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In pursuit of obtaining a Master's degree in Fundamental Microbiology under the theme :

The research of enteropathogenic bacteria in leafy green vegetables from farms and markets

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"In the name of **Allah**, the most gracious the most merciful"

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*Thank you, **Allah**, for giving me the strength and health to accomplish many things in life.*

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Sana

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List of abbreviations

ADH: Arginine dihydrolase**.**	**MEL:** Melibiose.
AMY: Amygdalin.	**MR:** Methyl Red.
API: Analytical Profile Index.	**MSA:** Mannitol Salt Agar.
ARA: Arabinose.	**NaCl:** Sodium Chloride.
BLEB: Buffered Listeria Enrichment Broth.	**NR:** Nitrate Reductase.
BP: Baird-Parker Agar.	**ODC:** Ornithine decarboxylase.
BPW: Buffered Peptone Water.	**ONPG:** β-galactosidase.
CA: Columbia Blood Agar.	**PALCAM:** Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol.
CIT: Citrate production.	**PCR:** Polymerous Chain Reaction.
EHEC: Enterohemorrhagic *Escherichia Coli*.	**RHA:** Rhamnose.
FDA: Food and Drug Administration.	**RVS:** Rappaport Vassiliadis Soya Peptone Broth.
GEL: Gelatinase.	**SAC:** Sucrose.
GLU: Glucose.	**SMAC:** MacConkey Sorbitol.
H_2S: Hydrogen Sulfide.	**STEC:** Shiga-like Producing *Escherichia coli.*
HUS: Hemolytic Uremic Syndrome.	**SOR:** Sorbitol.
IND: Indole.	**TDA:** Tryptophan-Deaminase.
INO: Inositol.	**TSA:** Trypticase Soy Agar.
KCL: Potassium Chloride.	**TSI:** Triple Sugar Iron Agar.
KOH: Potassium Hydroxide.	**URE:** Urease.
LB: Lactose Broth.	**VTEC:** Verocytotoxin Producing *Escherichia coli.*
LDC: Lysine decarboxylase.	**VP:** Voges-Proskauer.
MAN: Mannitol.	**WHO:** World Health Organization.

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Introduction

In the health conscious society of the 21st century, vegetables form an integral part of the human diet. Over the last three decades, the global consumption of fresh vegetables has increased significantly, thus expanding the market segment for fresh produce by more than 20%. They contain valuable food ingredients that are essential for the proper function of the body and contain various medicinal and therapeutic agents and are valued mainly for their high vitamin and mineral content **(Jaiswal and Sharnagat 2023)**. Regular daily consumption of them in sufficient amounts can help prevent some diseases such as cardiovascular diseases and certain cancers **(Taban & Halkman, 2011)**. For instance, the Food and Drug Administration (FDA) and World Health Organization (WHO) have recommended 5–9 servings of fruits and vegetables to be taken daily because correct fresh produce intake alone could save 2.7 million lives **(Jaiswal and Sharnagat 2023)**.

Fresh fruit and vegetables are now recognized to be a major route of entry for pathogenic enterobacteria into the food chain. *Salmonella*, *E. coli* and *Listeria* are among the most prevalent food-borne bacterial pathogens in the developed world and are able to enter the food chain at any point from farm to table **(Silva et al., 2014)**. These bacteria are known as causers of the diseases: salmonellosis, hemolytic uremic syndrome (HUS), and listeriosis **(Kljujev et al., 2018)**.

Reported outbreaks associated with the consumption of fresh vegetables have grown steadily. As most of these products are eaten raw or with minimal cooking, their microbial content may represent a risk factor for the consumer's health **(Jaiswal and Sharnagat 2023)**.

One of the largest outbreaks of verocytotoxin producing *E. coli* (VTEC) derived gastro enteritis occurred in Japan in 1996 as a result of contamination of radish sprouts with *E. coli* O157:H7. In 2006 an outbreak of this latter occurred across several US states as a result of the contamination of fresh spinach, with more than 200 reported cases of infection and three fatalities. In the latter half of 2007, *S. enterica serovar Paratyphi* associated with baby spinach and leafy vegetable salad infected at least 430 individuals in northern Europe **(Holden et al., 2009)**. Another outbreak of *E. coli* that dazed the world led to 50 deaths and hospitalizations of about 4,000 patients in about 16 countries **(Balali et al., 2020)**.

Large investigations on prevalence of pathogenic bacteria in fruits and vegetable were conducted in the UK, Ireland, Germany and the Netherlands in 2007. The proportion of produce samples that yielded *Salmonella* in these studies ranged from 0.1% to 2.3%, with pre-cut products having some of the highest proportions contaminated **(Berger et al., 2010)**.

Enterobacteriaceae is a large family of Gram negative bacteria that includes more familiar pathogens such as *Escherichia coli, Salmonella, Shigella, Klebsiella* and *Yersinia*. Most of the members of the family Enterobacteriaceae cause gastrointestinal problems in humans **(Jaiswal and Sharnagat 2023)**. They are a group of mesophilic bacteria that are found in a diverse variety of environments, terrestrial and aquatic, and in a broad range of host species, both plant and animal **(Holden et al., 2009)**.

Salmonella and *E. coli* are among the most prevalent food-borne enteric pathogens that frequently contaminate leafy greens. On one hand, the pathotype VTEC or shiga-like producing *E.coli* (STEC) causes gastrointestinal infections characterized by bloody diarrhea and produce shiga toxins that enter the bloodstream that can lead to hemolytic uremic syndrome, a serious complication of STEC infection that damages the kidneys **(Kintz et al., 2019)**. Enterohaemorrhagic *E. coli* (EHEC) strains can survive in fresh ground beef and on fresh leafy green vegetables, and it is well known that their main reservoirs are ruminants, which continually shed bacteria into the environment, contaminating food and water **(Luna-Guevara et al., 2019)**. In the USA, STEC was the pathotype most associated with outbreaks of foodborne illness, predominantly belonging to serogroup O157:H7, which accounted for 92% of cases between 1998 and 2013. In 2015, a new highly pathogenic strain of O157:H7 emerged in England and Wales, which has been identified in patients and was associated with the consumption of prepacked salad leaves **(Thomas et al., 2024)**.

And on the other, *Salmonella,* an etiologic agent of salmonellosis in humans, is a flagellated facultative anaerobe, rod-shaped bacterium **(Ehuwa et al., 2021)**. It is ubiquitous in soil, water, and vegetation and is part of the intestinal microbiota of many domestic and wild animals, including pigs, cattle, and poultry **(Quiroz-Santiago et al., 2009)**. This genus is composed of two species; *S. enterica* and *S. bongori. Salmonella enterica*, which is a leading cause of gastroenteritis, is subdivided into hundreds of serovars. It is the pathogen most frequently linked to consumption of fruit and vegetables. *S. enterica* serovars can colonize seeds, sprouted seeds, leaves, and fruit of a variety of plant species **(Berger et al., 2010)**. Outbreak reports between 2006 and 2023 in the USA, show that a range of *Salmonella* serovars

can contribute to outbreaks. Sprouted vegetables were a common vector for *Salmonella* spp., as well as papaya, melon/cantaloupe, cucumbers and tomatoes. Cucumber contamination included a large-scale outbreak of *S.* Poona in the USA, which led to 907 cases across 40 states and six fatalities **(Thomas et al., 2024)**.

Whereas *Salmonella* and *E. coli* are the two leading causes of bacterial outbreaks linked to the consumption of fresh fruit and vegetables, *L. monocytogenes* has caused comparatively fewer outbreaks, but a greater cost for the food industry. It is a persistent pathogenic organism that can survive under harsh conditions including low temperatures (freezing conditions), low pH, and even high salt concentrations **(Balali et al., 2020)**. Listeriosis results in the highest case fatality rate of the three bacterial pathogens discussed here, and ranks as one of the most frequent causes of death due to foodborne illness. *L. monocytogenes* can be subdivided into at least 13 serotypes differing in their pathogenicity. Serotype 4b is responsible for the majority of human listeriosis outbreaks, and led to 10 outbreaks, with a hospitalisation rate of 70%, and a case fatality rate of 13%. For example, between 2013 and 2014, 32 cases of listeriosis associated with ready-to-eat salads were reported in Switzerland and in 2011, a multi-state outbreak of *L. monocytogenes* on cantaloupe melons from a single farm in Colorado led to 147 cases across 28 states, causing 143 hospitalisations and 33 deaths **(Thomas et al., 2024)**.

Fruits and vegetables may be contaminated at any point in time during the production chain. Sources of contamination can be grouped into two broader groups, namely, preharvest and postharvest sources of contamination **(Balali et al., 2020)**. One of the first sources of contamination during the pre-harvest processes is the soil, especially if sites used for propagating fresh produce were previously used for animal production, waste disposal, or if manure was applied as fertiliser. *S. Typhimurium* can persist for up to 231 days, *E. coli* O157:H7 for up to 217 days, and *L. monocytogenes* for up to 360 days in soil microcosms **(Thomas et al., 2024)**.

Another well-known source of contamination is irrigation water, applied directly to crops during agricultural production. Water from rivers and lakes can introduce enteric pathogens on crops through contamination via runoff of sewage, soil, or animal faecal matter **(Thomas et al., 2024)**. It has been confirmed a few years ago that *E. coli* O157: H7 can be transmitted to lettuce through the soil and irrigation water and can persist throughout the life cycle of the plant **(Balali et al., 2020)**.

Animals are a common reservoir of enteric pathogens and can be either the source of contamination via their faeces which can be shed into soil, water or directly onto the foliage, or the vector of numerous pathogens, carrying pathogens from one area to another. The main reservoir for *E. coli* O157:H7 is in the intestine of healthy cattle, and both *Salmonella* and *L. monocytogenes* have also been detected in livestock. Birds may also act as longer distance routes of transmission of pathogens and have been shown to be potential vectors for all three pathogens. An additional source is manure from domestic animals which is often applied to agricultural soils as a form of fertiliser, which, when inadequately composted, can, in fact, provide a source of contamination and has led to previous outbreaks of *E. coli* in lettuce and spinach **(Thomas et al., 2024)**. For example, EHEC has the ability to adhere diffusely to the epidermis, with aggregation around the stomata, and penetration to a depth of 20 to 100 μm into the stomata and junction zones of cut lettuce leaves. In addition, it has been shown that *E. coli* O157 : H7 can move into the plant through the root system to reach the edible portion of lettuce. Insects could also be a source as contaminated flies have been shown to transfer *E. coli* to plant leaves or fruits. And finally, during the handling and harvesting of crops the workers hands as can become a vehicle for contamination **(Luna-Guevara et al., 2019)**.

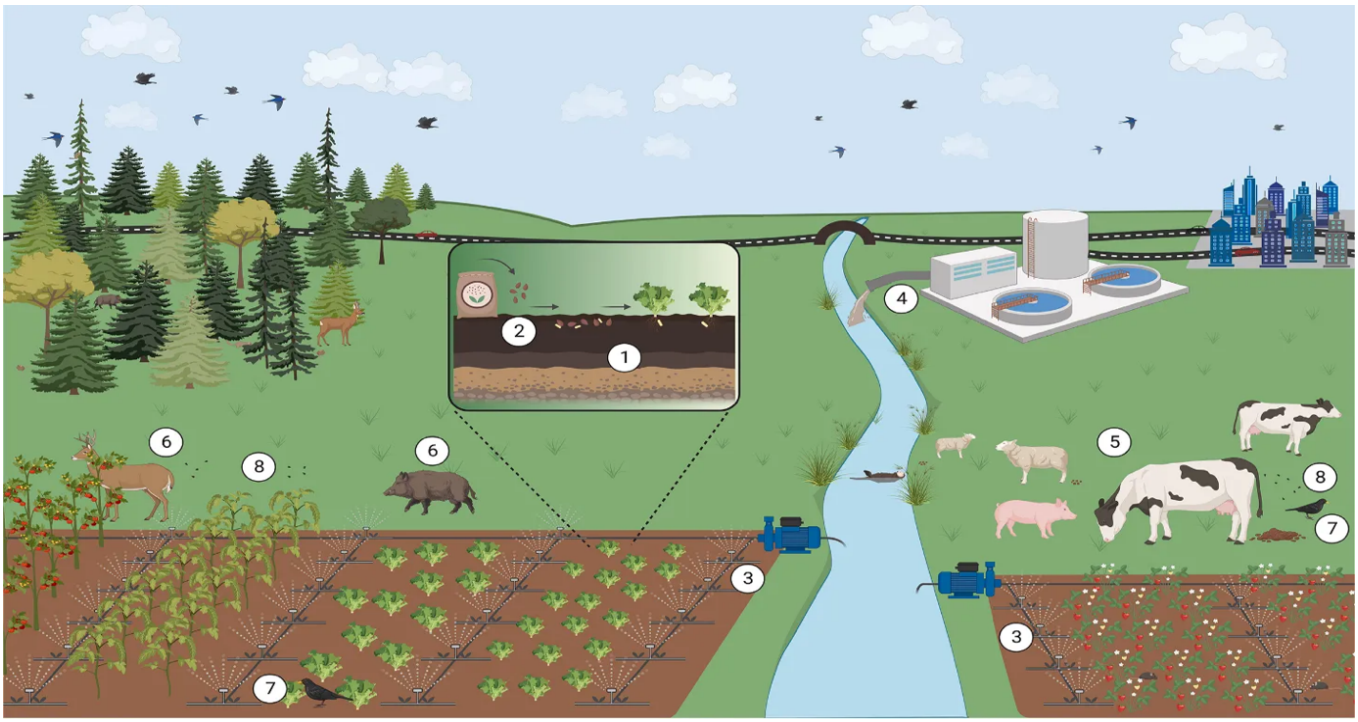


Figure 1. Pre-harvest sources of contamination: (1) soil (2) seeds (3) irrigation water (4) wastewater (5,6) domestic animals/wild animals (7) birds (8) insects. Source: **Thomas et. al. (2024).**

Post-harvest operations, including storage, preparation and packaging, can cause enteric pathogen contamination if not controlled correctly. Plant surfaces are stressful environments for enteric pathogens, since they are nutrient-poor compared to the gut of their usual warm-blooded hosts. Moreover, the micro-organisms are facing fluctuations in temperature, solar radiation, wind and rainfall, as well as the presence of indigenous populations of bacteria in the phyllosphere, which may be better adapted to survival on the leaf or fruit surface **(Thomas et al., 2024)**.

