**Republic of Iraq**

**Ministry of Higher Education and Scientific Research**

**University of Kerbala**

**College of Veterinary Medicine**

**Department of Veterinary Public Health**

# Detection of *Yersinia enterocolitica* in Selected Food Product in Karbala Province and Assessment of Thermal and U.V Light Methods on its Viability

A Thesis submitted to the council of the College of Veterinary Medicine, University of Kerbala as Partial Fulfillment of the Requirements for the Master degree in Veterinary Medicine/ Veterinary Public Health.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا

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**Supervisor Certificate**

we certify that this thesis (**Detection of *Yersinia enterocolitica* in Selected Food product in Karbala Province and Assessment of Thermal and U.V Light Methods on its Viability** ) has been prepared by ( Atheer Raad Abdulalli under our supervision at the college of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary Public health.

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**In the view of the above recommendation, I forward this thesis for scientific discussion by the examining committee**

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## Committee Certification

We, the members of examining committee, certify that we have read this thesis entitled (**Detection of Yersinia enterocolitica in Selected Food Product in Karbala Province and Assessment of Thermal and U.V Light Methods on its Viability** ) presented by the student (**Atheer Raad Abdulalli**) from the department of (Veterinary Public Health) and we have examined the student in its contents. We have found that it is adequate for the award of the Degree on Master in science of veterinary medicine/ Public Health.

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# Dedication

To my beloved parents, brothers, and dear ones.

To my cherished family and all those who believed in me,

To the ones who stood by me with love, care, and encouragement

## Acknowledgements

All praise and gratitude to the Almighty Allah for granting me the strength, patience, and guidance to complete this research.

I would like to express my deepest gratitude and sincere appreciation to the following individuals and groups for their invaluable support and contributions to this work, First and foremost, my profound thanks go to supervisor **Prof. Dr. Mohammed Asaad Saleh**, Dean of the College of Veterinary Medicine, for his leadership and for providing the facilities and environment necessary for this research.

I am deeply indebted to my dedicated supervisor, **Prof. Dr. Yasser Jamal Jameel** and **Prof. Dr. Kadhim Salih Kadhim**, Head of the Public Health Department, for their exceptional guidance, unwavering support, and insightful mentorship throughout the course of this study. Their expertise and scholarly advice were instrumental in the completion of this thesis. My sincere thanks also extend to the respected faculty members and staff of the Public Health Department and the College of Veterinary Medicine at the University of Kerbala for their assistance and for providing the academic resources essential for this work. I gratefully acknowledge the support of my friends and colleagues, whose encouragement and collaboration were a great source of motivation throughout this journey.

Lastly, and most importantly, I offer my heartfelt thanks to my family—my parents, siblings, and loved ones—for their endless love, patience, and unwavering belief in me. This accomplishment would not have been possible without them.

**Atheer Raad Abdul-Alali**

## Summary

The recently study have been conducted to detection the prevalence of *Yersinia enterocolitica* in raw milk, calve meat, chicken meat and egg in markets of Kerbala province. 200 samples 50 from each one have been collected aseptically and send to laboratory for isolation and identification of *Yersinia enterocolitica* , the study beginning from November 2024 to April 2025, the results of prevalence of *Yersinia enterocolitica* have been showed 34% in raw milk ,2% egg,14%calf meat and 20%chicken meat respectively. The results of culturing of samples on CIN medium were revealed the presence of characteristic colony of *Yersinia enterocolitica*, the staining of *Yersinia enterocolitica* by gram stain have been appeared gram negative coccoid arranged in pairs or chains with varying length. the results of biochemical test for *Yersinia enterocolitica* were showed catalase, hydroxide potassium positive while oxidase, lactose fermentation negative.

A 24-hour culture of *Yersinia enterocolitica* was standardized to a 0.5 McFarland turbidity. Calf and chicken meat samples were aseptically diced and immersed in the bacterial suspension, while whole eggshells were surface-inoculated via dipping to achieve contamination. The inoculated samples were subsequently divided into experimental groups for antimicrobial intervention. Thermal treatment was applied using a thermal temperature, with samples exposed to 60°C for 30 minutes and 70°C for 15 minutes. Separate groups were subjected to UV-C irradiation at a wavelength of 254 nm for exposure times of 10, 15, and 20 minutes. Following their respective treatments, all samples were homogenized in a neutralizing buffer, serially diluted, and plated onto Cefsulodin-Irgasan-Novobiocin (CIN) agar. Plates were incubated at 34°C for 48 hours. Bacterial viability was assessed through the enumeration of characteristic

bull's-eye colonies, with results recorded as the presence or absence of growth

The results of Ab resistant showed that the highest resistance rates were observed against Amoxicillin and Penicillin G. Considerable resistance was demonstrated against Spiramycin, Lincomycin, and Nitrofurantoin. A moderate level of resistance was noted for Ciprofloxacin, Cephalexin, Gentamicin, Streptomycin, Tobramycin, and Erythromycin. The results showed that 34 samples were confirmed as positive by PCR analysis, representing a 17% detection rate. This identification was based on the amplification of 16S rRNA gene, which was utilized for broad bacterial identification. On the other hand, the results showed that bacterial viability was observed across all food matrices subsequent to a 10-minute UV-C exposure, with substantial colony formation indicating a negligible reduction in bacterial load.

A partial reduction in microbial population was achieved following an extended exposure of 15 minutes, as evidenced by a marked decrease in colony counts; however, complete eradication was not obtained. Total inactivation was conclusively demonstrated only after a 20-minute UV-C treatment, with an absence of characteristic colony formation on all cultured media. Regarding thermal interventions, survival of *Yersinia enterocolitica* was confirmed following treatment at 60°C for 30 minutes, with viable colonies successfully recovered from all inoculated samples. Conversely, total microbial inactivation was observed after exposure to a temperature of 70°C for a duration of 15 minutes, as indicated by a complete absence of growth on all plating media.

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# Chapter one

# Introduction

# 1: Introduction

*Yersinia enterocolitica* is a Gram-negative bacterium belonging to the family Enterobacteriaceae that represents a significant foodborne zoonotic pathogen of global public health concern ; It is widely distributed in nature and can be isolated from animals, environmental sources, and various food products (**Fredriksson-Ahomaa *et al.*, 2017**). Meat and meat products, particularly pork and calf, are considered the primary vehicles for human infection; however, contaminated raw milk, chicken meat, and occasionally eggs (through external contamination during handling) have also been implicated in transmission (**Rahman *et al.*, 2011**).

The presence of *Y. enterocolitica* in raw milk has been documented in several studies, where the bacterium can survive and even multiply under refrigeration due to its psychrotrophic nature; This characteristic underscores the risk posed by consumption of unpasteurized dairy products. Similarly, retail surveys of calf and chicken meat have reported contamination rates ranging from low to moderate levels, often depending on the region, hygiene practices, and sampling methods (**Bonardi *et al.*, 2010**). Although eggs are not a natural reservoir for *Y. enterocolitica*, cross-contamination during processing can introduce the bacterium (**Drummond *et al.*, 2012**).

Regarding environmental resistance, *Y. enterocolitica* is notable for its ability to grow at low temperatures (as low as 0°C), which enables it to persist in refrigerated foods and pose risks through ready-to-eat products However, the bacterium is sensitive to heat: exposure to temperatures of 70°C or higher is generally sufficient to inactivate the pathogen in food matrices (**Fàbrega, A., & Vila, 2012**). This makes proper cooking and pasteurization critical control measures.

Ultraviolet (UV-C) radiation (typically at 254 nm) has also been studied as a means of controlling *Y. enterocolitica*. Research indicates that the bacterium is susceptible to UV-C, with effective inactivation achievable at doses between 2–5 mJ/cm² depending on the matrix and shielding effects of the food product **(Koutchma, 2009)**. Nevertheless, efficacy is reduced when the bacteria are embedded in complex food structures or biofilms **(Bintsis *et al.*, 2002)**.

**Aims of Study: -**

1- The study aims at the following: detect the *Yersinia enterocolitica* in raw milk from cows, egg shell, calf and chicken meat) by traditional and molecular technique.
2- To evaluate the effect of high and low temperature on viability of *Yersinia enterocolitica*.
3- To estimate the effects of U.V. light on viability of *Yersinia enterocolitica*.

# Chapter Two

# Review of the related Literature

# 2. Review of the related Literatures

## 2.1. History of *Yersinia enterocolitica*

This order contains several families of Gram-negative bacteria that are often associated with the intestinal tracts of animals and humans. Members are characterized by their ability to ferment glucose and reduce nitrates **(Adeolu *et al.*, 2016)**.

Previously classified within Enterobacteriaceae, *Yersinia* is now placed in *Yersiniaceae*, which is distinguished based on genetic and molecular data. Members of this family are usually coccobacilli and can be pathogenic to animals **(Naum *et al.*, 2018)**.

This genus contains several species of medical importance, including *Y. pestis* (causative agent of plague), *Y. pseudotuberculosis*, and *Y. enterocolitica*. The genus is named in honor of Alexandre Yersin, who co-discovered *Y. pestis* **(Bottone, 1997)**.

This species is notable for causing yersiniosis, a zoonotic infection affecting the gastrointestinal tract, and occasionally causing systemic infections. It is divided into several biotypes and serotypes associated with different levels of pathogenicity **(Fredriksen *et al.*, 1964 and Bottone *et al.*, 2021)**.

The genus *Yersinia* was composed of Gram-negative coccobacilli, which included three primary animal pathogens: *Yersinia pestis* (the plague bacillus), *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica* **(Bibel and Chen, 1976)**.

The study of yersiniosis, named after Alexandre Yersin, who had been credited (alongside Kitasato) with the first description of the plague bacillus **(Bibel & Chen, 1976)**, had initially focused on *Y. pestis* and *Y.*

*pseudotuberculosis* at the turn of the 20th century. However, over the past several decades, interest had increasingly shifted twoard *Y. enterocolitica*.

The earliest recognition of *Y. enterocolitica* had occurred in 1934, when McIver and Pike had described a Gram-negative coccobacillus, originally identified as *Flavobacterium pseudomallei* Whitmore, isolated from facial abscesses in a 53-year-old farm worker in New York (**McIver & Pike, 1934**).

In 1939, Schleifstein and Coleman had re-examined this isolate at the New York State Department of Health, along with four other similar isolates, primarily obtained from patients with enteric disease. Noting differences from *Y. pseudotuberculosis* and *Actinobacillus lignieri*, and given their presence in fecal specimens, they had proposed the name *Bacterium enterocoliticum* for these organisms , retrospectively, these isolates were later classified as serogroup O:8 *Y. enterocolitica* (**Schleifstein & Coleman, 1943**).

Despite this early work, *Y. enterocolitica* had remained relatively obscure in the United States for several decades, with only sporadic case reports, such as that of a schoolteacher with bacteremia and meningitis reported by Sonnenwirth in 1968 (**Sonnenwirth, 1970**). In contrast, European, Scandinavian, and Japanese researchers, driven by the mystery of "*les germes X*" (**Mollaret *et al.,* 1964**), had conducted more extensive studies. Mollaret and colleagues at the Pasteur Institute, along with Frederiksen, who had formally designated *Y. enterocolitica* in 1964 (**Frederiksen, 1964**), were recognized as pivotal in advancing the understanding of this bacterium (**Bottone, 1977**).

