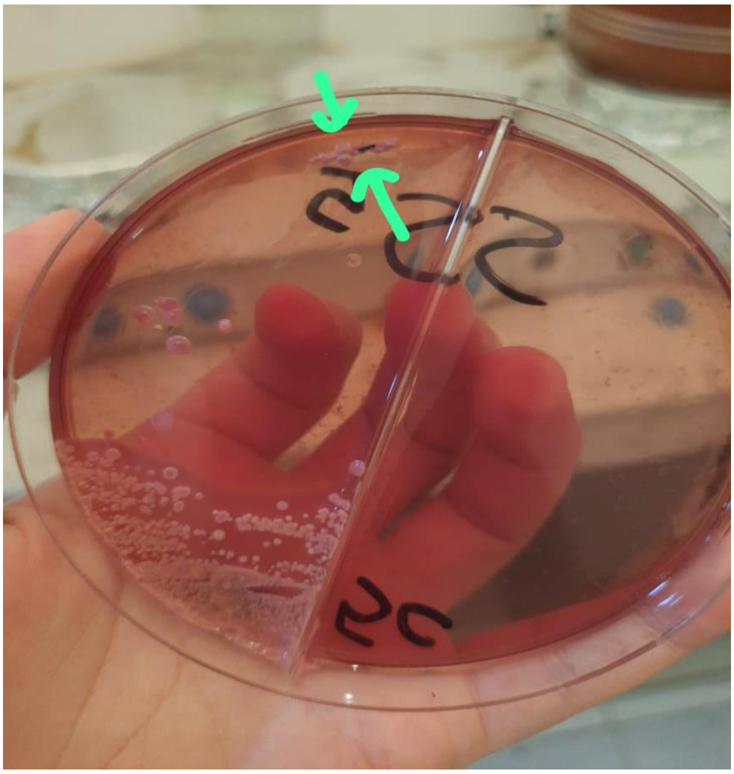


**Isolation on SS media:**

SS agar containing lactose, salicin, and bile salts shows colonies with characteristic features, such as clear to slightly translucent with a black center, indicating the presence of Salmonella spp.

The different capacities of SS agar can differentiate between Salmonella and Shigella, thereby improving the accuracy of isolation.

**Fig 8:** growth of salmonella colonies on the Fifth sample of coriander on SS



**On this table we have the results of the growing bacteria Among the 20 samples that we've worked on the selective media (XLD,SS) :**

| Number of sample | origin | gelose | aspect |
|---|---|---|---|
| 5 | Coriander | SS | typically appear as colorless or transparent colonies. may have a slightly translucent or opaque appearance. |
| 5 | Coriander | XLD | red colonies, usually have a black center |
| 6 | Coriander | XLD | |
| 7 | Coriander | XLD | |

# Conclusion

Enterobacteria hold a significant position in the minimally processed vegetable industry. This significance is due to the variety of bacterial species they encompass and their implications for public health.

In summary, this master's thesis provides valuable insights into the prevalence of Salmonella on minimally processed vegetables, especially parsley and coriander which are the 20 samples used in this experiment. Through thorough research and analysis, Salmonella contamination has been shown to be a potential risk to these popular culinary herbs, which are often eaten raw or minimally processed. The results highlight the importance of strict compliance with sanitation and hygiene practices throughout the supply chain from farm to fork to minimize the risk of foodborne illness associated with these vegetables.

Additionally, the study highlights the need for continued monitoring, surveillance, and research efforts to better understand the specific sources and factors that contribute to Salmonella contamination in minimally processed vegetables. This knowledge is essential for developing effective preventive measures and food safety regulations that protect public health.

As biologists, we remain committed to improving food safety and public health, and this research is a stepping stone to broader efforts to improve food supply security. By expanding our understanding of the prevalence of Salmonella in minimally processed vegetables, we can work to create a safer, healthier future for consumers around the world.

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## Resume

The primary objective of this study is to estimate the prevalence of Salmonella strains in minimally processed vegetables, specifically parsley and coriander as we did at the laboratory.

For this purpose, 20 samples of minimally processed parsley and coriander have been collected from various sources. Salmonella isolation was performed on selective agar ; The overall prevalence of Salmonella in minimally processed parsley and coriander was found in both gelose XLD and SS for the sample 5 but only in XLD for the 6th and 7th samples.

"It is crucial to take appropriate measures to minimize the prevalence of Salmonella in minimally processed vegetables, through strict control measures such as thorough food safety protocols and regular monitoring of product quality."

**Key Words :** *Salmonella,* prevalence, food safety.