A general model of leaf colonization by bacteria considers three stages: 1) bacteria arrive on leaves and adhere to the leaf surface, 2) bacteria multiply and form aggregates, and 3) bacteria internalise through open pores. The attachment of enteric pathogens to leaves is accomplished by several components of bacterial cell surfaces, including flagella, pili and fimbriae. Following adhesion to fresh produce, the ability of bacterial pathogens to survive and colonise produce surfaces is a key contributor to their ability to cause foodborne illness. Here, 'survival' is defined as the ability of the pathogen to survive on plant surfaces for extended periods of time, and 'colonisation' is the ability of the pathogen to multiply on the plant surface. Microbial biofilms can form on leaves, fruit and root surfaces and within plant tissue, providing an adaptive strategy for bacteria to persist on plants, and resist disinfection treatments **(Thomas et al., 2024)**.

And lastly, the ability of bacteria to internalise into plant tissue through natural openings on the surface enables them to avoid disinfection, which could provide one explanation as to why post-harvest processes may not be sufficient in reducing outbreaks. Stomatal pores present natural potential entry routes for enteric pathogens **(Thomas et al., 2024)**. It is now clear that enteric pathogens have acquired mechanisms to enter plants and reproduce inside of plants, a discovery that explains the failure of sanitizers to efficiently eradicate food-borne pathogens in produce **(Silva et al., 2014)**.

Since there is no bactericidal or killing agent for combating contaminations of spinach and lettuce with enteric bacterial pathogens such as *E. coli* and *Salmonella* spp., enterohemorrhagic *E.coli* during the harvesting, processing, and packing procedures, the pathogens tend to survive even better and stand the chance of human infection **(Balali et al., 2020)**.

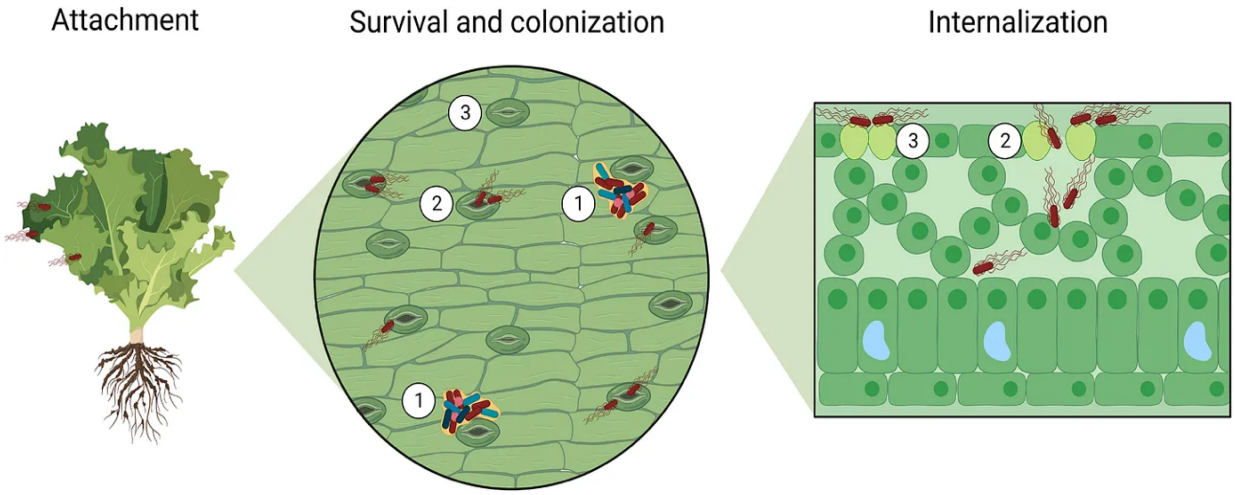


Figure 2. Stages of contamination of enteric pathogens of leaves, via attachment, colonisation, and internalisation. (1) Following initial attachment to the leaves, pathogens will colonise the surface by producing biofilms. (2) Whereas some bacteria can attach to the stomatal cells, (3) and invade the internal cavity, and some trigger plant immune responses inducing stomatal closure. Source: **Thomas et. al. (2024)**.

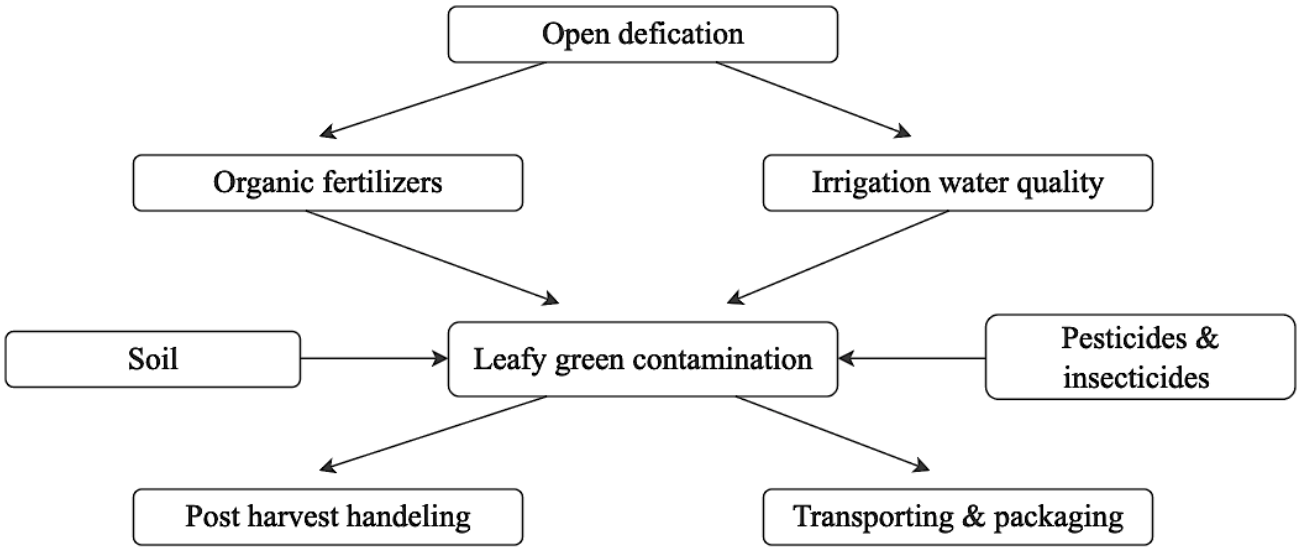


Figure 3. Factors contributing to the contamination of fruits and vegetables. Source: **Balali et al. (2020)**.

While many studies have evaluated leafy vegetable spoilage, our research incorporates two main distinctive elements that include; a geographical focus: the presence of enteric pathogens in leafy greens from farms and markets in Béjaïa, Algeria and a comparative analysis: the occurrence of contaminated greens between farms and markets.

Conducting research on the presence or absence of enteropathogens in herbs is crucial due to the lack of quality microbiological data on fresh produce in specific regions in Béjaïa. This gap in knowledge represents an important aspect that deserves further attention. This confronts consumers with potential health risks linked to the consumption of contaminated vegetables, highlighting the need for continuous monitoring and assessment.

This study aims to examine the presence of three most common pathogenic bacteria in leafy greens and to analyse the quality of water and soil which could be potential sources of contamination. This helps us address important public health concerns related to foodborne diseases caused by these pathogens and providing region specific information given that each region has unique agricultural and marketing practices. In addition to highlighting the prevalence and distribution of these pathogens in local Algerian produce.

Our hypothesis proposes that leafy greens from farms and markets are likely to be contaminated with enteric bacteria due to potontial exposure to contaminated water, soil, and handling practices. This dissertation is structured as follows:

- This general introduction that outlines the background and significance of our research.
- Material and methods that describe the sampling, pre-enrichment, enrichment, isolation, and re-isolation processes for strain isolation and biochemical tests used for strain identification.
- Results and discussions presenting our findings and providing recommendations for future research.
- A conclusion that summarizes the key insights and addresses the research question.

Material & Methods

I. Strain Isolation

I.1. Sample Collection

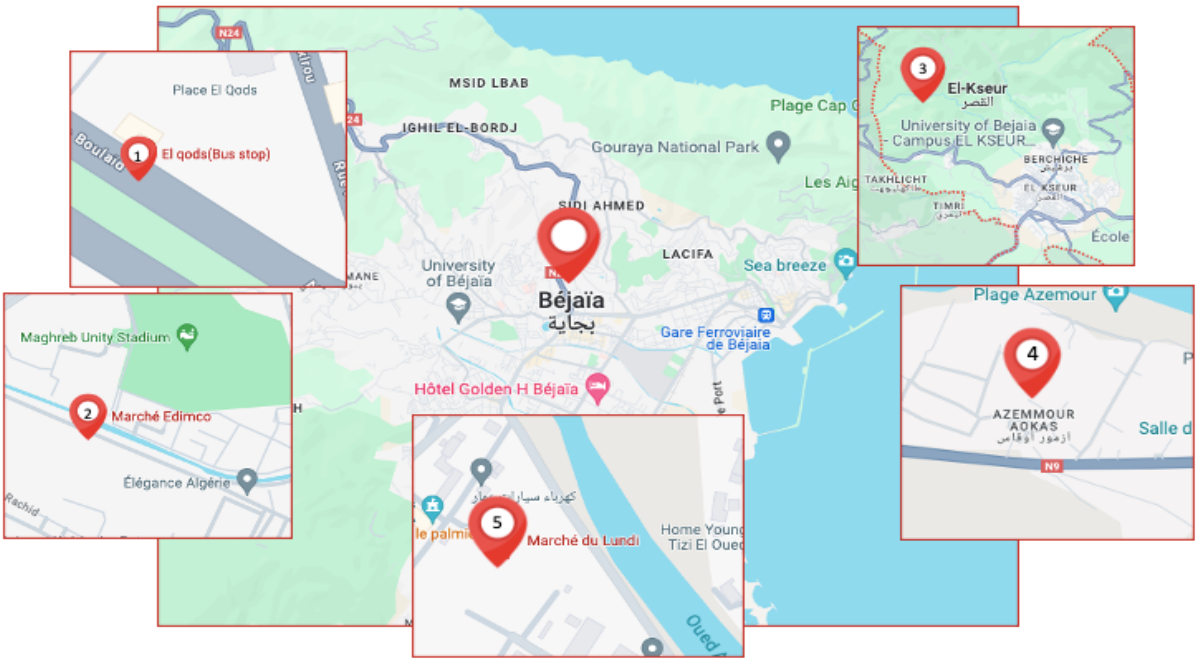


Figure 4. Leafy greens sampling locations with labelled cities. **1:** El Qods, **2:** Edimco, **3:** El Kseur (1) et (2), **4:** Aokas, **5:** Souk El Tenine.

Our research was conducted from March 4th through May 30th 2024 with a total of 175 samples consisting of parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*), celery (*Apium graveolens L.*), and mint (*Mentha*), purchased from various vegetable markets (n=154) at different times of the day (morning and afternoon), Irrigation water (water wells and rainfall), soil, and the same leafy greens previously mentioned, were also gathered from three farmlands (n=27) in Béjaïa, Algeria **(table 1)**.

The samples were collected using gloves, and within 2 hours, they were transported for laboratory analysis **(Touati et al., 2017)**. All samples examined in this study are listed in **(Tables 2,3,4, and 5)**.

I.2. Pre-enrichment

Upon arriving at the laboratory, we started by preparing a sufficient amount of Buffered Peptone water (BPW) for our samples. In an aseptic area, we weighed 25 g of each sample and added 225 ml of the BPW into it, inside sterile stomacher filter bags and shook them for 1 minute. We labeled and incubated the bags at 37 ºC for 24 hours **(Campos et al, 2013)**. This process was crucial to concentrate our target microorganisms and ameliorate bacterial identification. It was also carried out to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock **(Joseph A. Odumeru, 2012)**.