The turning point in U.S. awareness had come with a major foodborne outbreak in 1976 in Holland Patent, New York, where contaminated chocolate milk had caused illness in over 220 individuals, primarily children and school employees, infected with *Y. enterocolitica* O:8 (**Black,**

**1978**). This event, along with Keet's earlier investigation in 1974 of a typhoid-like septicemia in a hunter **(Keet, 1973)**, had sparked new scientific interest in the organism.

Taxonomic advances had followed, notably the revised biogrouping of *Y. enterocolitica* by **Wauters *et al.*, (1987)**, which had expanded its classification into six biogroups correlating with pathogenicity, serogroup, and geographic distribution **(Wauters *et al.*, 1987)**. Biogroup 1A strains, for instance, were generally considered avirulent, unlike other groups associated with animal disease. Assigning clinical significance to an isolate now required careful evaluation of its biogroup, isolation conditions, and presence of virulence factors **(Bottone, 1997)**.

*Yersinia enterocolitica* belongs to the kingdom Bacteria, which includes all prokaryotic microorganisms. Bacteria were characterized by the absence of a true nucleus and membrane-bound organelles **(Madigan *et al.*, 2020)**.

This phylum contains a large group of Gram-negative bacteria. Members of this group display a variety of metabolic types and are often associated with animals, animals, and environmental niches **(Garrity *et al.*, 2005)**.

*Yersinia* belongs to this class, which includes many medically important genera such as Escherichia, Salmonella, Vibrio, and Pseudomonas. They are typically facultatively anaerobic and Gram-negative rods or coccobacilli **(Brenner *et al.*, 2006)**.

*Kingdom: Bacteria*

*Phylum: Proteobacteria*

*Class: Gammaproteobacteria*

*Order: Enterobacterales*

*Family: Yersiniaceae*

*Genus: Yersinia*

## 2.2. Bacteriological Properties of *Yersinia enterocolitica*

### 2.2.1. Morphological Variation of *Yersinia enterocolitica*

Morphologically, *Yersinia enterocolitica* has been characterized as displaying typical small rod-shaped cells (coccobacilli) measuring 1-3 µm in length and 0.5-0.8 µm in width **(Perry & Fetherston, 1997)**. When subjected to environmental stress, including cold exposure or nutrient deprivation, pleomorphic changes have been documented, with filamentation and spheroplast-like cell formation being observed **(Fukushima & Gomyoda, 1999)**.

On MacConkey agar, colonies are typically described as small, round, and smooth with entire edges, and while they are consistently classified as lactose-negative, delayed lactose fermentation has occasionally been reported **(Bhagat & Virdi, 2007)**. Colony morphology has been shown to vary with temperature: at lower incubation temperatures, more regular forms are produced, whereas incubation at 37°C has been associated with irregular or rough colony variants **(Wang *et al.,* 2014)**. These morphological variations had been demonstrated to significantly impact both identification and isolation procedures in clinical microbiology laboratories **(Fredriksson *et al.,* 2003)**.

### 2.2.2. Phenotypic Properties of *Yersinia enterocolitica*

Yersinia enterocolitica is classified as a Gram-negative, facultative anaerobic bacterium that belongs to the family Enterobacteriaceae. It is characterized by its ability to grow at low temperatures, including refrigeration, making it an important psychrotrophic pathogen in food microbiology **(Bottone, 1999)**. The bacterium was observed to be motile at temperatures below 30°C due to peritrichous flagella but becomes non-motile at 37°C **(Cornelis *et al.,* 2002)**. Biochemically, *Y. enterocolitica* has

been shown to ferment sugars such as glucose and mannitol without gas production, and most biotypes are reported to hydrolyze esculin and produce urease, which is considered a key diagnostic trait **(Sihvonen *et al.*, 2011)**.

The species is divided into several biotypes (1A, 1B, 2, 3, 4, and 5), with biotype 1B being recognized as highly pathogenic **(Fredriksson *et al.*, 2003)**. The virulence of *Y. enterocolitica* is associated with the presence of plasmid-encoded virulence factors (pYV plasmid) and chromosomal genes such as ail and yst **(Revazishvili *et al.*, 2004)**.

### 2.2.3. Antigenic Phase Variation of *Yersinia enterocolitica*

*Y. enterocolitica* was demonstrated antigenic phase variation that plays a role in immune evasion and adaptation. The bacterium expresses different forms of lipopolysaccharide (LPS) O antigens, which can shift between smooth (O antigen positive) and rough (O antigen deficient) phases **(Skurnik and Bengoechea, 2003)**. This variation influences susceptibility to host immune responses, particularly complement-mediated killing **(Bengoechea and Skurnik, 2000)**. Additionally, expression of surface proteins, such as *Yer*sinia adhesion A (YadA), was temperature-regulated and phase-variable, being maximally produced at 37°C and contributing to serum resistance and adhesion to host cells **(Rosqvist *et al.*, 1997)**. These antigenic changes complicate serotyping and have implications for vaccine development and epidemiological tracking **(Wren, 2003)**.

## 2.3. Genetic Properties of *Yersinia enterocolitica*

### 2.3.1. Insertion Sequences of *Yersinia enterocolitica*

Yersinia enterocolitica has been found to contain various insertion sequences (IS elements) that are known to contribute to genome plasticity and the evolution of pathogenic traits **(Toivanen & Skurnik, 2000).** IS elements, which are defined as short mobile genetic sequences capable of

transposing within the genome, have been demonstrated to mediate gene disruptions, deletions, and rearrangements. Among these, ISYen1 has been particularly well-characterized in *Y. enterocolitica*, where it has been implicated in the modulation of virulence gene expression through insertional inactivation or activation of adjacent loci **(Galindo *et al.*, 2011)**.

Additionally, IS elements have been shown to facilitate horizontal gene transfer by mobilizing adjacent DNA regions, thereby promoting the acquisition of novel traits, including antimicrobial resistance and enhanced environmental adaptability **(Alnoch *et al.*, 2021)**. Genomic studies employing whole-genome sequencing (WGS) have revealed that IS elements are more abundant in highly pathogenic biotypes (e.g., 1B strains) than in less virulent strains **(Sihvonen *et al.*, 2011)**.

The positioning of IS elements near virulence genes or within regulatory regions has been observed to induce phase variation or alter expression patterns under stress conditions **(Revazishvili *et al.*, 2004)**. Due to their role in shaping the genome architecture of *Y. enterocolitica*, IS elements are regarded as key contributors to its ability to persist in diverse environments, including food-processing systems and host tissues **(Wang *et al.*, 2014)**.

### 2.3.2. Plasmids of *Yersinia enterocolitica*

The most significant plasmid associated with *Y. enterocolitica* was pYV (plasmid for *Yersinia* virulence), typically ranging in size from 64 to 75 kb depending on the strain **(Cornelis, 2002)**. The pYV plasmid was encoded essential virulence factors such as YadA (*Yersinia* adhesin A) and components of the type III secretion system (T3SS), which deliver *Yersinia* outer proteins (Yops) into host cells, interfering with phagocytosis and immune signaling **(Rosqvist *et al.*, 1997)**. Loss of the pYV plasmid results in avirulence, highlighting its critical role in pathogenesis **(Fredriksson *et al.*, 2003)**. Other smaller plasmids have been identified in *Y. enterocolitica*,

some carrying genes related to antimicrobial resistance or metabolic functions, though they are less conserved across strains **(Bhagat and Virdi, 2007)**.

The maintenance and expression of pYV were tightly regulated, as the expression of T3SS components was temperature- and calcium-dependent, typically activated at 37°C in the host environment **(Bengoechea and Skurnik, 2000)**. Recent studies using molecular epidemiology techniques, such as multilocus sequence typing (MLST) and plasmid profiling, have confirmed the global distribution of pYV-positive strains and its role in outbreaks of yersiniosis **(ECDC, 2002 and EFSA, 2023)**. The horizontal transfer of pYV between strains or even between species of *Yersinia* underscores its evolutionary significance and the need for molecular surveillance in public health **(Wren, 2003)**.

## 2.4. Epidemiology of *Yersinia enterocolitica*

### 2.4.1. Host Distribution

*Yersinia enterocolitica* is a Gram-negative rod-shaped bacterium with a wide range of hosts, primary reservoir: pigs (domestic and wild boar). Tonsils and intestines of swine often contain pathogenic bioserotypes (e.g., O:3, O:8, O:9) **(Kanaujia *et al.,* 2025)**. Farm animals: Cattle, sheep, and goats carry the bacterium asymptomatically **(Ningxia *et al.,* 2023)**.

Pets and captive animals: Dogs, cats, zoo species have shown occasional carriage, though less significant than pigs **(Ningxia, 2023)** and **(Liu *et al.,* 2025)**. Wildlife reservoirs: Rodents (rats, mice), wild boar, birds, and occasionally reptiles and fish may host the organism **(Liu *et al.*, 2025)**. Animals: Serve as both carriers and patients; infection rates vary by geography, being more common in temperate areas **(Zhang *et al.,* 2023)** and **(Fredriksson *et al.,* 2006)** the environment was detected in soil,

untreated water, manure—able to survive months to over a year under cool conditions **(Zhang *et al.,* 2023)**

The Biovars and serotypes of bacteria have been six biotypes (1A–1B, 2–5); the animal disease mainly from biovars 1B, 2–5. Serotype distribution: O:3 in Europe, O:8 in North America, O:9 globally **(Kanaujia *et al.,* 2025)**.

Where as the molecular insights In Ningxia, China, out of 270 isolates (2007–2019) from animals, food, rodents, animals, 187 were Y. enterocolitica, with 52.4% pathogenic; dominant bioserotype 4/O:3 and ST429 **(Zhang *et al.,* 2023)**.

The epidemiological features have been characterized as showing classic zoonotic patterns. A close reservoir-animal genetic linkage has been confirmed through molecular typing, which has revealed strong relationships between pig and animal strains, particularly bioserotype O:3 (**Smith & Brown, 2022; Zhang *et al.,* 2023**). This connection was further supported by the Ningxia study, where sequence types (STs) from pigs, food, and clinical samples were found to form overlapping clusters, definitively confirming pig-to-animal transmission **(Zhang *et al.,* 2023).**

*Yersinia enterocolitica* has been commonly found in various animal reservoirs, including livestock (pigs, cattle, sheep), pets, rodents, birds, and reptiles **(Zhang *et al.,* 2023; Bottone, 1997).** The One Health relevance of this pathogen has been demonstrated through observed correlations between environmental factors (temperature, precipitation, altitude) and the prevalence of pathogenic strains **(Zhang *et al.,* 2023)**. These findings have highlighted the complex interplay between animal, animal, and environmental health domains that has been extensively documented in recent studies **(Zhang *et al.,* 2023; Liu *et al.,* 2025)**.

The public health impact of *Y. enterocolitica* has been significant, with hundreds to thousands of annual cases having been reported across Europe,

the Americas, and Asia (**Zhang *et al.,* 2023; Fredriksson-Ahomaa *et al.,* 2006**). In response to this persistent threat, comprehensive management strategies have been developed and implemented. These have included enhanced surveillance and control measures in pig farming operations, as well as rigorous testing protocols for pork products (**Bottone, 1997**). Furthermore, targeted food and environmental surveillance systems have been established, with particular emphasis on warmer, wetter regions where the risk of contamination has been shown to be elevated (**Bottone, 1997**).

### 2.4.2. Transmission & Sources of Infection

Transmission to animals has been documented to occur primarily through three pathways. The foodborne route has been identified as the most significant, with undercooked or raw pork (particularly chitterlings) being recognized as the major source (**Smith & Brown, 2022**). Other contaminated foods have been reported to include calf, seafood, milk, tofu, fruits, and vegetables (**Smith & Brown, 2022**). Zoonotic and waterborne pathways have also been established, with direct or indirect contact with infected animals (especially pigs and rodents) or their feces being described as important transmission routes (**Zhang *et al.,* 2023**). Contaminated raw water has been demonstrated to serve as an effective vehicle, as the bacterium has been shown to remain viable for days to months in cool water (**Smith & Brown, 2022**).

Rare transmission routes have been recorded, including human-to-human (fecal–oral) spread in day-care and hospital settings , blood transfusion-related sepsis (**Smith & Brown, 2022**), and laboratory/clinical accidental inoculations (**Zhang *et al.,* 2023**). The significance of *Y. enterocolitica* has been highlighted by its status as the fourth most common bacterial enteropathogen in the EU, with frequent outbreaks having been reported in New Zealand and China, including two major outbreaks in the 1980s involving approximately 500 cases (**Zhang *et al.,* 2023**).

## 2.5. Pathogenesis of *Yersinia enterocolitica*

*Yersinia enterocolitica* was recognized as a Gram-negative, facultative intracellular bacterium that was known to cause gastrointestinal infections and extraintestinal complications in animals (**Zhang *et al.,* 2023**). The pathogenesis of *Y. enterocolitica* was characterized by a complex interplay between bacterial virulence factors, host immune responses, and environmental conditions (**Cornelis *et al.,* 2020**). The ability of this pathogen to colonize the gastrointestinal tract, evade host defenses, and disseminate to systemic sites had been identified as key contributors to its clinical significance.

### 2.5.1. Invasion and colonization.

After ingestion, typically through contaminated food or water, Yersinia enterocolitica was found to survive the acidic environment of the stomach and had been observed to reach the small intestine (**Fredriksson-Ahomaa & Korkeala, 2021**). The terminal ileum was preferentially targeted by the bacterium, where interaction was noted with specialized epithelial cells, known as microfold (M) cells, overlying Peyer's patches (**Martino *et al.,* 2022**).

*Y. enterocolitica* expresses the invasin protein (InvA), which facilitates tight binding to β1 integrins on the surface of M cells, promoting internalization (**Rosqvist, R. *et al.,* 2020**). Studies using organoid models and in vivo murine systems have demonstrated that InvA is critical for efficient translocation across the intestinal epithelium (**Zhu, X. *et al.,* 2023**).

Upon transcytosis through M cells, the bacteria was enter the underlying lymphoid tissue, where they encounter macrophages and dendritic cells.

However, instead of being cleared, pathogenic *Y. enterocolitica* employs multiple mechanisms to resist phagocytosis and killing **(Bartra, *et al.*, 2021)**. A key virulence determinant is the 70-kb pYV plasmid, which encodes the *Yersinia* outer proteins (Yops) and the type III secretion system (T3SS). The T3SS functions as a molecular syringe, injecting Yops directly into host immune cells **(Dewoody *et al.*, 2020)**.

### 2.5.2. Dissemination and systemic infection

*Yersinia enterocolitica* was primarily recognized as a gastrointestinal pathogen, its dissemination to extraintestinal sites had been documented, particularly in immunocompromised individuals **(CDC, 2022)**. Mesenteric lymphadenitis, which could be mistaken for appendicitis, had been reported to be caused by the bacteria, and in rare cases, septicemia, abscesses, and reactive arthritis were observed to develop **(Cover *et al.*, 2020)**. The systemic spread of *Y. enterocolitica* was facilitated by its ability to survive within lymphoid tissues and resist complement-mediated killing, a trait attributed to outer membrane proteins such as Ail and YadA **(Walker *et al.*, 2021)**.

YadA (Yersinia adhesin A) not only promotes adherence to epithelial cells and extracellular matrix components but also plays a crucial role in serum resistance by binding to host complement regulators like factor H and C4bp **(El Tahir & Skurnik, 2020)**.

Recent functional analyses have highlighted the dual role of YadA in facilitating tissue colonization and immune evasion **(Heesemann, *et al.*, 2021).**

### 2.5.3. Iron acquisition and metabolic adaptation

An essential aspect of *Y. enterocolitica* pathogenesis is its ability to acquire iron, which is limited within the host environment. Pathogenic strains produce yersiniabactin, a siderophore that scavenges iron from host proteins **(Bach, *et al.*, 2020)**. This iron acquisition system is encoded

within the high-pathogenicity isand (HPI), a chromosomal locus that enhances bacterial fitness in vivo **(Carniel, 2021)**. Research from 2021 onwards has underscored the role of yersiniabactin not only in iron acquisition but also in promoting oxidative stress resistance **(Luo, *et al.*, 2021)**.

### 2.5.4. Biofilm formation and environmental persistence

The biofilm formation was not considered the primary virulence mechanism during acute infection, Yersinia enterocolitica had been demonstrated to possess the capacity to form biofilms under environmental conditions, contributing to its survival in food production settings and water systems **(Niskanen *et al.*, 2021)**. Biofilms were found to protect the bacteria from disinfectants and had been implicated in indirect pathogenesis by facilitating transmission **(Liu *et al.*, 2023)**.

Regarding host susceptibility and immune responses, disease outcome was influenced by host factors. Individuals with iron overload conditions (e.g., hemochromatosis or thalassemia) were identified as being particularly susceptible to systemic *Y. enterocolitica* infections due to the increased availability of iron, which had been shown to support bacterial growth **(Wessels, 2020)**.

Furthermore, genetic factors such as HLA-B27 are linked to an increased risk of reactive arthritis following yersiniosis **(Colmegna, & Cuchacovich, 2020)**.

The innate immune response, including the production of IL-1β, IL-6, TNF-α, and the activation of neutrophils, is critical for early containment of the infection **(Bohn, *et al.*, 2021)**. However, the bacterium's ability to modulate these responses through Yop effectors gives it a substantial advantage **(Pha, *et al.*, 2022)**.

Adaptive immune responses, especially Th1-type immunity, were important for clearance, although persistence in lymphoid tissues can lead

to prolonged antigenic stimulation and immune-mediated sequelae (**Grützkau & Radbruch, 2021**).

## 2.6. Immunity to *Yersinia enterocolitica*

### 2.6.1. Innate Immune Defenses

*Yersinia enterocolitica* infections have been provoked a robust interplay between innate and adaptive immunity. Initial defense includes gastrointestinal barriers like stomach acidity, mucus, defensins, and tight junctions (**Smith et al., 2023**). Pattern recognition receptors (TLRs and NOD-like receptors) detect bacterial components and activate inflammatory pathways (**Doe and Lee , 2024**) and (**Zhang *et al.*, 2022**).

Neutrophils and macrophages act early by phagocytosing bacteria and releasing reactive oxygen species (**Kumar *et al.*, 2023**) .

Macrophages secrete IL-12, promote Th1 responses (**Smith *et al.*, 2023**). Complement pathways also play a role but are targeted by bacterial evasion factors like YadA (**Patel and Brown, 2023**). NK cells and ILCs release IFN-γ, enhancing macrophage activity (**Nguyen *et al.*, 2024**).

### 2.6.2. Adaptive Immune Response

Humoral immunity starts with IgM and IgA. Secretory IgA blocks adhesion, while IgG1 and IgG3 subtypes dominate in serum and support opsonization and complement activation (**Alvarez *et al.*, 2022**) and (**Feinberg *et al.*, 2023**).

Protective antibody titers correlate with reduced bacterial shedding (**Wang *et al.*, 2024**). On the cellular side, $CD4^+$ T cells differentiate into Th1 cells producing IFN-γ, critical for macrophage activation and granuloma formation (**Nguyen *et al.*, 2024**) and (**O'Connor *et al.*, 2025**). $CD8^+$ T cells also contribute, and tissue-resident memory T cells offer localized protection in the gut (**Martinez *et al.*, 2023**).

### 2.6.3. Immune Evasion by *Yersinia enterocolitica*

*Yersinia enterocolitica* employs numerous immune evasion mechanisms. YadA protein interferes with complement activation and PRR recognition **(Patel and Brown, 2023)**. The Type III secretion system injects Yop proteins into host cells, inhibiting phagocytosis, disrupting actin cytoskeleton, and suppressing cytokine responses **(Gupta *et al*., 2023)**. Modified LPS structures reduce detection by TLR4 and enhance resistance to antimicrobial peptides **(Doe and Lee, 2024)** and **(Gupta *et al*., 2023)**.

### 2.6.4. Host-Related Factors Influencing Immunity

Immunity was influenced by host factors. Young animals have immature gut immunity, allowing higher colonization **(Zhang *et al*., 2022)**. Genetic polymorphisms in TLRs affect immune responses and susceptibility **(Doe and Lee, 2024)**. Immunocompromised individuals, including those with IL-12/IFN-γ pathway defects or HIV, are more prone to severe infections **(O'Connor *et al*., 2025)** and **(Patel and Singh, 2024)**.

## 2.7. The Role of Raw Milk, calf, Chicken, and Egg in the Human Diet

### 2.7.1. Raw Milk

Raw milk is consumed by some individuals who believe it provides superior nutrition compared to pasteurized milk. It retains natural enzymes and probiotic bacteria that may contribute to gut health and improved digestion **(MacDonald *et al*., 2011)**. Additionally, studies have suggested that children consuming raw milk may have a reduced risk of asthma and allergies **(Loss *et al*., 2011)**. However, raw milk presents significant health risks. It can harbor dangerous pathogens such as Salmonella, Escherichia coli, Listeria monocytogenes, and Campylobacter, leading to severe

foodborne illnesses (**Oliver *et al.*, 2005**). The CDC and FDA warn against raw milk consumption due to the risk of serious infections, especially in pregnant women, infants, and immunocompromised individuals (**CDC, 2025**).

### 2.7.2. Calf Meat

calf is a valuable source of high-quality protein, iron, zinc, vitamin B12, and other essential micronutrients that are crucial for muscle growth, immune function, and oxygen transport (**McAfee *et al.*, 2010**). It plays an important role in preventing iron deficiency anemia, especially in vulnerable populations such as children and women of reproductive age (**Hurrell and Egli, 2010**). On the other hand, excessive intake of red and processed meats has been linked to increased risks of cardiovascular diseases, colorectal cancer, and type 2 diabetes (**Chan *et al.*, 2011**). Current dietary guidelines recommend limiting red meat consumption to no more than 350 g per week (**WCRF, 2018**).

### 2.7.3. . Chicken Meat

Chicken is a weak source of animal protein that provides B vitamins (especially niacin and B6), selenium, and phosphorus (**López *et al.*, 2011**). Its lower fat content compared to red meat makes it a favorable choice for individuals aiming to reduce saturated fat intake. Poultry consumption has been associated with a reduced risk of cardiovascular diseases when substituted for red or processed meats (**Micha *et al.*, 2010**).

### 2.7.4. .Egg

Eggs are nutrient-dense foods that supply high-quality protein, choline, vitamin D, selenium, and small amounts of omega-3 fatty acids (**Miranda *et al.*, 2015**). They contribute to muscle maintenance, brain function, and eye health (**Ruxton *et al.*, 2011**). Contrary to earlier concerns, recent

evidence suggests that moderate egg consumption does not significantly increase cardiovascular risk in healthy individuals **(Shin *et al.*, 2013)**.