I.3. Enrichment

After incubating the stomacher bags, we opened them in an aseptic zone and pipetted 1 ml of the solution into 10 ml of the Rappaport Vassiliadis Soya Peptone broth (RVS) test tubes, selective for *Salmonella*. Then into 10 ml of the Buffered *Listeria* Enrichment broth (BLEB)/Fraser broth, selective for *Listeria*, and 10 ml of the Lactose broth (LB) for EHEC. Lastly, we incubated the tubes accordingly at 42 ºC in a water bath, 37 ºC, and 42 ºC in an incubator for 24 hours **(Priyanka et al., 2021)**. This procedure was imperative 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)**.

I.4. Isolation

This step involved the inoculation from the RVS, Fraser/BLEB, and LB tubes respectively in the already prepared Xylose Lysine Deoxycholate agar (XLD) media selective for *Salmonella*, PALCAM media selective for *Listeria*, and MacConkey Sorbitol (SMAC) for EHEC in Petri dishes using the streak plate method aseptically. Finally, we incubated the plates at 37 ºC for 24 hours.

I.5. Re-Isolation

If present, suspected positive isolates were chosen from each bacterium and colonies were re-isolated from them. Aseptically, and using a sterile toothpick, we picked a red *Salmonella* isolated colony with a black center from the XLD agar, a grey-green with a black halo *Listeria* colony from PALCAM, and a colorless EHEC colony from the Trypticase Soy agar (TSA) and inoculated the collection tubes that contained 1 ml of physiological water and

agitated them. From this bacterial suspension and using the streak plate method, we streaked the SMAC, Mannitol Salt agar (MSA), and TSA mediums correspondingly and incubated them at 37 ºC for 24 hours.

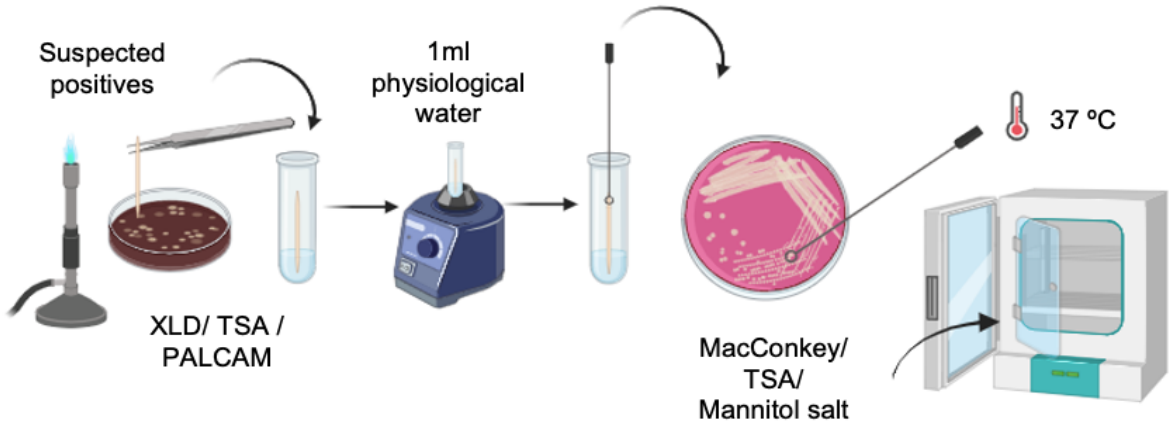


Figure 5. Re-isolation process. Figure was created in Biorender.

II. Strain Identification

To further identify the strains, a set of biochemical tests (including the IMViC tests), identification media, and the API Gallery 20E were performed:

II.1. Biochemical tests

II.1.1. Indole/Urea Broth

We distributed collection tubes that contained 0,5 ml of the urea/indole medium aseptically and inoculated them with the organism for the hydrolysis of urease. Then, we incubated the tubes at 37 ºC for 24 hours. The broth is then split into two tubes and 4 drops of the Kovacs reagent were added in one tube for detecting the production of indole, and 1 drop of TDA for its equal production in the other. **(Touati, 2023, pp. 156 & 319)**.

II.1.2. Methyl Red–Voges Proskauer Broth (MR-VP)

First, we inoculated the MR-VP tubes with the bacterium and incubated them at 37 ºC for 18 hours. After incubation, we split the broth into two tubes (for each strain) and added one drop of methyl red reagent in the MR tube, and shook it for 5 minutes to see if the bacteria fermented glucose using the mixed acid fermentation pathway. Whereas for the VP tube, 6 drops of the reagent alpha-naphthol and 2 drops of potassium hydroxide (KOH) were added to it to detect the fermentation of glucose through the butylene glycol pathway **(Touati, 2023, p. 329)**.

II.1.3. Simmons' Citrate Agar

We picked a loopful of a colony and gently streaked the surface of the medium's slope and incubated the tubes at 37 ºC for 18 hours. This helped detect whether the bacterium used citrate as a sole source of carbon or not **(Touati, 2023, p. 75)**.

II.1.4. Triple Sugar Iron Agar (TSI)

We inoculated the TSI agar slants by stabbing the butt of the medium, streaked the surface, and incubated the tubes at 37 ºC for 18 hours. This test detects the production of gas, hydrogen sulfide (H_2S), and the fermentation of three carbohydrates; lactose, glucose, and sucrose by enteric bacteria **(Touati, 2023, p. 310)**.

II.1.5. MEVAG Agar

In an aseptic area, we added 6 drops of our xylose and rhamnose solution in the MEVAG medium in 2 separate tubes (for each strain), shook the tubes, and placed them in cold water to cool off for a few minutes. With a loaded inoculum, we inoculated the tubes by stabbing the butt and streaking upwards circularly. We then added 4 drops of Vaseline oil in the first tube to create an anaerobic condition and closed it firmly, while the second tube was loosely closed with no Vaseline oil**,** and finally incubated at 37 ºC for 24 hours. MEVAG determines the path of attack of carbohydrates, namely oxidation (presence of oxygen) and fermentation (absence of oxygen) **(Touati, 2023, p. 155)**.

II.2. Identification media

II.2.1. Columbia Blood Agar (CA)

Using the 4-quadrant streak method aseptically, the strains were inoculated on the plate and incubated at 37 ºC for 48 hours. This medium is a general-purpose enriched medium often used to grow fastidious organisms and differentiate them based on their hemolytic properties **(Jaiswal and Sharnagat 2023)**.

II.2.2. Baird-Parker Agar (BP)

A loopful of a colony was inoculated on the medium using the streak plate method. The plates were then incubated at 37 ºC for 48 hours.

II.3. API Gallery 20E

To confirm the identification of our strains, the API Gallery 20E test kit was used as follows:

a. A bacterial suspension was prepared and added to the tubes using a sterile Pasteur pipette.
b. The tests CIT, VP, and GEL, were filled with the suspension completely (tube + cupule) to create aerobiosis, and only the (tubes) were filled for the remaining chambers.
c. The cupules of the tests; ADH, LDC, ODC, H_2S, and URE, were filled with paraffine oil to create an anaerobic condition.
d. We poured 5 ml of physiological water into the tray holes and added the strip on top, which stopped it from drying out during incubation.
e. The strip is then incubated at 37 ºC for 18 to 24 hours.
f. We then revealed the tests requiring the addition of a reagent (VP, TDA, indole, and glucose);
    - **VP:** One drop of 40% NaOH (VP_1), and one drop of alpha-naphthol (VP_2).
    - **TDA:** One drop of Tryptophan deaminase.
    - **IND:** One drop of Kovacs.
    - **GLU:** One drop of Nitrate Reductase (NR_1) and (NR_2).
g. All reactions were noted on our Biomérieux result sheet.
h. Identification was acquired using the official Biomérieux website.

Table I. Sample distribution from different vegetable markets and farms in Béjaïa.

	Site	Sample	No.
Markets	El Qods	Parsley **(P)**	20
		Coriander **(Co)**	14
		Celery **(Ce)**	12
	El Kseur (1)	Parsley	17
		Coriander	10
		Celery	10
	El Kseur (2)	Coriander	7
		Celery	7
	Souk El Tenine	Coriander	8
		Celery	8
	Edimco	Parsley	18
		Coriander	12
		Celery	11
Farms	Farm no. 1	Coriander	1
		Soil **(S)**	1
		Irrigation water **(e)**	1
	Farm no. 2	Celery	3
		Soil	3
		Irrigation water	3
	Farm no. 3	Mint **(Me)**	3
		Soil	3
		Irrigation water	3
	Total		**175**

Abbreviations. **P:** Parsley, **Ce:** Celery, **Co:** Coriander, **S:** Soil, **e:** Irrigation water, **Me:** Mint.

Table II. Leafy greens, irrigation water, and soil samples from different farms in Béjaïa.

Date	Site	Sample	Code
4/3/2024	Farm no. 1	Irrigation water	e1
		Soil	S1
		Coriander	Co1
	Farm no. 2	Irrigation water	e2
		Soil	S2
		Celery	Ce1
	Farm no. 3	Irrigation water	e3
		Soil	S3
		Mint	Me1
11/3/2024	Farm no. 2	Irrigation water	e4
		Soil	S4
		Celery	Ce2
	Farm no. 3	Irrigation water	e5
		Soil	S5
		Mint	Me2
18/3/2024	Farm no. 2	Irrigation water	e6
		Soil	S6
		Celery	Ce3
	Farm no. 3	Irrigation water	e7
		Soil	S7
		Mint	Me3

Abbreviations. **Ce:** Celery, **Co:** Coriander, **S:** Soil, **e:** Irrigation water, **Me:** Mint

Table III. Morning and afternoon "Parsley" samples from different vegetable markets in Béjaïa from the same vendors.

Date	Time	Site	Vendor	Code
4/3/2024	**8am**	Edimco	V1	P1
			V2	P2
			V3	P3
		El Kseur (1)	V1	P4
			V2	P5
			V3	P6
	12pm	Edimco	V1	P7
			V2	P8
			V3	P9
		El Kseur (1)	V1	P10
			V2	P11
			V3	P12
11/3/2024	**8am**	El Qods	V1	P13
			V2	P14
			V3	P15
			V4	P16
			V5	P17
		Edimco	V1	P18
			V2	P19
			V3	P20
		El Kseur (1)	V1	P29
			V2	P30
			V3	P31
	12pm	El Qods	V1	P21
			V2	P22
			V3	P23
			V4	P24
			V5	P25
		Edimco	V1	P26
			V2	P27
			V3	P28
		El Kseur (1)	V1	P32
			V2	P33
			V3	P34
18/3/2024	**8am**	Edimco	V1	P35
			V2	P36
			V3	P37
		El Qods	V1	P38
			V2	P39
			V3	P40
			V4	P41
			V5	P42
		El Kseur (1)	V1	P51
			V2	P52
			V3	P53
	12pm	Edimco	V1	P43
			V2	P44
			V3	P45
		El Qods	V1	P46
			V2	P47
			V3	P48
			V4	P49
			V5	P50
		El Kseur (1)	V1	P54
			V2	P55
Total				**55**

Table IV. Morning and afternoon "Celery" samples from different vegetable markets in Béjaïa from the same vendors.

Date	Time	Site	Vendor	Code
25/3/2024	**8am**	Edimco	V1	Ce4
			V2	Ce5
			V3	Ce6
		El Qods	V1	Ce7
			V2	Ce8
			V3	Ce9
			V4	Ce10
			V5	Ce11
			V6	Ce12
		Souk El Tenine	V1	Ce13
			V2	Ce14
		El Kseur (1)	V1	Ce20
			V2	Ce21
			V3	Ce22
		El Kseur (2)	V1	Ce26
			V2	Ce27
	12pm	Edimco	V1	Ce17
			V2	Ce18
			V3	Ce19
		El Qods	V1	Ce29
			V2	Ce30
			V3	Ce31
			V4	Ce32
			V5	Ce33
			V6	Ce34
		Souk El Tenine	V1	Ce15
			V2	Ce16
		El Kseur (1)	V1	Ce23
			V2	Ce24
			V3	Ce25
		El Kseur (2)	V1	Ce28
29/3/2024	**8am**	Edimco	V1	Ce35
			V2	Ce36
			V3	Ce37
		El Kseur (1)	V1	Ce38
			V2	Ce39
		El Kseur (2)	V1	Ce42
			V2	Ce43
		Souk El Tenine	V1	Ce46
			V2	Ce47
	12pm	El Kseur (1)	V1	Ce40
			V2	Ce41
		El Kseur (2)	V1	Ce44
			V2	Ce45
		Souk El Tenine	V1	Ce48
			V2	Ce49
		Edimco	V1	Ce50
			V2	Ce51
		Total		**48**

Table V. Morning and afternoon "Coriander" samples from different vegetable markets in Béjaïa from the same vendors.