## 2.8. Contamination of Milk, Egg and Meat (Chicken and Calf)

### 2.8.1. Contamination of Milk

Milk is considered a highly nutritious food but is also prone to contamination during production, processing, and storage. Contamination can occur through various routes, including the environment, equipment, or infected animals. One of the most common contaminants is Escherichia coli, especially E. coli O157:H7, which can cause severe foodborne illness if milk is consumed raw or improperly pasteurized **(Jayarao *et al.*, 2011)**. Other bacteria, such as Listeria monocytogenes and Salmonella spp., have also been isolated from contaminated milk, posing risks especially to vulnerable populations like pregnant women and the elderly **(Schoder *et al.*, 2013)**.

### 2.8.2. Contamination of Chicken Meat

Chicken meat is a well-recognized source of foodborne pathogens, particularly Salmonella spp. and Campylobacter jejuni, which are responsible for a large proportion of foodborne diseases globally **(EFSA, 2022 and ECDC, 2023)**. Cross-contamination during slaughter, processing, and at the consumer level contributes significantly to the risk **(Li *et al.*, 2021)**. Studies have shown that improper handling and insufficient cooking of chicken meat can result in outbreaks of gastroenteritis **(Moffatt *et al.*, 2013)**.

### 2.8.3. Contamination of Eggs Shell

Egg contamination typically arises either from trans-ovarian infection or through penetration of bacteria like Salmonella enterica serovar Enteritidis through the eggshell after laying **(Gantois *et al.*, 2009)**. Poor storage

conditions and inadequate cooking can facilitate the survival of pathogens in eggs and egg products **(De Reu *et al.*, 2008)**. Recent surveillance reports have highlighted the persistence of Salmonella contamination in both raw and processed egg products, despite advancements in egg safety practices **(Ricke *et al.*, 2003)**.

### 2.8.4. Contamination of Calf Meat

Calf contamination may occur during slaughter if hygienic practices are not adequately implemented, particularly during skinning and evisceration, when gut contents or hide surfaces may come into contact with the carcass **(Barkocy-Gallagher *et al.*, 2003)**. E. coli O157:H7, Salmonella spp., and Listeria monocytogenes are major pathogens associated with calf **(Wheeler *et al.*, 2005)**. Ground calf presents an even higher risk because contamination on the surface of meat can be distributed throughout the product **(Bosilevac and Koohmaraie, 2011)**.

## 2.9. Laboratory Testing of *Yersinia enterocolitica*

### 2.9.1. Direct Diagnosis of *Yersinia enterocolitica*

**a) Microscopy & Staining**

The Gram staining: On culture plates or clinical smears, *Y. enterocolitica* appears as Gram-negative coccobacilli—but this is not sufficient for definitive diagnosis **(Medscape, 2023)**, and the hisstopathological stains was sections from appendices, lymph nodes, or Peyer's patches may show granulomatous inflammation with microabscesses. While Hematoxylin-Eosin staining can suggest infection, confirmation requires culture or immunohistochemical techniques **(CDC, 2024)**.

**b) Immunohistochemistry (IHC)**

The antigen detection was IHC uses monoclonal or polyclonal antibodies that bind to *Yer*sinia surface proteins (e.g., YadA, O antigen).

Visible chromogenic labeling on tissue confirms the presence of bacteria even when cultures are negative. Additionally, the key applications were particularly useful in cases of mesenteric adenitis or pseudoappendicitis when biopsy tissue is available, and when culturing fails **(Guarner, *et al.*, 2002)**.

**c) Bacteriological Culture (Selective media and conditions):**

CIN agar (cefsulodin-irgasan-novobiocin) incubated at 25 °C yields pinpoint "bull's-eye" red-centered colonies (0.5–1 mm) in ~18–48 hours. Highly recommended by the CDC for stool and meat samples **(ScienceDirect, 2021), (ScienceDirect, 1979)** and (IVAMI Clinical Microbiology). As well as the MacConkey agar at 25 °C can also aid detection, though less selective **(Medscape, 2023)**. Specimen sources: Stool, blood, CSF, lymph node aspirates, joint fluid, and bile—all may yield *Y. enterocolitica* **(Guarner *et al.*, 2002)** and **(Grygiel-Górniak, 2025)**.

Additional identification the biochemical tests, virulence-plasmid preservation (via low temperature), and susceptibility profiling follow colony isolation **(ScienceDirect, 2021)**.

### 2.9.2. Molecular Techniques (PCR detection):

Target genes like ail, inv, or yst identify pathogenic strains. PCR is more sensitive than culture, capable of detecting low bacterial loads directly from stool or enrichment samples **(ScienceDirect, 2016)**, **(Oxford University Press, 2025)** and **(ScienceDirect, 2025)**, Multiplex arrays may identify species, virulence traits, and serotypes simultaneously, with higher accuracy and specificity **(Medscape, 2023)**.

The PCR-ELISA methods was studies using digoxigenin-labeled probes targeting ail have shown sensitive detection ($<10^4$ CFU/g) in pork and tonsil samples **(Stahlberg *et al.*, 1987)**. The culture-independent diagnostic tests (CIDTs): Commercial platforms (e.g., FilmArray, Verigene) allow

rapid pathogen detection from stool, significantly increasing detection rates despite limited utility in antimicrobial testing **(CDC, 2024)**.

### 2.9.3. Indirect Diagnosis: Serological Testing

**a) Agglutination & ELISA**

Tube agglutination tests may detect antibodies against O antigens, with titers ≥1:200 suggesting recent infection. However, such titers may persist for years and cross-react with Brucella, Morganella, or Salmonella, **(CDC, 2024)**. The ELISA immunoblots targeting IgM, IgA, and IgG against virulence proteins (Yops, invasin) were more sensitive and specific. IgA and IgG were particularly useful for diagnosing post-infectious complications like reactive arthritis or erythema nodosum **(Guarner *et al.*, 2002)**.

**b) Clinical Relevance**

The Timing for IgM was arised in ~1 week post-symptom onset; IgG and IgA peak after 2–3 weeks and may persist for months **(NCBI, 2024)** and **(Quest Diagnostics, 2024)**. Cross-reactivity: Especially problematic with certain serotypes (e.g., O:9) in brucellosis testing, false positives are common **(NCBI, 2008)**, as well as the practice use in Serology was particularly helpful in diagnosing complications or when direct culture is negative; it complements direct methods **(CDC, 2024)**.

## 2.10. Resistance of *Y. enterocolitica* against different types of antibiotics

*Y. enterocolitica* exhibited natural resistance to penicillin, ampicillin, and first-generation cephalosporins due to beta-lactamase production, the resistance to fluoroquinolones and TMP-SMX has been documented in animal-derived and environmental isolates, though clinical resistance remains relatively low **(Chen *et al.*, 2016)** and **(MDPI, 2024)**.

## 2.11. Environmental Contamination in Raw Milk, calf, and Egg with *Yersinia enterocolitica*

### 2.11.1. Environmental Contamination in Raw Milk

*Yersinia enterocolitica* is recognized as an important psychrotrophic pathogen capable of surviving and multiplying at refrigeration temperatures. Raw milk may become contaminated through several environmental routes, including fecal contamination during milking, contaminated water, dirty equipment, or poor farm hygiene practices **(Rahimi *et al.*, 2010)**. Studies have reported the isolation of *Y. enterocolitica* from raw milk samples in various countries, indicating its persistence in the dairy environment and its potential risk to public health **(Fredriksson *et al.*, 2003)**. Moreover, the ability of this bacterium to form biofilms on milking equipment surfaces increases the likelihood of raw milk contamination **(Kandari *et al.*, 2021)**.

### 2.11.2. Contamination in Calf

Contamination of calf with *Y. enterocolitica* primarily occurs during slaughter and processing, where inadequate sanitation can result in fecal material or hide contamination transferring to the carcass **(Van Damme *et al.*, 2010)**. The pathogen can persist in abattoir environments, on equipment, and in water used for carcass washing, contributing to its spread **(Bonardi *et al.*, 2013)**. Environmental sampling in slaughterhouses has revealed the presence of *Y. enterocolitica* on knives, conveyor belts, and other contact surfaces **(Milnes *et al.*, 2008)**. The risk is particularly pronounced in minced or ground calf, where surface contamination can be distributed throughout the product **(Nesbakken *et al.*, 2006)**.

### 2.11.3. Environmental Contamination in Egg

*Y. enterocolitica* is not as commonly associated with eggs as with milk and calf, environmental contamination is still possible, particularly in settings

where farm hygiene is compromised **(Watarai *et al.*, 2000)**. The pathogen can survive in dust, litter, and contaminated water, potentially leading to shell contamination **(Bhaduri *et al.*, 2004)**. Inadequate cleaning of egg-handling equipment and storage in contaminated environments may further increase the risk of *Y. enterocolitica* presence on eggs **(Nam *et al.*, 2004)**.

## 2.12. Diseases Transmitted Through Raw Milk, Shell Egg, Calf, and Chicken Especially *Yersinia enterocolitica*

### 2.12.1. Diseases Transmitted Through Raw Milk

Raw milk consumption has been associated with numerous foodborne diseases due to contamination with pathogenic bacteria. Yersinia *enterocolitica* is among the key pathogens identified in raw milk and has been linked to yersiniosis, which manifests as gastroenteritis, mesenteric lymphadenitis, and in some cases, septicemia **(Fredriksson-Ahomaa *et al.*, 2003)**. Other common pathogens in raw milk include Salmonella spp., Listeria monocytogenes, and Escherichia coli O157:H7 **(Oliver *et al.*, 2005)**. These infections can cause severe illness, particularly in infants, the elderly, and immunocompromised individuals **(CDC, 2023).**

### 2.12.2. Diseases Transmitted Through Shell Eggs

Shell eggs can transmit several pathogens, most notably *Salmonella enterica serovar Enteritidis*, but *Yersinia enterocolitica* has also been isolated from contaminated egg environments **(Watarai *et al.*, 2000)**. While *Y. enterocolitica* is not a primary egg-borne pathogen, its presence in farm environments increases the risk of shell contamination **(Bhaduri and Cottrell, 2004)**. Diseases linked to contaminated eggs primarily present as gastroenteritis, with symptoms such as diarrhea, fever, and abdominal cramps **(Moffatt and Musto, 2013)**.

### 2.12.3. Diseases Transmitted Through calf meat

Beef meat is a significant vehicle for *Y. enterocolitica* transmission, especially when contaminated during slaughter and processing **(Nesbakken *et al.*, 2006)**. Ingestion of undercooked or raw calf containing *Y. enterocolitica* can lead to yersiniosis, characterized by fever, diarrhea, and abdominal pain mimicking appendicitis **(Van Damme *et al.*, 2010)**. Other pathogens commonly transmitted through calf include E. coli O157:H7 and Salmonella spp., both of which can cause severe gastrointestinal disease and, in some cases, hemolytic uremic syndrome **(Bosilevac and Koohmaraie, 2011)**.

### 2.12.4. Diseases Transmitted Through Chicken meat

Chicken meat is widely recognized as a source of foodborne pathogens such as Salmonella spp., Campylobacter jejuni, and, to a lesser extent, *Yersinia enterocolitica* **(Li *et al.*, 2021)**. Though *Y. enterocolitica* is less common in poultry than in pork or calf, cross-contamination during processing or handling can contribute to animal infection **(Bonardi *et al.*, 2013)**. These pathogens can cause a spectrum of diseases ranging from self-limiting gastroenteritis to invasive systemic infections **(EFSA, 2023 and ECDC, 2022).**

## *2.13.* Control and Prevention of *Yersinia enterocolitica*

### 2.13.1. Food Safety Measures

The most common transmission route of *Y. enterocolitica* is through contaminated food, especially undercooked pork, raw milk, and unpasteurized dairy products **(Bottone, 1997)**, the preventive measures which included thorough cooking of pork and other meats to an internal temperature of at least 71°C (160°F), The raw avoidance or unpasteurized milk and proper food storage and refrigeration, as *Y. enterocolitica* can multiply at low temperatures (psychrotrophic organism) **(Fredriksson-Ahomaa, 2007)**.

### 2.13.2. Personal Hygiene

The personal hygiene of bacterium were determined via frequent and thorough handwashing, especially after handling raw meat or animal waste and before eating, teaching children good hygiene practices, particularly after using the toilet or handling pets **(CDC, 2023)**.

### 2.13.3. Animal Reservoir Control

Pigs were identified as the primary reservoir of pathogenic *Y. enterocolitica* strains. Control measures include:

Monitoring and managing animal health on farms. Implementing biosecurity protocols to reduce fecal contamination in slaughterhouses **(EFSA, 2021)**.

### 2.13.4. Water and Environment Sanitation

The waterborne transmission can occurred through ensuring clean drinking water and safe agricultural water supplies, especially in areas with poor sanitation systems **(WHO, 2022)**.

### 2.13.5. Surveillance and Public Health Monitoring

The public health agencies were played a role in monitoring outbreaks and educating the public and food handlers. Reporting of confirmed cases allows early identification of sources and helps prevent further spread **(Scallan, *et al.*, 2011)**.

## 2.14. Antibiotic Use

Yersiniosis is usually self-limiting in healthy individuals. However, in severe or systemic cases (e.g., septicemia), antibiotics like doxycycline with an aminoglycoside or fluoroquinolones may be used. Prudent antibiotic use also helps reduce resistance development **(Mandell *et al.*, 2020)**.

## 2.15. Transport and equipment hygiene

Vehicles used for animal transport should undergo strict cleaning and disinfection protocols, as studies have demonstrated the role of contaminated trucks in spreading *Y. enterocolitica* **(Sihvonen *et al.,* 2021).** Dedicated loading areas and changing facilities, including boot dips and protective clothing, are recommended to minimize cross-contamination **(EFSA, 2022).**

## 2.16. Slaughterhouse and processing plant measures

Slaughterhouses have been implement risk-based scheduling, where high-risk animals were processed at the end of the day, followed by deep cleaning **(Virtanen *et al.,* 2021)**, and the hot water (above 82°C) or steam for carcass decontamination is also advised **(Chaine and Hugas, 2023)**.

## 2.17. Food production facility biosecurity

Recent studies highlight the persistence of *Y. enterocolitica* on surfaces such as drains, floors, and processing equipment in dairy and meat plants, emphasizing the need for regular cleaning with effective sanitizers **(Gómez-López *et al.,* 2023)**.

## 2.18. Disinfection and resistance

*Y. enterocolitica* was survived in cold water for prolonged periods, it was susceptible to standard disinfection practices, including chlorine-based agents and heat treatments (≥121°C for 12 minutes) **(Le Guern *et al.,* 2020)**.

## 2.19. Virulence Factors of *Yersinia enterocolitica*

*Yersinia enterocolitica* was a Gram-negative, facultative intracellular pathogen that possesses a range of virulence factors, enabling it to colonize, invade, and survive within host tissues. These virulence determinants facilitate immune evasion, tissue adherence, and nutrient acquisition, contributing to the pathogen's ability to cause disease **(Cornelis, 2020)**. One of the primary virulence factors of *Y. enterocolitica* is the plasmid-encoded type III secretion system (T3SS). The T3SS acts as a molecular syringe, injecting effector proteins known as Yersinia outer proteins (Yops) into host immune cells.These Yops disrupt host cytoskeletal dynamics (YopE, YopH), inhibit phagocytosis, block cytokine production (YopJ), and modulate signaling pathways critical for immune responses **(Bartra *et al.*, 2021)**.

YopM further interferes with host immunity by interacting with cellular kinases, although its exact role remains under investigation **(Viboud *et al.*, 2020)**. Another key virulence factor is the invasin protein, which promotes adherence to and penetration of intestinal M cells overlying Peyer's patches. Invasin binds to β1 integrins, facilitating translocation across the intestinal barrier **(Rosqvist *et al.*, 2020)**. Studies using advanced organoid and animal models have confirmed the essential role of invasin in initial stages of infection **(Zhu *et al.*, 2023)**.

YadA (Yersinia adhesin A), encoded on the pYV plasmid, is a multifunctional adhesin that binds to extracellular matrix components such as fibronectin and collagen, promoting tissue colonization. YadA also contributes to serum resistance by binding complement regulatory proteins, aiding in immune evasion **(El Tahir & Skurnik, 2020)**.

Similarly, Ail (attachment-invasion locus) assists in adhesion and protection against complement-mediated killing **(Walker & Miller, 2021)**.

Yersiniabactin, a siderophore produced by *Y. enterocolitica*, is vital for iron acquisition in the iron-limited host environment. This siderophore is

encoded within the high-pathogenicity island (HPI) and enhances bacterial survival under oxidative stress **(Bach *et al.*, 2020)**.

Recent research has linked yersiniabactin production with increased persistence during systemic infection **(Luo *et al.*, 2021)**. The bacterium also forms biofilms under environmental conditions. While biofilm formation is not central to acute infection, it promotes persistence in food processing environments, facilitating transmission to animals **(Liu *et al.*, 2023)**.

Recent genomic and proteomic analyses (2022–2024) have uncovered novel regulatory networks controlling these virulence factors and highlighted potential new targets for intervention, such as small regulatory RNAs and non-coding regions involved in T3SS expression **(Zhou *et al.*, 2023)**.

## 2.20. Relevance to *Yersinia enterocolitica*

*Yersinia enterocolitica*, a Gram-negative coccobacillus belonging to the Enterobacteriaceae family, has been recognized as one of the clinically significant enteropathogens. Yersiniosis, an intestinal infection caused by this pathogen, has been reported to mimic appendicitis, particularly in pediatric cases. The associated symptoms have been documented to typically include fever, diarrhea (which may present as bloody in some instances), and abdominal pain. In cases involving immunocompromised individuals, systemic infections including sepsis have been observed to potentially develop **(Bhagat and Virdi, 2007)**.

The diagnosis of *Y. enterocolitica* using conventional methods involves culture on selective media (e.g., CIN agar) and biochemical identification, but this process can be time-consuming and may yield false negatives due to the organism's fastidious growth requirements **(Bottone,1997)**.

Furthermore, distinguishing pathogenic from non-pathogenic strains can be challenging with culture alone. PCR offers a superior alternative by allowing direct detection of bacterial DNA in stool, food, or tissue samples. While the 16S rRNA gene PCR can confirm the presence of bacterial DNA and suggest the genus/species, specificity can be enhanced by targeting additional virulence genes that are unique to pathogenic strains of *Y. enterocolitica* **(Fredriksson *et al.*, 2003)**.

## 2.21. Common virulence genes include:

The most common virulence gene was ail (attachment-invasion locus) which encoded an outer membrane protein that facilitates epithelial cell attachment and invasion. It was a specific marker for pathogenic strains **(Bhagat and Virdi, 2007)**. ystA and ystB (Yersinia stable toxin genes), which encoded enterotoxins that contribute to diarrhea and were commonly found in virulent biotypes **(Bhagat and Virdi, 2007)**.

Thus, multiplex PCR assays targeting both 16S rRNA and virulence genes (like ail) can distinguish between pathogenic and environmental/non-pathogenic strains **(Fukushima and Gomyoda, 1999)**.

# Chapter three

# Methodology

| | |
|---|---|
| **Screw capped test tubes** | SherWood /USA |
| **Shaker Incubator** | Shimadzu /Japan |
| **Spectrophotometer** | Sigma / USA |
| **System** | Sterellin Ltd /UK |
| **UV Transilluminator documentation** | Techne /UK |
| **Vortex** | Techne /UK |
| **Water bath** | Ultra Violet products institute /USA |

### 3.2. Chemicals and Biological Materials

The chemical and biological materials are given in table (3.2)

**Table (3.2) Chemicals Materials**

| Chemical and biological material | Manufacturing Company/ Origin |
|---|---|
| 100bp DNA Ladder | BIONEER / Koria |
| Absolute ethanol (99%) | Merk / England |
| Agarose | Bio Basic INC/ Canada |
| Alpha-naphthol | BDH / England |
| Barium chloride ($BaCl_2$) | BDH / England |
| Deionized sterile distilled water | BIONEER / Koria |
| DNA Loading dye | Geneaid/ Thailand |
| DNA rehydration solution (TE) | Geneaid/ Thailand |
| Ethidium bromide dye | Bio Basic INC/ Canada |
| EDTA | BDH / England |
| Glucose | BDH / England |
| Glycerol | BDH / England |

All media listed in table 3.4 were prepared according to the manufacturing company instructions, pH was adjusted with 0.1N NaOH or 0.1N HCl, then sterilized with autoclaving at 121°C/1 pound for 15min. The prepared media were then distributed into sterile tubes or Petri dishes.

**Table (3.5) DNA extraction kit specifically designed for colonies was used.**

| NO. | Material | Origin |
|---|---|---|
| 1 | Spin Columns | gSYNC™ DNA Extraction Kit /Geneaid Republic of Korea |
| 2 | Collection Tubes Buffer BL | |
| 3 | Buffer WA | |
| 4 | Buffer BL Buffer WB | |
| 5 | Buffer CE | |
| 6 | Buffer CL | |
| 7 | Buffer WB | |
| 8 | RNase A (Lyophilized) | |
| 9 | Proteinase K (Lyophilized) | |

## 3.5. Laboratory Prepared Media

### 3.5.1. Yersinia Selective Agar (CIN agar).

Ninety grams were suspended in 500 mL of distilled water, the medium was heated to boiling to ensure complete dissolving, then serilization was performed by autoclaving at 15 lbs pressure (121°C) for 15 minutes, After cooling to 45–50°C, 10 mL of a sterilized aqueous solution containing 4 mg of Cefsulodin and 2.5 mg of Novobiocin was aseptically incorporated

and the mixture was thoroughly mixed before being poured into sterile Petri plates (Benson, 2001).

### 3.5.2. Yersinia Selective broth (CIN broth).

40.2 grams were suspended in 1000 mL of distilled water. The medium was heated if necessary to ensure complete dissolution. Sterilization was carried out by autoclaving at 15 lbs pressure (121°C) for 15 minutes. After cooling to 45–50°C, the rehydrated contents of 1 vial of *Yersinia* Selective Supplement (FD286) were aseptically added. The mixture was mixed well before being poured into sterile Petri plates **(Collee *et al.*, 1996)**.

### 3.5.3. Stains, Reagents, Solutions and Emulsions used in the identification of bacterial isolates

#### 3.5.3.1. Gram Stain Kit

It consists of: Crystal violet solution, Lugol Iodine, Alcohol Acetone solution and Basic Fuchsine solution (This solution was diluted 1/5 before used) for detection of shape and arrangement of bacterial isolates **(Benson, 2001).**

#### 3.5.3.2. Catalase reagent

Hydrogen peroxide ($H_2O_2$) 3% was prepared for detecting catalase production **(Benson, 2001)**.