Date	Time	Site	Vendor	Code
25/3/2024	**8am**	Edimco	V1	Co2
			V2	Co3
			V3	Co4
		El Qods	V1	Co5
			V2	Co6
			V3	Co7
			V4	Co8
			V5	Co9
			V6	Co10
			V7	Co11
		Souk El Tenine	V1	Co12
			V2	Co13
		El Kseur (1)	V1	Co19
			V2	Co20
			V3	Co21
		El Kseur (2)	V1	Co25
			V2	Co26
	12pm	Edimco	V1	Co16
			V2	Co17
			V3	Co18
		El Qods	V1	Co28
			V2	Co29
			V3	Co30
			V4	Co31
			V5	Co32
			V6	Co33
			V7	Co34
		Souk El Tenine	V1	Co14
			V2	Co15
		El Kseur (1)	V1	Co22
			V2	Co23
			V3	Co24
		El Kseur (2)	V1	Co27
29/3/2024	**8am**	Edimco	V1	Co35
			V2	Co36
			V3	Co37
		El Kseur (1)	V1	Co38
			V2	Co39
		El Kseur (2)	V1	Co42
			V2	Co43
		Souk El Tenine	V1	Co46
			V2	Co47
	12pm	El Kseur (1)	V1	Co40
			V2	Co41
		El Kseur (2)	V1	Co44
			V2	Co45
		Souk El Tenine	V1	Co48
			V2	Co49
		Edimco	V1	Co50
			V2	Co51
			V3	Co52
		Total		**51**

Results & Discussions

I. Strain Isolation

1. Sample Collection

During our study, a total of 175 samples were collected, in which; parsley (n=55), celery (n=51), coriander (n=52), mint (n=3), irrigation water (n=7), and soil (n=7) were taken from different vegetable markets and farms for laboratory analysis.

2. Isolation

Isolation on the selection mediums allowed us to select 47 strains of our targeted bacteria including 17 EHEC, 8 *Listeria*, and 22 *Salmonella* suspected species **(Table 6)**.

Table VI. Positive suspected isolates from the isolation and re-isolation steps and their suspected species results.

Code	XLD *(Salmonella)*	PALCAM *(Listeria)*	MacConkey (EHEC)	Colony Aspect
Co1			+	Colorless
e2			+	Colorless
Ce1	+			Red with a black center
e3			+	Colorless
S3	+			Red with a black center
Me1			+	Colorless
Ce2			+	Colorless
Me3	+			Red with a black center
P2	+			Red with a black center
P3			+	Colorless
P4			+	Colorless
P5			+	Colorless
P6			+	Colorless
P7			+	Colorless
P8			+	Colorless
P9			+	Colorless
P10	+			Red with a black center
P12		+		Grey-green with a black halo
P22		+		Grey-green with a black halo
P23		+		Grey-green with a black halo
P52	+			Red with a black center
P54		+		Grey-green with a black halo
P24		+		Grey-green with a black halo
P32		+		Grey-green with a black halo
P37		+		Grey-green with a black halo

P41	+			Red with a black center
Ce5	+			Red with a black center
Ce7	+			Red with a black center
Ce8		+		Grey-green with a black halo
Ce10	+			Red with a black center
Ce11	+			Red with a black center
Ce21	+			Red with a black center
Ce29		+		Grey-green with a black halo
Ce30	+			Red with a black center
Ce33	+			Red with a black center
Ce23	+			Red with a black center
Co2			+	Colorless
Co4			+	Colorless
Co5	+			Red with a black center
Co7			+	Colorless
Co8	+			Red with a black center
Co10	+			Red with a black center
Co11	+			Red with a black center
Co19	+			Red with a black center
Co17			+	Colorless
Co18	+			Red with a black center
Co29	+			Red with a black center
Co22	+			Red with a black center
Co23	+			Red with a black center
Co45			+	Colorless

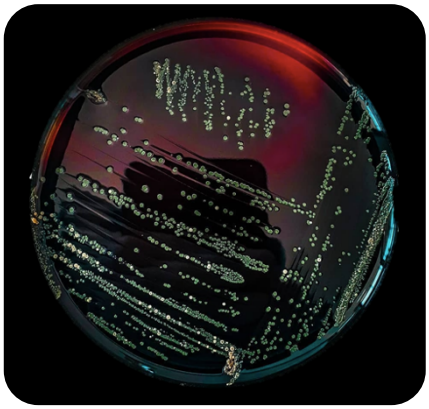


Figure 6. *Listeria* colonies on PALCAM agar.

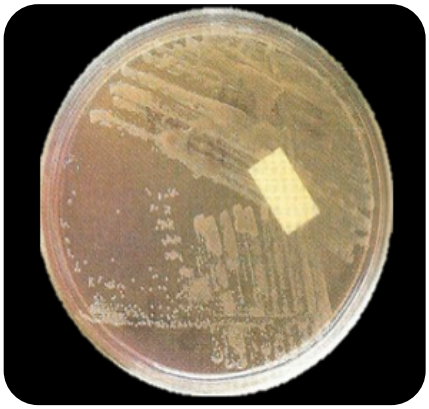


Figure 7. EHEC colonies on MacConkey agar.

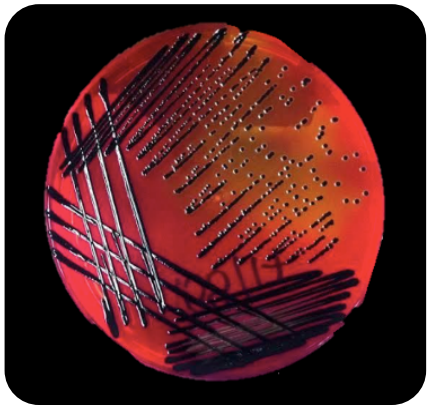


Figure 8. *Salmonella* colonies on XLD agar.

3. Comparative Study

a) Between morning and afternoon samples from markets

We observed a total count for suspected microbes in samples taken in the morning of (21:154) with a rate of 13,63% which is slightly higher than the afternoon samples (18:154) with 11,68% and a percentile difference between the two, of 1,95% **(Tables 1,2,3,4 and 5, Annexes I).**

In their research on the microbial safety of raw mixed salad, Ameko et al., reported the presence of enteric pathogens in both morning and afternoon samples, however, contamination was significantly higher ($p < 0,05$) from the afternoon samples than in the morning. This could be a result of unclean implements, poor hygiene in hands, cross-contamination (preparation or storage), and the processing equipment of the sellers **(Luna-Guevara et al., 2019b).**

a) Between markets and farms

Our findings revealed that the suspected pathogens count was higher in vegetable markets with a ratio of 39:154, on the other hand, it was found to be significantly lower in farms at 8:27 **(Table 6, Annexes I)**. Ameko et al., implied in their study that vendors did not take conscious precautions to avoid contamination of the raw greens during preparation and sale, and this is due to the ignorance of the majority of them on the causes of food contamination.

The uneven number of our samples between farms and markets can influence and introduce variability in our outcomes, nevertheless, this was intentionally done due to Algerian consumers primarily purchasing vegetables from markets rather than farms, as the latter typically distribute their produce solely in large quantities to markets and do not sell directly to consumers.

II. Strain Identification

1. Biochemical tests

Table VII. Biochemical tests results.

Isolates	Biochemical Tests											Suspected for
	Urea Indole			TSI			MEVAG		MR	VP	Citrate	
	Urease	Indole	TDA	Lac	Glu	H_2S	Xylose	Rhamnose				
Ce2	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
Me3	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
P52	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
P54	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
P37	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
Ce5	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce7	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce10	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce11	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce21	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce29	-	+	-	+	+	-	/	/	+	-	-	EHEC
Ce30	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce33	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce23	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co4	-	+	-	+	+	-	/	/	+	-	-	EHEC
Co8	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co10	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co29	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co22	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co23	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*

(/): unknown, (+): positive, (-): negative.

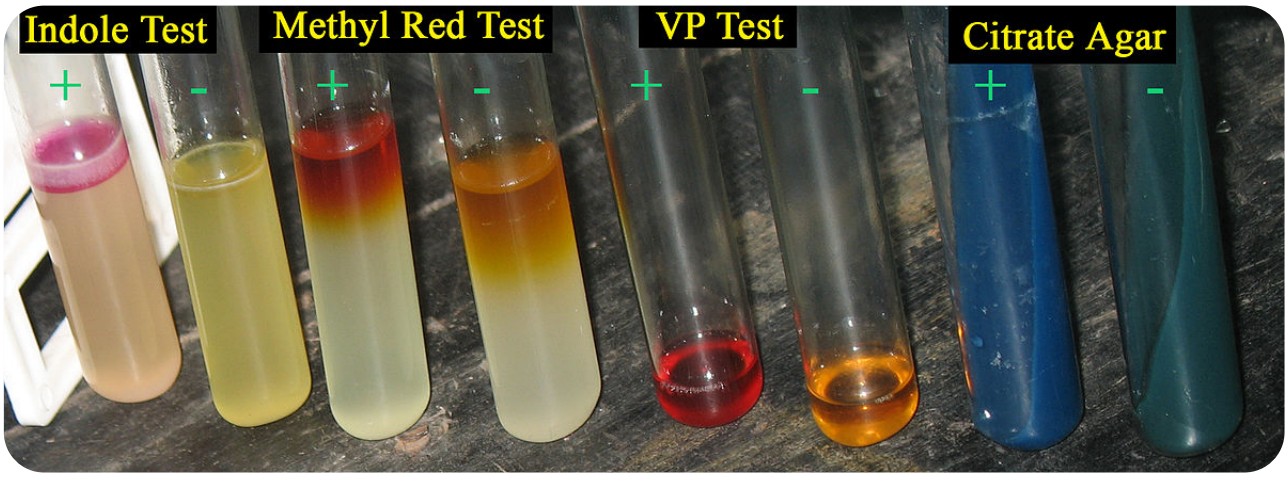


Figure 9. IMViC test results; Indole, MR, VP, and Citrate.

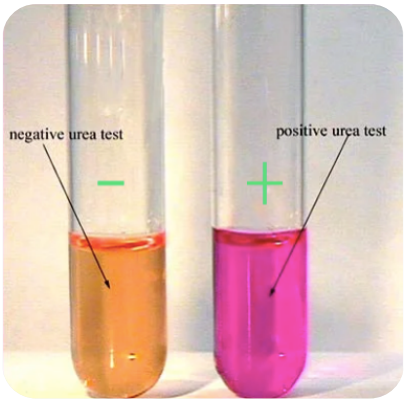


Figure 10. Urea test results.

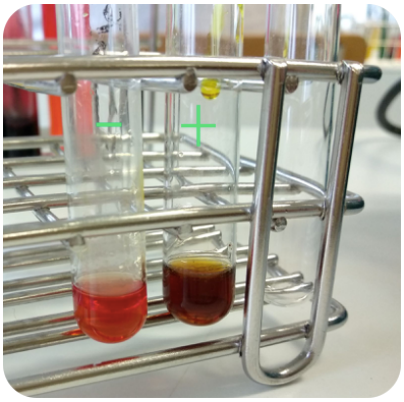


Figure 11. TDA test results.

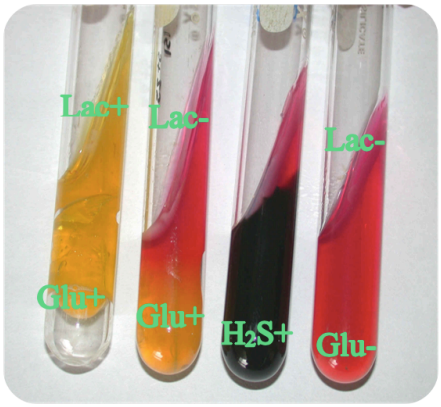


Figure 12. TSI results. *Left to right* (yellow /yellow): Lac+, Glu+, (red /yellow): Lac-, Glu+, (black precipitate): H_2S+, (bubbles): Gaz+,(red/red): Lac-,Glu-.

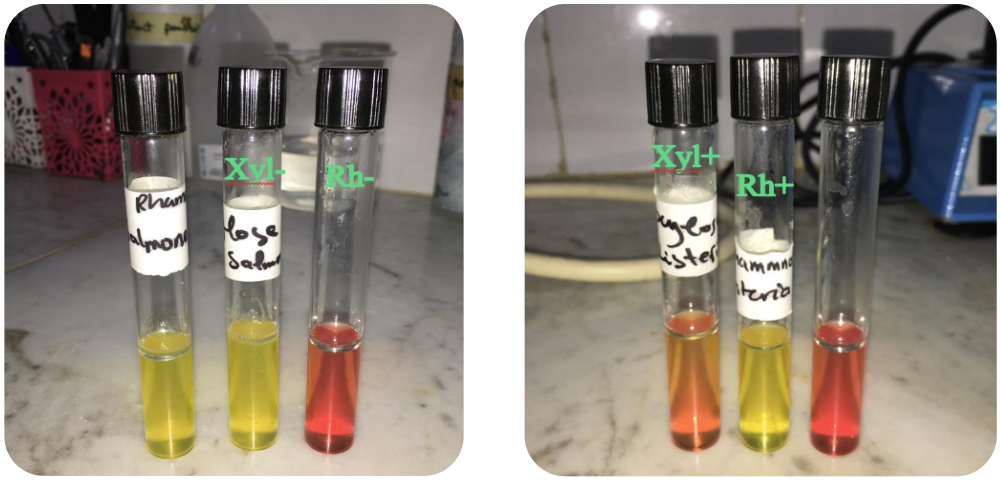


Figure 13. MEVAG test results. **Xyl:** xylose and **Rh:** rhamnose.

2.Identification Media

a) Columbia Blood Agar (CA)

The isolates P37 and Ce2 were characterized as small, grey colonies surrounded by a zone of clear beta hemolysis on the Columbia blood agar **(Figure 14)**. A β-hemolytic reaction implies complete lysis of the red blood cells, causing a clear zone on the agar surrounding the colony **(Jaiswal and Sharnagat 2023)**.