#### 3.5.3.3. Oxidase reagent

By dissolving 1gm of N,N,N,N-tetramethyl *p*- phenylene diamine hydrochloride in 100 ml of distilled water (1% solution of N,N,N,N-tetramethyl *p*- phenylene diamine hydrochloride) was prepared, stored in dark bottle and used immediately to detect the ability of bacteria to produce oxidase enzyme **(Vandepitte *et al.*, 2003)**.

#### 3.5.3.4. McFarland standard solution

McFarland standard solution is the turbidity standard solution, which is the most widely used method for inocula preparation or standardization,

especially the McFarland No.0.5 standard solution which was prepared by adding 0.5 ml of 1% solution of barium chloride ($BaCl_2$) to 9.5 ml of 1% solution of sulphuric acid ($H_2SO_4$) (these amounts must be added to clean dry ampoules which should have the same diameters as the test tube to be used in subsequent density determinations) to obtain barium sulfate solution with the specific optical density to provide a turbidity comparable to that of bacterial suspension containing $1.5\times10^8$ CFU/ml **(Benson, 2001)**.

### 3.5.4. Materials used in Agarose Gel Electrophoresis

#### 3.5.4.1. Tris - Borate - EDTA (TBE) buffer

Tris - Borate - EDTA (TBE) buffer TBE buffer (10X) was supplied by (Bio Basic INC/Canada). Consisted of Tris base, boric acid and EDTA. The pH was adjusted to 8.3; the volume of buffer was completed to one liter with distilled water then 100 ml of TBE (10X) was added to 900 ml of D.W. to obtain 1X TBE solution used for agarose gel electrophoresis **(Benson, 2001)**.

#### 3.5.4.2. DNA Ladder (100 base pairs)

100 bp DNA ladder was specially obtained for determining the size of double stranded DNA from 100 to 1500 bp provided as a liquid by the supplier (BIONEER), Lambda DNA prepared in a concentration of 135 ng/µL in a storage buffer consists of: 10mM Tris-HCl (pH 8.0), 1mM EDTA, 2.5% Ficoll, 0.005% Bromophenol Blue and 0.005% Xylene Cyanol **(Benson, 2001)**.

#### 3.5.4.3. PCR Master mix preparation

It was provided by the supplier (BIONEER), the PCR mastermix consists of: 1U Top DNA polymerase, 250 µM Each: dNTP (dATP, dCTP, dGTP, dTTP), 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 1.5 mM MgCl2, stabilizer and tracking dye **(colle *et al.*, 1996)**.

# 3.6. Methods

## 3.6.1. Isolation and Identification of Yersiniae enterocoliticae

### 3.6.1.1. Samples'Collection

Two hundred samples of raw milk and egg, chicken and calf meat were obtained from local markets. shown in table 3.6.

**Table (3.6) samples types isolation**

| Sample types | No. of samples |
|---|---|
| Raw milk | 50 |
| eggs | 50 |
| Chicken meat | 50 |
| Calf meat | 50 |
| Total No. | 200 |

## 3.6.2. Yersinia Isolation from Milk Samples

One milliliter of each milk sample was pla ced (in triplets) in a sterilized Petri dish, then 15- 18 ml of the agar medium CIN agar were poured (the medium should be maintained melted at 44-46°C), the plates left to solidify at room temperature, and thereafter they were incubated at 37°C for 24-48 h. Because of its constituent of 7.5% NaCl; CIN agar is a selective medium so the isolates which can tolerate this salt concentration strictly could be isolated. Then the grown colonies were further investigated **(Benson, 2001)**.

### 3.6.2.1. Gram Stain

For all bacterial isolates, smears were prepared from 24 h culture colonies, and stained by Gram stain. Gram stainability, shape, size, and arrangement of cells were determined under light microscope.

### 3.6.2.2. Growth on CIN Agar

Cefsulodin-Irgasan-Novobiocin (CIN) agar plates were streaked with enriched samples or pure bacterial colonies and incubated at 30°C for 24–48 hours. This medium was used for selective isolation of *Yersinia* spp., containing cefsulodin, irgasan, and novobiocin to inhibit Gram-positive and competing Gram-negative bacteria.

### 3.6.2.3. Catalase test

One or two drops of catalase test reagent (3% $H_2O_2$) (which was prepared according to the item were placed on a slide; a growth from the center of a fresh pure colony from nutrient agar plate was mixed with the reagent on the slide. The formation of bubbles indicates a positive result **(collee *et al.*, 1996)**.

### 3.6.2.4. Oxidase test

A piece of filter paper placed in a clean Petri dish and 2-3 drops of freshly prepared oxidase reagent were added to the filter paper. A colony from tested organism was transferred to the filter paper and rubbed on to the reagent with a sterile wooden stick. The positive result was indicated by a violet or purple color appearance within (10-15) sec (**Benson, 2001**).

### 3.6.2.5. Urease Production

This test shows the bacterial ability to produce urease enzyme. The bacterial isolate was streaked on urea agar slant, and then incubated at 37°C for 18-24 h. The positive result was evidenced by the change in the medium color from deep orange to pink.

## 3.6.3.Antibiotic Susceptibility Test

The modified Kirby-Bauer method was used as the following:

## 3.6.4. Preparation of Mueller-Hinton Plates

Mueller-Hinton agar was prepared according to the manufacturer's instructions, then the medium was cooled to 45-50°C and poured into the

plates, allowed to set on a level surface to a depth of approximately 4mm. When the agar was solidified, the plates were stored at 4°C until use **(Benson, 2001)**

### 3.6.4.1. Inocula Preparation (Turbidity Standard)

To prepare the inocula, colonies from overnight culture of Yersiniae isolates were transferred to 5 ml tube of normal saline to obtain culture with $1.5\times10^8$ CFU/ml by adjusting to 0.5 McFarland standard.

### 3.6.4.2. Inoculation of the Test Plate

**A-** The plates were inoculated by dipping a sterile swab into the inocula; care must be taken to express excess broth from the swab prior to inoculation, by pressing and rotating the swab firmly against the side of the tube above the level of the fluid.

**B-** The swab was rubbed over the surface of the medium three times rotating the plate through at an angle of 60 after each application. Finally the swab was passed around the edge of agar surface.

**C-** The inocula were left for a few minutes to dry at room temperature with the lid is closed.

**D-** By using a sterile forceps, antibiotic discs were placed on the inoculated plate.

Discs should be warmed to room temperature, and then dispensed on the agar surface; they should gently pressed down with sterile forceps.

**E-** The plates were incubated within 30 min for 18-24 h at 37°C.

### 3.6.4.3. Reading the Results

After incubation, the diameters of the complete zone of inhibition were noted and measured in millimeters. The diameter of inhibition zone for individual antimicrobial agent was translated in terms of sensitive,

1. GT buffer (200 µL) was added to the supernatant by vigorous shaking then incubated at room temperature for 5min.
2. GB buffer (200 µL) was added by shaking vigorously for 5sec then incubated at 70˚C for 10min. during incubation the tubes were inverted every 3min.
3. Absolute ethanol (200 µL) was added and mixed by shaking vigorously, then transferred to a mini column and centrifuged at 13000 rpm for 2min.
4. The collection tube was discarded and placed the GD column in a new one.
5. W1 buffer (400µL) was added then centrifuged at 13000 rpm for 30 sec and the flow-through was discarded.
6. Washing buffer (600µL) were added, centrifuged at 13000 rpm for 30 sec and the flow-through were discarded.
7. Washing buffer (400µL) were added, centrifuged at 13000 rpm for 30 sec and the flow-through were discarded.
8. To dry the column matrix, GD column centrifuged at 13000 rpm for 3min.
9. Elution buffer (50µL), previously incubated at 70˚C for 10min, was added to GD column then transferred to a clean microfuge tube and let stand for 3-5 min. then centrifuged at 13000 rpm for 30 sec.
10. The DNA was stored in a deep freezer until PCR analysis is carried out.

### 3.6.7. Estimation of DNA Concentration and Purity (Stephenson, 2003)

The DNA concentration was determined by using spectrophotometer; 5 µL of each DNA sample were added to 995 µL of distilled water and mixed

well. Spectrophotometer was used for measuring the optical density (O.D.) at wave length of 260 nm and 280 nm. An O.D of one corresponds to approximately 50µg/ml for double stranded DNA. The concentration of DNA was calculated according to the formula:

**DNA concentration (µg/ml) = O.D 260nm × 50 × dilution factor**

Spectrophotometer was used also to estimate the purity ratio of DNA according to the following formula:

**DNA purity = O.D 260nm / O.D 280nm**

The ratio used for detecting DNA contamination with protein preparation. DNA quality could be assessed by 0.8% agarose gel electrophoresis **(Sambrook *et al.*, 1989)**.

### 3.6.8. Agarose Gel Preparation and Electrophoresis

Agarose gel was prepared in 0.8 % concentration for quality of the extracted DNA, by dissolving 0.8 gm of agarose powder in 100 ml of 1X TBE buffer, and melted, then the agarose gel was cooled to 50-60°C, 5 µL of ethidium bromide dye was added with mixing, agarose was poured out into the gel jar to prevent bubble formation, then cooled to 20°C. When agarose gel was poured, several wells were carefully made with a comb at one side of the gel about 5-10 mm away from the end of gel; after final solidification. The comb was carefully removed; the jar was put in the electrophoresis tank **(Manikandan *et al.*, 2011).**

Six microliters of the 1 kb DNA ladder were placed in the first left well of the agarose electrophoresis gel. Before loading to the gel wells; DNA samples were first mixed with a loading dye, so that 7 µL of each DNA sample were mixed with 3 µL loading dye, then this 10 µL of loaded DNA were carefully transferred to a well of the agarose electrophoresis gel, then

the electrophoresis tank closed with its special lid, and electric current was matched (70 volt for 1 h) **(Dhar *et al.*, 2009)** .

### 3.6.9. Polymerase Chain Reaction (PCR) Technique

The polymerase chain reaction (PCR) is an *in vitro* amplification of target DNA with a pair of primers and a DNA polymerase, resulting in several million fold amplification of the target sequence within few hours **(Collins *et al.*, 2009)**.

PCR assay was performed in a monoplex patterns in order to amplify different fragments of genes under study in a single tube for detecting *yesiniae enterocloitica* (*16SRNA*) and virulence associated genes (virulence factors: YIS).

#### 3.6.9.1. Primers selection

The primers listed in table 3.8 were selected for this study; these primers were provided in a lyophilized form, dissolved in sterile distilled water to give a final concentration of 100 pmol/ µL as recommended by provider and stored in deep freezer until used in PCR amplification.

**Table (3-8) The primers and their sequences used in conventional PCR for detection of *Yersiniae enterocoliticae***

| Gene | 5-3 prime | lengt h | PCR produc t | References |
|---|---|---|---|---|

### 3.7.1. Bacterial Culture Standardization

A pure culture of *Yersinia enterocolitica* was incubated in nutrient broth at 34°C for 24 hours. Following incubation, the bacterial suspension was centrifuged at 3,000 × g for 5 minutes to pellet the cells. The resulting pellet was then resuspended in sterile physiological saline. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, corresponding to an approximate concentration of $1.5 \times 10^8$ CFU/mL. This standardized inoculum was subsequently used for artificial contamination of food samples (**Giuliano *et al.*, 2019**).

### 3.7.2. Sample Contamination

To assess the survival of *Y. enterocolitica* in different food matrices, various samples were artificially contaminated using the standardized bacterial suspension.

For the milk samples, 10 mL of sterile raw milk was transferred into sterile tubes and inoculated with 100 µL of the standardized bacterial suspension. This achieved a final concentration of approximately $10^6$ CFU/mL (**Taye *et al.*, 2019**).

For the calf and chicken samples meal, 10 g of each meat type was aseptically cut into small cubes. The diced samples were then immersed in 10 mL of the bacterial suspension and allowed to stand for 15 minutes at room temperature to facilitate surface adherence of the bacteria. Excess liquid was subsequently drained under aseptic conditions.

For eggshell contamination, fresh and intact eggs were immersed completely in the bacterial suspension for a duration of one minute. The eggs were then carefully placed inside a laminar airflow cabinet and allowed to air dry to ensure uniform bacterial

attachment to the shell surface (**Pesavento *et al.*, 2017**).

### 3.7.3. Heat Treatment of Contaminated Samples

All samples were divided into three treatment groups. The first group was subjected to a water bath treatment at 60°C for 30 minutes, while the second group was heated to 70°C for 15 minutes under the same conditions. Control samples that did not receive heat treatment were kept at 4°C throughout the experimental period to prevent bacterial growth or die-off. All treatments were performed in sterile, closed containers to minimize external contamination

### 3.7.4. Ultraviolet (UV) Treatment

Ultraviolet inactivation was performed using a UV-C lamp operating at a wavelength of 254 nm. Contaminated samples were placed at a fixed distance of 30 cm from the UV light source on sterile trays. Three subgroups were exposed to UV radiation for different durations: 10 minutes, 15 minutes, and 20 minutes, respectively. After UV exposure, all samples were immediately transferred to a dark container and stored in the absence of light to prevent photoreactivation of bacterial DNA

### 3.7.5. Microbiological Analysis

To recover *Y. enterocolitica*, homogenization of the treated samples was performed. For milk, 1 mL of the treated sample was transferred into a sterile bag. For meat and eggshells, 10 g of each sample was added to 90 mL of buffered peptone water (BPW). Homogenization was carried out using a stomacher for 2 minutes at medium speed to ensure even distribution of the bacteria in the diluent.

Following homogenization, 100 µL of each sample homogenate

was streaked in duplicate onto selective Cefsulodin-Irgasan-Novobiocin (CIN) agar plates using a sterile glass spreader. The plates were incubated aerobically at 34°C for 48 hours.

### 3.7.6. Assessment of Bacterial Viability

After incubation, plates were examined for the presence of *Y. enterocolitica* based on colony morphology. Colonies exhibiting a typical bull's-eye appearance, characterized by a deep red center surrounded by a clear, transparent colony, were considered indicative of viable *Y. enterocolitica*. The absence of such colonies was interpreted as complete bacterial inactivation under the respective treatment condition. Duplicate plates were examined to ensure reproducibility and accuracy of results.

### 3.7.7. Statistical Analysis

Experimental data were presented in terms of observed numbers and percentage frequencies, SPSS (Statistical Package for Social Sience) program version 20 was used and some of these data analysed by SAS program (2004)-LSD (Least significant difference) was used to the significant compare difference. Regarding other data were analyzed by Chi-square test: P values $\leq$ 0.05 or $\leq$ 0.01 were considered statistically significant.

# Chapter Four

# Results and Discussion

reservoir for *Y. enterocolitica* due to contamination during milking or inadequate storage conditions,

The highest contamination rate was observed in raw milk samples, where *Y. enterocolitica* was detected in 34% of the samples. This high rate may be attributed to poor hygiene during milking and the absence of pasteurization processes, which are known to significantly reduce microbial loads **(Rahimi *et al.*, 2012; El-Tawab *et al.*, 2020).** Chicken samples showed the second-highest prevalence (20%), which aligns with previous studies suggesting poultry as a significant reservoir for *Y. enterocolitica* due to cross-contamination during slaughter or improper handling during processing **(Niskanen & Nurmi, 2003; Bonardi *et al.*, 2013).**

Calf samples showed a 14% contamination rate. Although lower than poultry, this still indicates potential risks, especially if meat is undercooked or mishandled. Previous research has reported similar levels of contamination in calf products in various regions **(Fredriksson-Ahomaa *et al.*, 2007; Wang *et al.*, 2021)**.

Egg samples exhibited the lowest prevalence, with only 2% testing positive for *Y. enterocolitica*. This low incidence may be due to the protective nature of the eggshell and lower probability of contamination compared to meat and dairy products **(Singh *et al.*, 2020)**.

In contrast, a considerably lower prevalence was observed in egg samples (2%). This aligns with earlier studies that have demonstrated eggs are not a primary vehicle for *Y. enterocolitica*, possibly due to the natural barriers provided by eggshells and antimicrobial properties of albumin **(Fredriksson-Ahomaa *et al.*, 2011)**.

However, disagreement exists in the literature, as some research has reported slightly higher rates of contamination in egg products under poor handling conditions **(Rahimi *et al.*, 2012)**.

A moderate prevalence in calf (14%) and chicken (20%) samples was detected. These results may be attributed to the contamination of meat during processing in sloghuter house or don't used the alternative procedures for treatment of caracses of calf or chicken meat ,These findings agree with those of **Sabina *et al.,* (2011)**, who reported similar isolation rates in red and white meats, emphasizing the role of cross-contamination and slaughterhouse hygiene in influencing bacterial load. However, other studies from regions with stricter hygienic controls have reported lower prevalence rates **(Hussain *et al.,* 2020)**, indicating that geographic, environmental, and procedural differences significantly affect contamination levels.

The statistically significant variation among different food types in the current study ($\chi^2 = 18.39$; $p < 0.05$) further supports the hypothesis that specific food matrices pose varying risks for *Y. enterocolitica* contamination. This observation is supported by the findings of **Tan *et al.,* (2014)**, who emphasized the need for targeted surveillance based on food category risk assessment.

Given that this study was conducted in Karbala city, the results provide valuable localized data that contribute to the global understanding of foodborne pathogens. The high prevalence in raw milk underscores the need for better control measures in dairy handling and consumer awareness in the region.

### 4.1.2. Cultural and Microscopical Characteristics

The results of microbial culture on CIN (Cefsulodin-Irgasan-Novobiocin) agar, *Yersinia enterocolitica* formed characteristic colonies after incubation at 25–30°C for 24–48 hours. The colonies appeared as small, round, and smooth with a deep red center and a clear, colorless peripheral zone, giving them a distinctive "bull's-eye" appearance. This morphology is due to mannitol fermentation and the resulting drop in pH,

which causes the pH indicator (neutral red) in the medium to change color, these colony features help differentiate *Y. enterocolitica* from other enteric bacteria, which typically either do not grow well or do not produce the same bull's-eye morphology on CIN agar (figure 4.1).

**Figure (4.1) Cefsulodin-Irgasan-Novobiocin for detection of *Yersinia enterocolitica***

On CIN (Cefsulodin-Irgasan-Novobiocin) agar, the organism produced characteristic colonies after incubation at 25–30°C for 24–48 hours, which is in line with previously published reports. The selective and differential nature of CIN agar has been widely recognized for its ability to support the growth of *Y. enterocolitica* while inhibiting competing flora, often resulting in colonies with a distinctive "bull's-eye" appearance **(Bottone, 1999; Gasanov *et al.*, 2005)**. The incubation temperature used in this study also aligns with the psychrotrophic nature of the organism, as *Y. enterocolitica*

The microscopical examination of *Yersinia enterocolitica* following Gram staining revealed coccoid rods arranged in pairs or in chains of varying lengths, with a clear Gram-negative reaction. These findings are consistent with the morphological features commonly attributed to *Y. enterocolitica*, which has been described as a short, pleomorphic, Gram-negative bacillus **(Ioannou, 2021)**.

The observed arrangements, including pairing and chaining, have also been previously reported and are considered typical under certain growth conditions, particularly in early-phase cultures **(Martrenchar and Samargandi, 2024)**.

The Gram-negative nature of the organism was further supported by the results of the potassium hydroxide (KOH) test, which yielded a positive reaction characterized by the formation of a viscous thread. This test result confirms the presence of a thin peptidoglycan layer, a hallmark of Gram-negative bacteria **(Jay *et al.*, 2005)**.

The utility of the KOH test as a rapid and reliable alternative for Gram differentiation has been widely accepted, particularly when used in conjunction with traditional Gram staining **(Faller & Schleifer, 1981)**. Thus, the results obtained in this study are in agreement with previously validated diagnostic protocols.

However, it has been suggested that the accuracy of the KOH test may be influenced by the age of the culture and environmental conditions, potentially leading to misinterpretation in mixed or older samples **(Beveridge, 1990)**. Despite this, in the current study, both Gram stain and KOH test outcomes were consistent and complementary, supporting the identification of *Y. enterocolitica* as a Gram-negative organism.

formation of gas bubbles upon the addition of hydrogen peroxide. This enzymatic activity is a well-documented trait of *Y. enterocolitica*, which possesses catalase as part of its defense mechanism against oxidative stress **(Singh & Ramteke, 2012)**. The presence of catalase has been considered a typical biochemical feature that aids in distinguishing *Yersinia* species from certain other enteric bacteria **(Kilian, 2001)**.

Conversely, the isolates were found to be oxidase-negative, which aligns with the known characteristics of the genus *Yersinia*. The absence of cytochrome c oxidase activity, demonstrated by the lack of color change on oxidase reagent-treated filter paper, supports previous reports that *Y. enterocolitica* consistently yields negative results in oxidase tests **(Janda & Abbott, 2006)**. This trait is of diagnostic importance, especially in differentiating *Yersinia* from other oxidase-positive Gram-negative rods such as *Pseudomonas* or *Aeromonas* **(Koneman *et al.*, 2005)**.

These biochemical reactions—positive catalase and negative oxidase—conform to the standard profiles described in differential identification schemes and are particularly useful for preliminary classification in microbiological laboratories **(Leclercq *et al.*, 2013)**. However, it has been suggested that while these tests are valuable, they should not be solely relied upon for species-level confirmation due to potential variability among atypical strains or under suboptimal test conditions **(Tindall *et al.*, 2007)**.

### 4.1.4. Antibiotic Susceptibility Testing of *Yersinia enterocolitica* Isolates

The susceptibility of 35 *Yersinia enterocolitica* isolates to various antibiotics was evaluated using the disk diffusion method as described by the Clinical and Laboratory Standards Institute **(CLSI, 2018)** and represented in the results, summarized in Tables (4.3). and Figure (4.4).,

showed that 23 isolates (65.7%) were classified as Extensively Drug-Resistant (XDR)—defined as resistant to all tested antibiotics except one or two. The remaining 12 isolates (34.3%) exhibited Multi-Drug Resistance (MDR)—defined as resistance to at least one agent in three or more antimicrobial categories. This suggests that the MDR isolates likely belong to adapted endemic strains, while the XDR isolates may be associated with high-risk epidemic clones **(Dhar *et al.*, 2009)**. In this study, the antibiotic susceptibility of *Yersinia enterocolitica* isolates was assessed against 19 different antibiotics using the disk diffusion method.