A **B** **C**

Figure 14. **A, B:** Suspected strains for *Listeria* morphology on Columbia blood agar.
C: *Listeria* strain of reference.

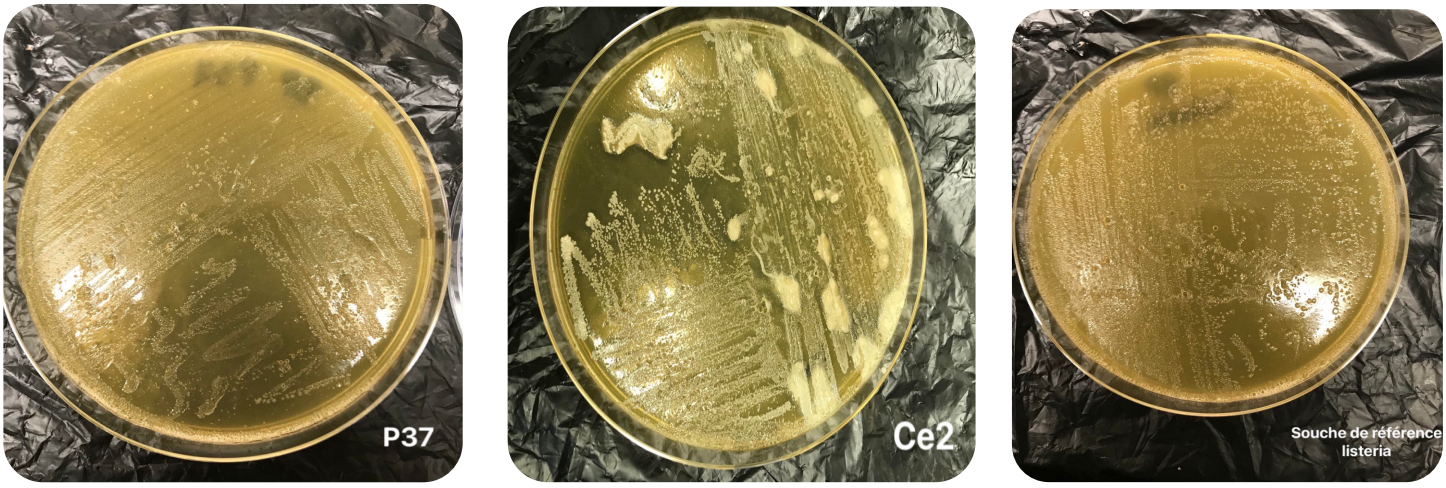
Co4 and Ce29 appeared as medium round-sized colonies with a greyish-white color with no apparent hemolysis **(Figure 15)**.

A **B** **C**

Figure 15. **A, B:** Suspected strains for EHEC morphology on Columbia blood agar.
C: EHEC strain of reference.

b) Baird-Parker Agar (BP)

P37 and Ce2 showed transparent small isolated colonies were characterized and suspected for *Listeria* on the Baird Parker agar with no Lecithinase halo **(Figure 16)**.



A **B** **C**

Figure 16. **A, B:** Suspected strains for *Listeria* morphology on Baird Parker agar.
C: *Listeria* strain of reference.

3. API Gallery 20E

Table VIII. API Gallery 20E results for each suspected strain.

	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
P37	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Ce2	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Co4	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	+
Ce29	-	+	-	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-

Abbreviations: **ONPG:** β-galactosidase. **ADH:** Arginine dihydrolase**. LDC:** Lysine decarboxylase. **ODC:** Ornithine decarboxylase. **CIT:** Citrate production. **H_2S:** Hydrogen Sulfide. **URE:** Urease. **TDA:** Tryptophan-Deaminase. **IND:** Indole. **VP:** Voges-Proskauer. **GEL:** Gelatinase. **GLU:** Glucose. **MAN:** Mannitol. **INO:** Inositol. **SOR:** Sorbitol. **RHA:** Rhamnose. **SAC:** Sucrose. **MEL:** Melibiose. **AMY:** Amygdalin. **ARA:** Arabinose.

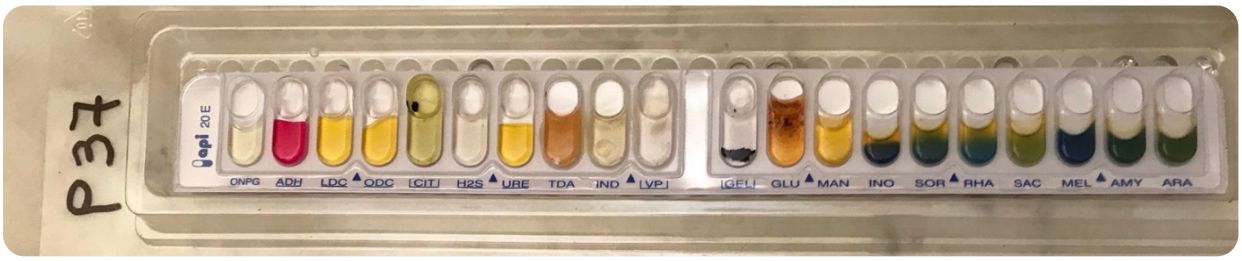


Figure 17. Strain P37 results on the API Gallery 20E: ***Aeromonas salmonicida ssp salmonicida.***

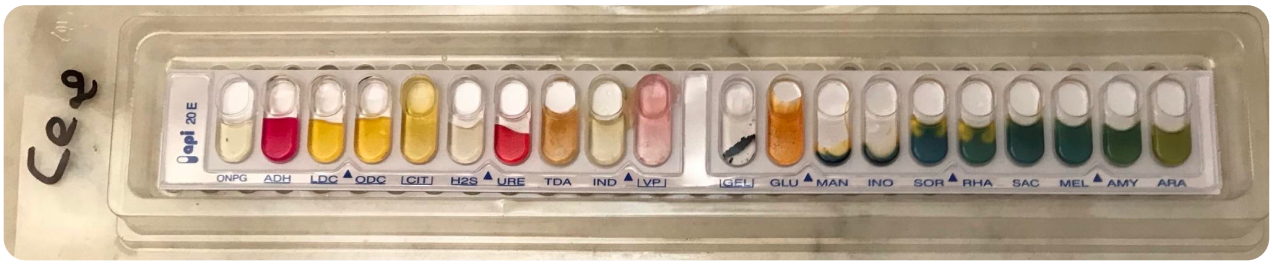


Figure 18. Strain Ce2 results on the API Gallery 20E: ***Photobacterium damsela.***

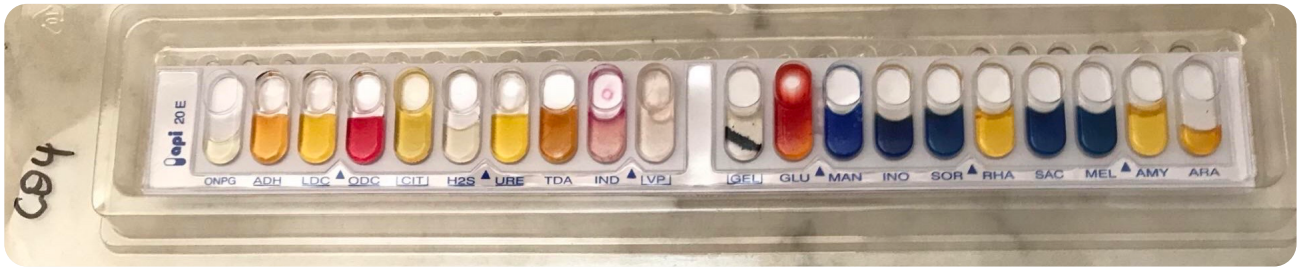


Figure 19. Strain Co4 results on the API Gallery 20E: ***Escherichia hermannii.***

Figure 20. Strain Ce29 results on the API Gallery 20E: ***Cedecea davisae.***

Table IX. Identified species on the API Gallery 20E.

Strain	Suspected for	API Identification
P37	*Listeria*	*Aeromonas salmonicida ssp salmonicida*
Ce2	*Listeria*	*Photobacterium damselae*
Co4	EHEC	*Escherichia hermannii*
Ce29	EHEC	*Cedecea davisae*

The bacteria detected in this study share a common trait of being opportunistic pathogens **(Table 9)**. *Aeromonas salmonicida ssp salmonicida* and *Photobacterium damselae* are both commonly found in marine environments and primarily affect fish. *Escherichia hermannii* can be found in water, soil, human wounds, and stool. While *Cedecea davisae* is rarely found and not very well-studied. All the bacteria we identified are not associated with enteric infections nor are they commonly found in leafy greens or vegetables.

The odds of finding these opportunistic pathogens in leafy greens and vegetables are very low, however, a reason for this discovery could be a result of cross-contamination from water, soil, equipment used, storage and transportation, or the diverse microflora surrounding the environment in which the herbs were grown.

III. Prevalence

Table X. Bacterial prevalence in leafy greens, irrigation water, and soil from farms and markets in Béjaïa.

Sample	**No.**	**Prevalence**			**Bacteria found**
		Salmonella	*Listeria*	EHEC	
Parsley	55	0%	0%	0%	*Aeromonas salmonicida ssp salmonicida* 1,8% (1/55)
Coriander	52	0%	0%	0%	*Escherichia hermannii* 1,9% (1/52)
Celery	51	0%	0%	0%	*Photobacterium damselae* 1,96% (1/51) & *Cedecea davisae* 1,96% (1/51)
Soil	7	0%	0%	0%	None 0%
Irrigation Water	7	0%	0%	0%	None 0%

E.coli O157:H7, *Salmonella*, and *Listeria* were not detected in any of our samples thus, indicating an absence of their prevalence in this study **(Table 10)**. That being noted, these findings did not match our initial expectations.

The absence of these bacteria may be due to incomplete irrigation water analysis (in which filtration should have been added to our method) or that they are pure and uncontaminated as they were collected during the winter, and incorrect media preparation that might have affected its selective properties. This shows how complex and sensitive microbiological methods are and how they should be done vigilantly.

Table XI. Comparative analysis of bacterial contamination in leafy greens in different countries.

Country	Sample	No. of sample	Positive No. of bacteria			Prevalence			Study
			Salmonella	*E.coli*	*Listeria*	*Salmonella*	*E.coli*	*Listeria*	
Algeria	Leafy greens	175	0	0	0	0%	0%	0%	**This study**
Czech Republic	Vegetables	91	/	24	/	/	26,40%	/	**(Skockova et al., 2013)**
Northern Ireland	Vegetables	86	0	0	0	0%	0%	0%	**(McMahon and Wilson, 2001)**
Spain	Vegetables	345	26	297	/	7,50%	86,10%	/	**(Ruiz et al., 1987)**
United States	Leafy greens	605	2	48	/	0,40%	11,30%	/	**(Mukherjee et al., 2004)**
Malaysia	Vegetables	306	/	/	171	/	/	55,80%	**(Ponniah et al., 2010)**
Algeria	Vegetables	491	0	/	/	0%	/	/	**(Zekar et al., 2017)**

Comparing our research to other similar studies, our findings align with results obtained by both McMahon and Wilson (2001) and Zekar et al.,(2017) with no detection of all three bacteria with a prevalence rate of 0% from 86 samples and 0% *salmonella* from 491 samples respectively. Unlike the farms in Algeria from this study and Zekar et al.,(2017), which use water from wells or rainfall, others like Ruiz et al., (1987) that obtained 26/345 (7,50%) *salmonella,* might have isolated them from farms that use treated wastewater **(Zekar et al., 2017)**.

Furthermore, *E.coli* was remarkably prevalent at 86,10% (297/345) in Spain and 26,40% in the Czech Republic **(Table 11)**. Shedding light on our research question on the factors contributing to the contamination of leafy greens, this could be due to the likelihood that the plants were contaminated indirectly by fecal bacteria from animals during the

fertilization process or through direct contact with humans during harvesting, handling, and packaging of products due to insufficient hygiene measures **(Zekar et al., 2017)**. During cultivation and processing, natural fertilizers such as animal manure are used where no chemical treatments are employed to reduce the microbiological load of the raw product or to extend its shelf life which represents an increased risk to public health. The pre-harvest contamination is considered to be the most common way of contaminating vegetables, as it is extremely difficult to prevent **(Skockova et al., 2013)**.

It appeared that the microbial counts were lower during the winter and higher during the summer which could be due to the greater use of contaminated irrigation water, as well as to the higher temperatures favoring the development of microorganisms in particular during spring and summer **(Ruiz et al., 1987)**. Our samples were collected during the winter, which could explain the lack of results, as pathogenic bacteria tend to find better growth conditions during the summer with higher temperatures and humidity rates than during the cold season.

Among the 605 samples, Mukherjee et al., (2004) identified zero *E.coli* O157:H7 (0%) which is consistent with our study. These results could have been influenced by the unbalanced numbers of samples among produce varieties, the potential effects of weather and geographic location, and the natural fluctuations that may occur in microbial populations **(Mukherjee et al., 2004)**. 171/306 positive *Listeria* strains (55,80%) were identified by Ponniah et al., (2010) in Malaysia. It has been suggested that a warm humid environment may allow *L. monocytogenes* to grow to detectable levels in vegetables.

Our results might be very different from other studies done in other countries, but this is possibly due to the geographic location and different practices that the farmers and vendors conform to, which could have contributed to improved hygiene, hence the absence of pathogenic bacteria both in this study and the other one done by Zekar et al.,(2017) in Algeria. Referring this back to our initial hypothesis, which suggests that leafy greens are likely to be contaminated with enteropathogens due to the potential exposure to contaminated water, soil, and handling practices, our results disagree. However, it can be suggested like previously mentioned, that effective practices and elevated hygiene measures were taken into consideration by cultivators and retailers.

Due to our inability to detect any enteropathogenic bacteria, several recommendations can be provided for future studies to ensure an improved and better understanding of the research of enteric bacteria in herbs, namely:

- Increasing the sample size and diversifying the types of leafy greens and vegetables, thereby increasing the chances of the detection of contaminated bacteria.
- Performing seasonal sampling to study the variations of contaminants during the 4 different seasons.
- Conducting several surveys and asking farmers and sellers about the food chain processing.
- Using molecular detection methods like PCR (Polymerous Chain Reaction) and antibiotic sensitivity testing besides the traditional techniques used in this study.

Conclusions

In this study, we aimed to identify three of the most common enteropathogenic bacteria that cause urinary tract and gastrointestinal infections in humans which can be found in foods and leafy green vegetables, which are *Salmonella*, *Listeria,* and Enterohemorrhagic *E.coli* (EHEC) from farms and markets in Béjaïa, Algeria. We also analysed the water and soil used on the vegetables to test as potential primary contamination causers.

Despite using a suitable quality control protocol, we were unable to detect any enteric bacteria and rather discovered opportunistic Gram-negative bacteria in celery, parsley and coriander. This outcome suggests good hygienic practices and handling methods by farmers and vendors, moreover, the variations in contamination including the quantity and types of samples, and seasonal differences which may all have contributed to the absence of the targeted microorganisms.

Although our research has proven proper sanitation from markets and fields in Béjaïa, food safety and hygiene standards remain to be improved. Nonetheless, farmers should analyse and test the water, soil, and organic fertilizers used for potential contaminants before planting, using clean utensils during the pre-harvest process, ensuring the storage of the herbs in dry, clean, well-ventilated areas with proper temperatures and humidity to maintain freshness, and transporting them in sanitised vehicles while packing them in clean baskets throughout the post-harvest procedure. Likewise, market vendors ought to properly pack the vegetables using clean gloves, keep them stored in appropriate temperatures, inspect for fungal infections, and provide educational resources on handling practices and food safety to both the sellers and consumers. The latter should also follow guidelines in their homes by washing the fresh produce thoroughly after the purchase with sanitised hands, keeping their kitchen and utensils clean, and storing the greens directly in the refrigerator. After all, it remains crucial to always be informed about current outbreaks in your country to protect oneself from different diseases and food poisoning as it is the least you can do.

Through our research, we have been able to make a few contributions to the scientific industry in Béjaïa, such as understanding the prevalence and distribution of pathogenic bacteria

in agricultural fields and various famous vegetable markets visited by many Algerian consumers weekly which helps in developing data on pathogen persistence and transmission in each region. In addition, our comparative study between markets and farms brought insights to the different contamination levels that helps in identifying the diverse stages of microbial infections from fields to markets.

In conclusion, this study highlights the importance of addressing the risks of foodborne illnesses from herbs and how this knowledge can lead to the improvement of food security measures to ensure public health safety.

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Review Article

Investigating Enteropathogenic bacteria in leafy greens from vegetable markets and agricultural land

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ABSTRACT

Aim: Examine the presence of enteric pathogens; *Salmonella* and Enterohemorrhagic *Escherichia Coli*, and the pathogenic *Listeria* in leafy greens.

Background: Fresh fruit and vegetables are now recognized to be a major route of entry for pathogenic enterobacteria into the food chain. *Salmonella*, *E. coli* and *Listeria* are among the most prevalent food-borne bacterial pathogens in the developed world and are able to enter the food chain at any point from farm to table.

Methods: A total of 175 samples of lettuce, parsley, coriander, celery and mint; irrigation water and soil were collected from farms and greenmarkets. After isolation, the strains were identified using a set of biochemical tests, identification mediums, and the gallery API 20E.

Results: 0% prevalence was found of enteric bacteria specifically EHEC, *Listeria*, and *Salmonella* from the samples analysed. Instead, four opportunistic bacteria were identified; *Aeromonas salmonicida*, *E.hermannii*, *Phtobacterium damselae*, and *Cedecea davisae* found in parsley, coriander, and 2 celery samples. A 0% occurrence was also observed in soil and irrigation water.

Conclusion: Despite using a suitable quality control protocol, we were unable to detect any enteric bacteria and rather discovered opportunistic Gram-negative bacteria in celery, parsley and coriander. This outcome suggests good hygienic practices and handling methods by farmers and vendors, moreover, the variations in contamination including the quantity and types of samples, and seasonal differences which may all have contributed to the absence of the targeted microorganisms.

Keywords: Enteric pathogens, Leafy greens, Contaminated vegetables, Human health risk.

1. Introduction

In the health conscious society of the 21st century, vegetables form an integral part of the human diet. Over the last three decades, the global consumption of fresh vegetables has increased significantly, thus expanding the market segment for fresh produce by more than 20%. They contain valuable food ingredients that are essential for the proper function of the body and contain various medicinal and therapeutic agents and are valued mainly for their high vitamin and mineral content **(Jaiswal and Sharnagat 2023)**. Regular daily consumption of them in sufficient amounts can help prevent some diseases such as cardiovascular diseases and certain cancers **(Taban & Halkman, 2011)**. Fresh fruit and vegetables are now recognized to be a major route of entry for pathogenic enterobacteria into the food chain. *Salmonella*, *E. coli* and *Listeria* are among the most prevalent food-borne bacterial pathogens in the developed world and are able to enter the food chain at any point from farm to table **(Silva et al., 2014)**. These bacteria are known as causers of diseases: salmonellosis, hemolytic uremic syndrome (HUS), and listeriosis **(Kljujev et al., 2018)**.

Reported outbreaks associated with the consumption of fresh vegetables have grown steadily. As most of these products are eaten raw or with minimal cooking, their microbial content

may represent a risk factor for the consumer's health **(Jaiswal and Sharnagat 2023)**.

This study is focused on examining the presence of *Salmonella*, *Listeria*, and EHEC (Enterohemorrhagic *E.coli*) in leafy greens from markets and farms in Béjaïa, Algeria and analysing water and soil used for growing them.

2. Material & Methods

I.2. Pre-enrichment

Upon arriving at the laboratory, we started by preparing a sufficient amount of Buffered Peptone water (BPW) for our samples. In an aseptic area, we weighed 25 g of each sample and added 225 ml of the BPW into it, inside sterile stomacher filter bags and shook them for 1 minute. We labeled and incubated the bags at 37 ºC for 24 hours **(Campos et al, 2013)**. This process was crucial to concentrate our target microorganisms and ameliorate bacterial identification. It was also carried out to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock **(Joseph A. Odumeru, 2012)**.

I.3. Enrichment

After incubating the stomacher bags, we opened them in an aseptic zone and pipetted 1 ml of the solution into 10 ml of the Rappaport Vassiliadis Soya Peptone broth (RVS) test tubes, selective for *Salmonella*. Then into 10 ml of the Buffered *Listeria* Enrichment broth (BLEB)/Fraser broth, selective for *Listeria*, and 10 ml of the Lactose broth (LB) for EHEC. Lastly, we incubated the tubes accordingly at 42 ºC in a water bath, 37 ºC, and 42 ºC in an incubator for 24 hours **(Priyanka et al., 2021)**. This procedure was imperative 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)**.

I.4. Isolation

This step involved the inoculation from the RVS, Fraser/BLEB, and LB tubes respectively in the already prepared Xylose Lysine Deoxycholate agar (XLD) media selective for *Salmonella*, PALCAM media selective for *Listeria*, and MacConkey Sorbitol (SMAC) for EHEC in Petri dishes using the streak plate method aseptically. Finally, we incubated the plates at 37 ºC for 24 hours.

I.5. Re-Isolation

If present, suspected positive isolates were chosen from each bacterium and colonies were re-isolated from them. Aseptically, and using a sterile toothpick, we picked a red *Salmonella* isolated colony with a black center from the XLD agar, a grey-green with a black halo *Listeria* colony from PALCAM, and a colorless EHEC colony from the Trypticase Soy agar (TSA) and inoculated the collection tubes that contained 1 ml of physiological water and agitated them. From this bacterial suspension and using the streak plate method, we streaked the SMAC, Mannitol Salt agar (MSA), and TSA mediums correspondingly and incubated them at 37 ºC for 24 hours.

II. Strain Identification

To further identify the strains, a set of biochemical tests (including the IMViC tests) and identification media were preformed:

II.2. Identification media

II.2.1. Columbia Blood Agar (CA)

This medium is a general-purpose enriched medium often used to grow fastidious organisms and differentiate them based on their hemolytic properties (Jaiswal and Sharnagat 2023).

II.2.2. Baird-Parker Agar (BP)

It is recommended for use in the examination of foods and other materials by the Food and Drug Administration (FDA) (*Bacteriological Analytical Manual Chapter 23: Methods for Cosmetics*, n.d.).

API Gallery 20E was also performed.

3. Results & Discussions

I. Strain Isolation

1. Sample Collection

During our study, a total of 175 samples were collected, in which; parsley (n=55), celery (n=51), coriander (n=52), mint (n=3), irrigation water (n=7), and soil (n=7) were taken from different vegetable markets and farms for laboratory analysis.

2. Isolation

Isolation on the selection mediums allowed us to select 47 strains of our targeted bacteria including

17 EHEC, 8 *Listeria*, and 22 *Salmonella* suspected species **(Table I)**.

Table I. Positive suspected isolates from the isolation and re-isolation steps and their suspected species

Code	XLD *(Salmonella)*	PALCAM *(Listeria)*	MacConkey (EHEC)	Colony Aspect
Co1			+	Colorless
e2			+	Colorless
Ce1	+			Red with a black center
e3			+	Colorless
S3	+			Red with a black center
Me1			+	Colorless
Ce2			+	Colorless
Me3	+			Red with a black center
P2	+			Red with a black center
P3			+	Colorless
P4			+	Colorless
P5			+	Colorless
P6			+	Colorless
P7			+	Colorless
P8			+	Colorless
P9			+	Colorless
P10	+			Red with a black center
P12		+		Grey-green with a black halo
P22		+		Grey-green with a black halo
P23		+		Grey-green with a black halo
P52	+			Red with a black center
P54		+		Grey-green with a black halo
P24		+		Grey-green with a black halo
P32		+		Grey-green with a black halo
P37		+		Grey-green with a black halo
P41	+			Red with a black center
Ce5	+			Red with a black center
Ce7	+			Red with a black center
Ce8		+		Grey-green with a black halo
Ce10	+			Red with a black center
Ce11	+			Red with a black center
Ce21	+			Red with a black center
Ce29		+		Grey-green with a black halo
Ce30	+			Red with a black center
Ce33	+			Red with a black center
Ce23	+			Red with a black center
Co2			+	Colorless
Co4			+	Colorless
Co5	+			Red with a black center
Co7			+	Colorless
Co8	+			Red with a black center
Co10	+			Red with a black center
Co11	+			Red with a black center
Co19	+			Red with a black center
Co17			+	Colorless
Co18	+			Red with a black center
Co29	+			Red with a black center
Co22	+			Red with a black center
Co23	+			Red with a black center
Co45			+	Colorless

3.Comparative Study

a) Between morning and afternoon samples from markets

We observed a total count for suspected microbes in samples taken in the morning of (21:154) with a rate of 13,63% which is slightly higher than the afternoon samples (18:154) with 11,68% and a percentile difference between the two, of 1,95%.

In their research on the microbial safety of raw mixed salad, Ameko et al., reported the presence of enteric pathogens in both morning and afternoon samples, however, contamination was significantly higher ($p < 0,05$) from the afternoon samples than in the morning. This could be a result of unclean implements, poor hygiene in hands, cross-contamination (preparation or storage), and the processing equipment of the sellers **(Luna-Guevara et al., 2019)**.

This contradiction with our results could be explained by errors during bacterial isolation and re-isolation that might have contributed to our failure in obtaining a higher microbial count during the evening samples rather than morning.

b) Between markets and farms

Our findings revealed that the suspected pathogens count was higher in vegetable markets with a ratio of 39:154, on the other hand, it was found to be significantly lower in farms at 8:27. Ameko et al., implied in their study that vendors did not take conscious precautions to avoid contamination of the raw greens during preparation and sale, and this is due to the ignorance of the majority of them on the causes of food contamination.

The uneven number of our samples between farms and markets can influence and introduce variability in our outcomes, nevertheless, this was intentionally done due to Algerian consumers primarily purchasing vegetables from markets rather than farms, as the latter typically distribute their produce solely in large quantities to markets and do not sell directly to consumers.

II. Strain Identification

1. Biochemical tests

Table II. Biochemical tests results.

Isolates	Biochemical Tests											Suspected for
	Urea Indole			TSI			MEVAG		MR	VP	Citrate	
	Urease	Indole	TDA	Lac	Glu	H_2S	Xylose	Rhamnose				
Ce2	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
Me3	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
P52	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
P54	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
P37	-	-	/	+	+	-	-	+	+	+	+	*Listeria*
Ce5	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce7	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce10	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce11	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce21	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce29	-	+	-	+	+	-	/	/	+	-	-	EHEC
Ce30	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce33	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Ce23	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co4	-	+	-	+	+	-	/	/	+	-	-	EHEC
Co8	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co10	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co29	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co22	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*
Co23	-	-	-	-	+	-	+	+	+	-	+	*Salmonella*

2. Identification Media

a) Columbia Blood Agar (CA)

The isolates P37 and Ce2 were characterized as small, grey colonies surrounded by a zone of clear beta hemolysis on the Columbia blood agar.

Co4 and Ce29 appeared as medium round-sized colonies with a greyish-white color with no apparent hemolysis **(Figures 1 & 2)**.

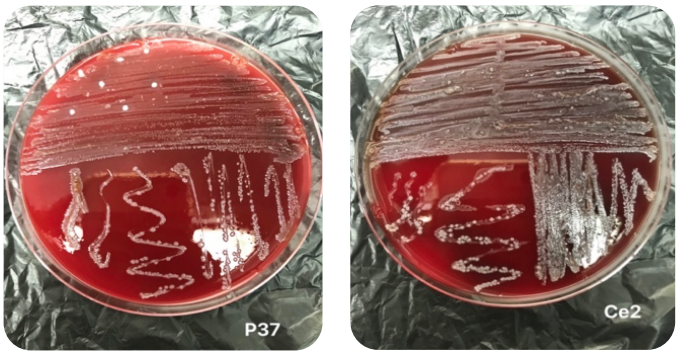

Figure 1. Suspected strains for *Listeria* morphology on Columbia blood agar.

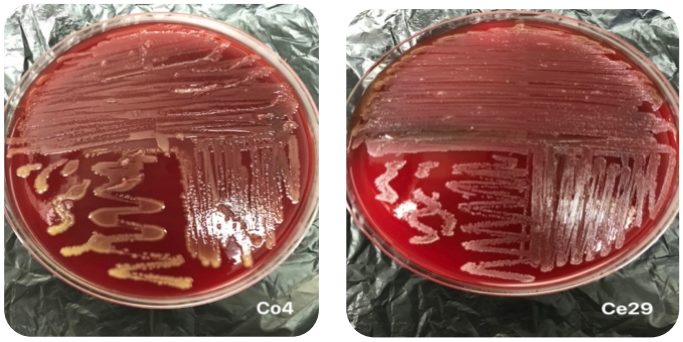

Figure 2. Suspected strains for EHEC morphology on Columbia blood agar.

b) Baird-Parker Agar (BP)

P37 and Ce2 looked transparent small isolated colonies were characterized and suspected for *Listeria* on the Baird Parker agar with no Lecithinase halo **(figure 3)**.

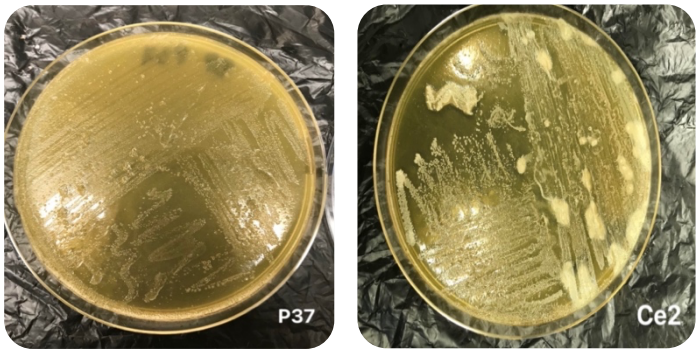

Figure 3. Suspected strains for *Listeria* morphology on Baird Parker agar.

3. API Gallery 20E

Table III. API Gallery 20E results for each suspected strain.

	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
P37	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Ce2	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Co4	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	+
Ce29	-	+	-	+	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-

Table IV. Identified species on the API Gallery 20E.

Strain	Suspected for	API Identification
P37	*Listeria*	*Aeromonas salmonicida ssp salmonicida*
Ce2	*Listeria*	*Photobacterium damselae*
Co4	EHEC	*Escherichia hermannii*
Ce29	EHEC	*Cedecea davisae*

The bacteria detected in this study share a common trait of being opportunistic pathogens **(Table IV)**. *Aeromonas salmonicida ssp salmonicida* and *Photobacterium damselae* are both commonly found in marine environments and primarily affect fish. *Escherichia hermannii* can be found in water, soil, human wounds, and stool. While *Cedecea davisae* is rarely found and not very well-studied. All the bacteria we identified are not associated with enteric infections nor are they commonly found in leafy greens or vegetables.

The odds of finding these opportunistic pathogens in leafy greens and vegetables are very low, however, a reason for this discovery could be a result of cross-contamination from water, soil, equipment used, storage and transportation, or the diverse microflora surrounding the environment in which the herbs were grown.

III.Prevalence

Table V. Bacterial prevalence in leafy greens, irrigation water, and soil from farms and markets in Béjaïa.

Sample	No.	Prevalence			Bacteria found
		Salmonella	*Listeria*	EHEC	
Parsley	55	0%	0%	0%	*Aeromonas salmonicida ssp salmonicida* 1,8% (1/55)
Coriander	52	0%	0%	0%	*Escherichia hermannii* 1,9% (1/52)
Celery	51	0%	0%	0%	*Photobacterium damselae* 1,96% (1/51) & *Cedecea davisae* 1,96% (1/51)
Soil	7	0%	0%	0%	None 0%
Irrigation Water	7	0%	0%	0%	None 0%

E.coli O157:H7, *Salmonella*, and *Listeria* were not detected in any of our samples thus, indicating an absence of their prevalence in this study **(Table V)**. That being noted, these findings did not match our initial expectations.

The absence of these bacteria may be due to contamination with other bacteria, incomplete irrigation water analysis (in which filtration should have been added to our method) or that they are pure and uncontaminated as they were collected during the winter, and incorrect media preparation that might have affected its selective properties. This shows how complex and sensitive microbiological methods are and how they should be done vigilantly.

Comparing our research to other similar studies, our findings align with results obtained by both McMahon and Wilson (2001) and Zekar et al.,(2017) with no detection of all three bacteria with a prevalence rate of 0% from 86 samples and 0% *salmonella* from 491 samples respectively. Unlike the farms in Algeria from this study and Zekar et al.,(2017), which use water from wells or rainfall, others like Ruiz et al., (1987) that obtained 26/345 (7,50%) *salmonella,* might have isolated them from farms that use treated wastewater **(Zekar et al., 2017)**.

Furthermore, *E.coli* was remarkably prevalent at 86,10% (297/345) in Spain and 26,40% in the Czech Republic. Shedding light on our research question on the factors contributing to the contamination of leafy greens, this could be due to the likelihood that the plants were contaminated indirectly by fecal bacteria from animals during the fertilization process or through direct contact with humans during harvesting, handling, and packaging of products due to insufficient hygiene measures **(Zekar et al., 2017)**. During cultivation and processing, natural fertilizers such as animal manure are used where no chemical treatments are employed to reduce the microbiological load of the raw product or to extend its shelf life which represents an increased risk to public health. The pre-harvest contamination is considered to be the most common way of contaminating vegetables, as it is extremely difficult to prevent **(Skockova et al., 2013)**.

It appeared that the microbial counts were lower during the winter and higher during the summer which could be due to the greater use of contaminated irrigation water, as well as to the higher temperatures favoring the development of microorganisms in particular during spring and summer **(Ruiz et al., 1987)**. Our samples were collected during the winter, which could explain the lack of results, as pathogenic bacteria tend to find better growth conditions during the summer with higher temperatures and humidity rates than during the cold season.

Among the 605 samples, Mukherjee et al., (2004) identified zero *E.coli* O157:H7 (0%) which is consistent with our study. These results could have been influenced by the unbalanced numbers of samples among produce varieties, the potential effects of weather and geographic location, and the natural fluctuations that may occur in microbial populations **(Mukherjee et al., 2004)**. 171/306 positive *Listeria* strains (55,80%) were identified by Ponniah et al., (2010) in Malaysia. It has been suggested that a warm humid environment may allow *L. monocytogenes* to grow to detectable levels in vegetables.

Our results might be very different from other studies done in other countries, but this is possibly due to the geographic location and different practices that the farmers and vendors conform to, which could have contributed to improved hygiene, hence the absence of pathogenic bacteria both in this study and the other one done by Zekar et al.,(2017) in Algeria. Referring this back to our initial hypothesis, which suggests that leafy greens are likely to be contaminated with enteropathogens due to the potential exposure to contaminated water, soil, and handling practices, our results disagree. However, it can be suggested like previously mentioned, that effective practices and elevated hygiene measures were taken into consideration by cultivators and retailers.

Due to our inability to detect any enteropathogenic bacteria, several recommendations can be provided for future studies to ensure an improved and better understanding of the research of enteric bacteria in herbs, namely: Increasing the sample size and diversifying the types of leafy greens and

vegetables, thereby increasing the chances of the detection of contaminated bacteria. Performing seasonal sampling to study the variations of contaminants during the 4 different seasons. Conducting several surveys and asking farmers and sellers about the food chain processing. And using molecular detection methods like PCR (Polymerous Chain Reaction) and antibiotic sensitivity testing besides the traditional techniques used in this study.

4. Conclusions

In this study, we aimed to identify three of the most common enteropathogenic bacteria that cause urinary tract and gastrointestinal infections in humans which can be found in foods and leafy greens, which are *Salmonella*, *Listeria,* and Enterohemorrhagic *E.coli* (EHEC) from farms and markets in Béjaïa, Algeria.

Despite using a suitable quality control protocol, we were unable to detect any enteric bacteria and rather discovered opportunistic Gram-negative bacteria in celery, parsley and coriander. This outcome suggests good hygienic practices and handling methods by farmers and vendors, moreover, the variations in contamination including the quantity and types of samples, and seasonal differences which may all have contributed to the absence of the targeted microorganisms.

Although our research has proven proper sanitation from markets and fields in Béjaïa, food safety and hygiene standards remain to be improved. Nonetheless, farmers should analyse and test the water, soil, and organic fertilizers used for potential contaminants before planting, using clean utensils during the pre-harvest process, ensuring the storage of the herbs in dry, clean, well-ventilated areas with proper temperatures and humidity to maintain freshness, and transporting them in sanitised vehicles while packing them in clean baskets throughout the post-harvest procedure. Likewise, market vendors ought to properly pack the vegetables using clean gloves, keep them stored in appropriate temperatures, inspect for fungal infections, and provide educational resources on handling practices and food safety to both the sellers and consumers. The latter should also follow guidelines in their homes by washing the fresh produce thoroughly after the purchase with sanitised hands, keeping their kitchen and utensils clean, and storing the greens directly in the refrigerator. After all, it remains crucial to always be informed about current outbreaks in your country to protect oneself from different diseases and food poisoning as it is the least you can do.

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ANNEXES I

Table I.1. Suspected and eliminated strains of morning and afternoon samples from Edimco market.

Code	Time	Vendor	Results
P1	**Morning**	V1	Eliminated
P2		V2	**Suspected**
P3		V3	**Suspected**
P18		V1	Eliminated
P19		V2	Eliminated
P20		V3	Eliminated
P35		V1	Eliminated
P36		V2	Eliminated
P37		V3	**Suspected**
P7	**Afternoon**	V1	**Suspected**
P8		V2	**Suspected**
P9		V3	**Suspected**
P26		V1	Eliminated
P27		V2	Eliminated
P28		V3	Eliminated
P43		V1	Eliminated
P44		V2	Eliminated
P45		V3	Eliminated
Ce 4	**Morning**	V1	Eliminated
Ce5		V2	**Suspected**
Ce6		V3	Eliminated
Ce35		V1	Eliminated
Ce36		V2	Eliminated
Ce37		V3	Eliminated
Ce17	**Afternoon**	V1	Eliminated
Ce18		V2	Eliminated
Ce19		V3	Eliminated
Ce50		V1	Eliminated
Ce51		V2	Eliminated
Co2	**Morning**	V1	**Suspected**
Co3		V2	Eliminated
Co4		V3	**Suspected**
Co35		V1	Eliminated
Co36		V2	Eliminated
Co37		V3	Eliminated
Co16	**Afternoon**	V1	Eliminated
Co17		V2	**Suspected**
Co18		V3	**Suspected**
Co50		V1	Eliminated
Co51		V2	Eliminated
Co52		V3	Eliminated

Table I.2. Suspected and eliminated strains of morning and afternoon samples from El Kseur1 market.

Code	Time	Vendor	Results
P4	**Morning**	V1	**Suspected**
P5		V2	**Suspected**
P6		V3	**Suspected**
P29		V1	Eliminated
P30		V2	Eliminated
P31		V3	Eliminated
P51		V1	Eliminated
P52		V2	**Suspected**
P53		V3	Eliminated
P10	**Afternoon**	V1	**Suspected**
P11		V2	Eliminated
P12		V3	**Suspected**
P32		V1	**Suspected**
P33		V2	Eliminated
P34		V3	Eliminated
P54		V1	**Suspected**
P55		V2	Eliminated
Ce20	**Morning**	V1	Eliminated
Ce21		V2	**Suspected**
Ce22		V3	Eliminated
Ce38		V1	Eliminated
Ce39		V2	Eliminated
Ce23	**Afternoon**	V1	**Suspected**
Ce24		V2	Eliminated
Ce25		V3	Eliminated
Ce40		V1	Eliminated
Ce41		V2	Eliminated
Co19	**Morning**	V1	**Suspected**
Co20		V2	Eliminated
Co21		V3	Eliminated
Co38		V1	Eliminated
Co39		V2	Eliminated
Co22	**Afternoon**	V1	**Suspected**
Co23		V2	**Suspected**
Co24		V3	Eliminated
Co40		V1	Eliminated
Co41		V2	Eliminated

Table I.3. Suspected and eliminated strains of morning and afternoon samples from El Kseur2 market.

Code	Time	Vendor	Results
Ce26	**Morning**	V1	Eliminated
Ce27		V2	Eliminated
Ce42		V1	Eliminated
Ce43		V2	Eliminated
Ce28	**Afternoon**	V1	Eliminated
Ce44		V1	Eliminated
Ce45		V2	Eliminated
Co25	**Morning**	V1	Eliminated
Co26		V2	Eliminated
Co42		V1	Eliminated
Co43		V2	Eliminated
Co27	**Afternoon**	V1	Eliminated
Co44		V1	Eliminated
Co45		V2	**Suspected**

Table I.4. Suspected and eliminated strains of morning and afternoon samples from Souk El Tenine market.

Code	Time	Vendor	Results
Ce13	**Morning**	V1	Eliminated
Ce14		V2	Eliminated
Ce46		V1	Eliminated
Ce47		V2	Eliminated
Ce15	**Afternoon**	V1	Eliminated
Ce16		V2	Eliminated
Ce48		V1	Eliminated
Ce49		V2	Eliminated
Co12	**Morning**	V1	Eliminated
Co13		V2	Eliminated
Co46		V1	Eliminated
Co47		V2	Eliminated
Co14	**Afternoon**	V1	Eliminated
Co15		V2	Eliminated
Co48		V1	Eliminated
Co49		V2	Eliminated

Table I.5. Suspected and eliminated strains of morning and afternoon samples from Kouds market.

Code	Time	Vendor	Results
P13	**Morning**	V1	Eliminated
P14		V2	Eliminated
P15		V3	Eliminated
P16		V4	Eliminated
P17		V5	Eliminated
P38		V1	Eliminated
P39		V2	Eliminated
P40		V3	Eliminated
P41		V4	Eliminated
P42		V5	Eliminated
P21	**Afternoon**	V1	Eliminated
P22		V2	**Suspected**
P23		V3	**Suspected**
P24		V4	Eliminated
P25		V5	Eliminated
P46		V1	Eliminated

P47		V2	Eliminated
P48		V3	Eliminated
P49		V4	Eliminated
P50		V5	Eliminated
Ce7	**Morning**	V1	**Suspected**
Ce8		V2	**Suspected**
Ce9		V3	Eliminated
Ce10		V4	**Suspected**
Ce11		V5	**Suspected**
Ce12		V6	Eliminated
Ce29	**Afternoon**	V1	**Suspected**
Ce30		V2	**Suspected**
Ce31		V3	Eliminated
Ce32		V4	Eliminated
Ce33		V5	**Suspected**
Ce34		V6	Eliminated
Co5	**Morning**	V1	**Suspected**
Co6		V2	Eliminated
Co7		V3	**Suspected**
Co8		V4	**Suspected**
Co9		V5	Eliminated
Co10		V6	**Suspected**
Co11		V7	**Suspected**
Co28	**Afternoon**	V1	**Suspected**
Co29		V2	Eliminated
Co30		V3	Eliminated
Co31		V4	Eliminated
Co32		V5	Eliminated
Co33		V6	Eliminated
Co34		V7	Eliminated

Table I.6. Suspected and eliminated strains from farms.

Code	Results
e1	Eliminated
S1	Eliminated
Co1	**Suspected**
e2	**Suspected**
S2	Eliminated
Ce1	**Suspected**
e3	**Suspected**
S3	**Suspected**
Me1	**Suspected**
e4	Eliminated
S4	Eliminated
Ce2	**Suspected**
e5	Eliminated
S5	Eliminated
Me2	Eliminated
e6	Eliminated
S6	Eliminated
Ce3	Eliminated
e7	Eliminated
S7	Eliminated
Me3	**Suspected**

ANNEXES II

Culture Media (g/1L distilled water)

Baird-Parker Agar

Meat Extract……..……...…….05
Yeast Extract……..……....01
K-Tellurite solution (1%)…….....10ml
Egg Yolk…….....50ml
Lithium-Chloride……....05
Sodium-Pyruvate…….…….....10
Glycine……12
Agar…….....……....17

pH 7,0 ± 0,2 at 25 ºC

Buffered Listeria Enrichment Broth

Trypticase Soy Broth……..……30
Yeast Extract……..……...…..…06
Monopotassium Phosphate……..…1,35
Disodium Phosphate…….....9,6
Sodium Pyruvate……...……...1,11

pH 7,3 ± 0,2 at 25 ºC

Buffered Peptone Water

Peptone……10
NaCl……...……..…….……..…05
Disodium Hydrogen Phosphate……...……...09
Potassium Dihydrogen Phosphate……..1,5

pH 7,2 ± 0,2 at 25 ºC

Columbia Blood Agar

Tryptone…….……...…..…10
Peptone Proteose……..…….....05
Yeast Extract…….....05
Beef Heart Digestion.…….....……...03
Corn Starch……...……...…01
Sodium Chloride…….……05
Agar…….……..……...…15
human Blood……...…….……..5%
pH 7,3 ± 0,2 at 25 ºC

Fraser Broth

Peptone……..……..…05
Casein Enzymic Hydrolysate……..…05
Yeast Extract……...……05
Meat Extract…….....05

Lactose Broth

Peptone……...…05
Beef Extract……...……...…..03
Lactose……..…...05

pH 6,9 ± 0,2 at 25 ºC

MacConkey Sorbitol Agar

Peptone……20
Sorbitol……...…..……10
Bile Salts…….……....1,5
NaCl…..……..05
Neutral Red……...…..……..0,03
Crystal Violet…….…….0,001
Agar……..……...15

pH 7,1 ± 0,2 at 25 ºC

Mannitol Salt Agar

Proteose Peptone…….……....10
NaCl.…….…….……..75
D- Mannitol…..……...10
Beef Extract…….....…….....01
Phenol Red……..0,025
Agar……...……..15

pH 7,4 ± 0,2 at 25 ºC

Methyl Red Voges Proskauer Broth

Buffered Peptone…….……...……07
Dextrose……05
Dipotassium Phosphate…….....…05

pH 6,9 ± 0,2 at 25 ºC

MEVAG Agar

Macerated Meat……..……50
KCL…….…….……...……05
Agar…….……09
Phenol Red……...…...1,5

pH 7,2 ± 0,2 at 25 ºC

NaCl……...…….20
Disodium Hydrogen Phosphate……..…12
Potassium Dihydrogen Phosphate……1,35
Esculin…….....01
Lithium Chloride…….…….....03

pH 7,2 ± 0,2 at 25 ºC

PALCAM Agar

Yeast Extract……..…...……..…….…03
Glucose……...…….……..0,5
Esculin…..…….……...…..0,8
Ferric Ammonium Citrate…….....0,5
Mannitol…….…10
Phenol Red……..….0,08
Lithium Chloride…….……..….15

pH 7,2 ± 0,2 at 25 ºC

Physiological Water

Sodium Chloride…..……..……...……9g/100ml

pH 7,0 ± 0,2 at 25 ºC

Rappaport Vassiliadis Broth

Soy Peptone…….……..4,5
NaCl…… ……...…..……7,2
Potassium Dihydrogen Phosphate.…..…1,26
Potassium Hydrogen Phosphate……...0,18
Magnesium Chloride.… ……13,58
Malachite Green…….0,036

pH 5,2 ± 0,2 at 25 ºC

Simmons' Citrate Agar

Magnesium Sulphate……..0,2
Ammonium dihydrogen phosphate.…….....01
Dipotassium phosphate…..……..01
Sodium citrate.……...…...……..02
NaCl……05
Bromothymol blue.……...……0,08
Agar…….…….....15

pH 6,8 ± 0,2 at 25 ºC

Tryptic Soy Agar

Casein Peptone……15
Soya Peptone…….……..05
NaCl……...……..05
Agar…….....15

pH 7,3 ± 0,2 at 25 ºC

Urea/Indole Broth

Potassium Phosphate Monobasic …..…..10
NaCl……...……..05
Tryptone……...30
Phenol Red.……..…..… 0.004
Urea…….13

pH 6,8 ± 0,2 at 25 ºC

Xylose Lysine Deoxycholate Agar

Yeast Extract……..…..03
L-Lysine Hydrochloride……..…....05
Xylose……..…….3,75
Lactose…….…...……....7,5
Sucrose.… …….....7,5
Sodium Deoxycholate.…….…...1,0
NaCl…….……...…….…...05
Thiosulfate Sodium.…..……..…6,8
Ammoniacal Iron Citrate.… ……..0,8
Phenol Red.… …….....0,08
Agar……..….....12,5

pH 7,4 ± 0,2 at 25 ºC

Triple Sugar Iron Agar

Pancreatic Digest of Casein……..…10
Peptic Digest of Animal Tissue……...10
Glucose……...……01
Lactose.….....10
Sucrose.……....10
Ferric Ammonium Sulfate.……..……...0,2
NaCl.……...……05
Sodium Thiosulfate.…….……..…0,3
Phenol Red.…….…....0,024
Agar…….....13

pH 7,4 ± 0,2 at 25 ºC

Reagents

Kovacs

p-Dimethylaminobenzaldehyde……...05g
Amyl Alcohol…….....75ml
Concentrated Hydrochloric Acid…….....25ml

Voges-Proskauer (reagent A)

Alpha-Naphthol, 5%…….....……...05g
Absolute Ethanol……..……..…100ml

Voges-Proskauer (reagent B)

Potassium Hydroxide…….....40g
Deionized Water……..……100ml

Tryptophan-Deaminase

Ferric Chloride…….....…..1g/10ml

Methyl Red

ABSTRACT

Aim : Examine the presence or absence of enteric pathogens; *Salmonella* and Enterohemorrhagic *Escherichia Coli*, and the pathogenic *Listeria* in leafy greens from markets and farms in Béjaïa and analyze the quality of water and soil which could be potential sources of contamination.

Methods : A total of 175 samples of lettuce, parsley, coriander, celery and mint; irrigation water and soil were collected from farms and greenmarkets. After isolation, the strains were identified using a set of biochemical tests, identification mediums, and the gallery API 20E.

Results : 0% prevalence was found of enteric bacteria specifically EHEC, *Listeria*, and *Salmonella* from the samples analysed. Instead, four opportunistic bacteria were identified; *Aeromonas salmonicida*, *E.hermannii*, *Phtobacterium damselae*, and *Cedecea davisae* found in parsley, coriander, and 2 celery samples. A 0% occurrence was also observed in soil and irrigation water.

Conclusion : Despite using a suitable quality control protocol, we were unable to detect any enteric bacteria and rather discovered opportunistic Gram-negative bacteria in celery, parsley and coriander. This outcome suggests good hygienic practices and handling methods by farmers and vendors, moreover, the variations in contamination including the quantity and types of samples, and seasonal differences which may all have contributed to the absence of the targeted microorganisms.

Keywords : Enteric pathogens, Leafy greens, Contaminated vegetables, Human health risk.

RÉSUMÉ

Objectif : Examiner la présence ou non des enteropathogènes ; *Salmonella* et *Escherichia Coli* entérohémorragiques, ainsi que la pathogène *Listeria* dans les herbes des marchés et des fermes de Béjaïa et analyser la qualité d'eau et sol, qui peuvent être des sources de contamination.

Méthodes : Un total de 175 échantillons de persil, coriandre, céleri et menthe ; l'eau d'irrigation et le sol ont été collectés d'après les fermes et les marchés. Après l'isolement, les souches ont été identifiées à l'aide des tests biochimiques, des milieux d'identification, et des galeries API 20E.

Résultats : Une prévalence de 0 % a été trouvée pour les bactéries entériques, en particulier EHEC, *Listeria* et *Salmonella*, dans les échantillons analysés. Au lieu de cela, quatre bactéries opportunistes ont été identifiées ; *Aeromonas salmonicida*, *E.hermannii*, *Phtobacterium damselae* et *Cedecea davisae* trouvés dans des échantillons de persil, de coriandre et de 2 céleris. Un taux de 0 % a également été observée dans le sol et l'eau d'irrigation.

Conclusion : Malgré l'utilisation d'un protocole de contrôle qualité adapté, nous n'avons pu détecter aucune bactérie entérique et avons plutôt découvert des bactéries Gram-négatives opportunistes dans le céleri, le persil et la coriandre. Ce résultat suggère de bonnes pratiques d'hygiène et méthodes de manipulation de la part des agriculteurs et des vendeurs, en outre, les variations de contamination, y compris la quantité et les types d'échantillons, et les différences saisonnières qui peuvent toutes avoir contribué à l'absence des micro-organismes ciblés.

Mots-clés : Enteropathogènes, Légumes-feuilles, Légumes contaminés, Risque pour la santé humaine.