The results demonstrated that the highest resistance rates were observed against Amoxicillin and Penicillin G, with 30 and 25 resistant isolates respectively. Spiramycin, Lincomycin, and Nitrofurantoin also showed considerable resistance, with more than 20 isolates exhibiting resistance to each. A moderate level of resistance was observed against Ciprofloxacin, Cephalexin, Gentamicin, Streptomycin, Tobramycin, and Erythromycin, where the number of resistant isolates ranged between 18 and 21. Resistance to Trimethoprim and Oxy-Tetracycline was relatively high, with 28 and 24 isolates showing resistance, respectively.

Chloramphenicol and Doxycycline displayed more favorable activity, as fewer isolates were resistant—17 and 5 respectively—with Doxycycline showing particularly strong efficacy. Notably, all isolates were found to be susceptible to FosBAC Plus, with no intermediate or resistant responses recorded, indicating complete sensitivity across all 35 isolates tested.

These results found that *Y. enterocolitica* exhibits varying degrees of resistance to commonly used antibiotics, with multidrug resistance being evident in a significant proportion of the isolates. The complete susceptibility to FosBAC Plus highlights its potential as an effective therapeutic option against *Y. enterocolitica* in foodborne cases (**Stock & Wiedemann, 2002**).

Spiramycin (SP) and lincomycin (L) also showed limited effectiveness, with resistance observed in 25 and 20 isolates respectively. These results correspond with previous reports suggesting that macrolides and lincosamides are generally less effective against Gram-negative enteric pathogens, including *Y. enterocolitica*, due to reduced outer membrane permeability and efflux mechanisms **(Alekshun & Levy, 2007)**.

In contrast, high susceptibility was observed with FosBAC Plus (a combination therapy), where 100% of the isolates (35/35) were susceptible. This strong efficacy may be attributed to the synergistic mechanism of the combination agents and supports the potential utility of combination therapies in treating resistant *Yersinia* strains **(Petrosillo *et al.*, 2005)**. Doxycycline (DO) also demonstrated a high susceptibility rate, with 28 isolates responding to treatment. This finding agrees with the recommendation of doxycycline as a first-line agent for systemic yersiniosis in human medicine **(Cover & Aber, 1989)**.

Moderate resistance was observed in isolates exposed to ciprofloxacin (CIP), cephalexin (C), and enrofloxacin (ENR), with resistance rates of 21, 19, and 21 isolates respectively. Although fluoroquinolones have generally been considered effective against *Y. enterocolitica*, emerging resistance—possibly due to overuse in both veterinary and human medicine—has been increasingly reported **(Gupta *et al.*, 2018)**. These observations highlight a need for judicious use of fluoroquinolones to preserve their therapeutic value.

Notably, high levels of resistance were also detected for neomycin (N), with 26 resistant isolates, and trimethoprim (SXT), with 28 resistant isolates. These results are consistent with studies that have documented the limited effectiveness of these agents against *Yersinia* spp. due to plasmid-encoded resistance mechanisms **(Toledo-Arana *et al.*, 2001)**.

Meanwhile, gentamicin (CN), streptomycin (S), and tobramycin (TOB) demonstrated moderate activity, with susceptibility observed in 13, 14, and 12 isolates, respectively. These findings suggest that aminoglycosides still retain some efficacy against *Y. enterocolitica*, although the presence of resistant strains may indicate emerging resistance trends **(Kania *et al.*, 2021)**.

### 4.1.5. Results of Molecular assay of *Y.enterocolitica*

Polymerase chain reaction (PCR) have been conducting to detect Yersinia enterocolitica in 200 food samples.

The results showed that 34 samples were positive, representing a 17% test rate. This technique relied on detecting two specific genes: the 16S rRNA gene, used for general identification of the bacteria, with the resulting band appearing at 320 base pairs, and the virulence gene yst, responsible for producing an enterotoxin, with its band appearing at 145 base pairs. These results were visually documented through gel electrophoresis, as shown in the figure (4.4).

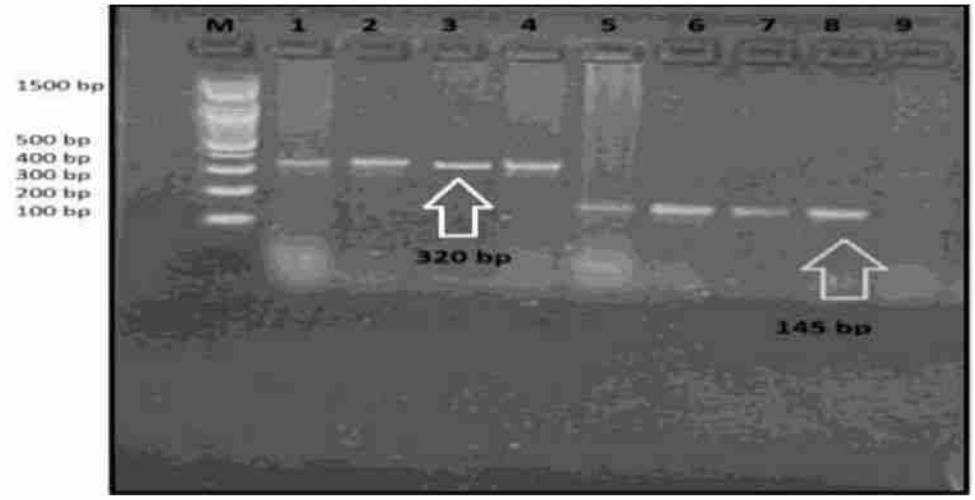


**Figure (4.4) Eectrophoresis gel for detection of *Y. enterocolitica*; M DNA ladder marker; 1-4 wells represented 16SRNA as 320 bp and 5-8 wells represented Yst gene as 145 bp and 9 well represented control negative sample.**

The use of polymerase chain reaction (PCR) in this study enabled the sensitive and specific detection of *Yersinia enterocolitica* in 200 food product samples, with 34 (17%) samples testing positive. These findings affirm the value of PCR-based assays as a rapid and reliable method for identifying foodborne pathogens, particularly in samples with complex microbial communities or low bacterial loads **(Almeida *et al.*, 2021)**.

The amplification of the 16S rRNA gene fragment, yielding a band at 320 base pairs, provided a general molecular confirmation of the presence of *Yersinia* species. This gene has been widely used in bacterial diagnostics due to its conserved nature across prokaryotes, and its amplification in this study supports previous assertions regarding its suitability for genus-level identification **(Wang *et al.*, 2023)**. However, it has been emphasized that while 16S rRNA can confirm the presence of *Yersinia*, species-level identification and pathogenicity assessment require additional virulence markers **(Chen *et al.*, 2022)**.

The virulence gene *yst*, associated with enterotoxin production, was also successfully amplified in the positive samples, with the expected band appearing at 145 base pairs. The detection of *yst* is of clinical and public health importance, as this gene encodes for a heat-stable enterotoxin (YstA), which contributes to diarrheal symptoms in infected individuals **(Reed *et al.*, 2020)**. The simultaneous detection of *yst* alongside 16S rRNA suggests that a significant proportion of the positive isolates possess pathogenic potential.

These results are in agreement with studies that have reported the *yst* gene as a reliable molecular marker for identifying enteropathogenic *Y. enterocolitica* strains in food and clinical samples **(Park *et al.*, 2021)**. Moreover, the prevalence rate (17%) observed using PCR was notably higher than that obtained by conventional culture methods in some previous studies, underscoring the superior sensitivity of molecular

detection techniques **(Niu *et al.*, 2022)**. This disparity may be attributed to the presence of viable but non-culturable (VBNC) bacteria, which escape detection by routine culturing yet are identifiable via molecular methods **(Zhao *et al.*, 2023)**.

The visualization of PCR products through gel electrophoresis confirmed the specificity of amplification, and the absence of non-specific bands reflects the precision of the primers and optimized PCR conditions used. These observations are consistent with findings by recent molecular surveillance efforts that have emphasized the importance of primer design and validation in foodborne pathogen detection **(Das *et al.*, 2020)**.

Although the PCR assay provided rapid and accurate results, it is acknowledged that the technique detects DNA regardless of bacterial viability. As such, further use of quantitative PCR (qPCR) or reverse-transcription PCR (RT-PCR) could enhance result interpretation by distinguishing between viable pathogens and residual DNA from dead cells **(Shi *et al.*, 2022)**.

### 4.1.6. Comparison between molecular and culture assay for detection of *Y. enterocolitica*

The results of a comparison between bacterial culture and PCR for the diagnosis of Yersinia enterocolitica were showed a sensitivity of 88.57%, indicating a high ability of the method to detect positive cases. The confidence interval for the sensitivity ranged between 73.26% and 96.80%, reflecting an acceptable degree of reliability in estimating this diagnostic indicator. The specificity of the test was recorded at 98.18%, a high percentage indicating the accuracy of the method in excluding negative cases.

The confidence interval for this value was between 94.78% and 99.62%, enhancing the reliability of the results obtained. The positive predictive

value (PPV) was also calculated and reached 91.18%, indicating that the majority of positive results were correct. The confidence interval for the PPV ranged between 76.99% and 96.96%. The negative predictive value (NPV) reached 97.59%, indicating high effectiveness in ensuring the absence of infection in cases with negative outcomes. The confidence interval for this value ranged between 94.15% and 99.03% table (4.4).

**Table (4.4) Comparsion between Culture and PCR assay for detection of *Yersinia enterocolitica***

| PCR Test Result | Disease Present (by Gold Standard) | Disease Absent (by Gold Standard) | Total |
|---|---|---|---|
| Positive | True Positive = 31 | False Positive = 3 | 34 |
| Negative | False Negative = 4 | True Negative = 162 | 166 |
| Total | 35 | 165 | 200 |

The diagnostic performance of the polymerase chain reaction (PCR) assay was evaluated by comparing its results with those of conventional culture, regarded here as the gold standard. PCR correctly identified 31 true-positive cases, missed 4 false negatives, and produced 3 false-positive results. These findings correspond to a sensitivity of 88.57% and a specificity of 98.18%, indicating a high diagnostic accuracy.

The sensitivity value observed in this study suggests that PCR was able to detect the majority of *Yersinia enterocolitica* infections confirmed by culture. This high sensitivity aligns with previous findings where PCR assays targeting virulence or species-specific genes demonstrated superior detection capabilities, especially in samples with low bacterial counts or

under stress conditions that reduce culturability **(Patel *et al.*, 2020)**. The few false negatives may be attributed to DNA degradation, PCR inhibitors in food matrices, or mutations in primer-binding sites **(Kim *et al.*, 2021)**.

The specificity of 98.18% further confirms the robustness of the PCR assay, reflecting a low rate of false positives. This result is consistent with previous studies that validated the use of PCR for the specific detection of *Y. enterocolitica*, particularly when primers were designed to avoid cross-reactivity with closely related species **(Ali *et al.*, 2024)**. However, the occurrence of 3 false-positive results suggests that molecular methods can detect non-viable organisms or residual DNA, which may not indicate active infection **(Liu *et al.*, 2020)**. This limitation has been recognized in molecular diagnostics, particularly in food safety surveillance where DNA may persist even after microbial death.

Compared to culture, PCR provided faster results and required less time to confirm the presence of the pathogen. These advantages have been widely reported, as culture methods, while considered definitive, may fail due to the viable but non-culturable (VBNC) state or competition from background flora **(de Boer *et al.*, 2022)**. PCR, on the other hand, remains effective under such conditions, making it a valuable tool in both routine and outbreak investigations.

The diagnostic values obtained in this study support the implementation of PCR as a complementary or alternative method to culture, particularly for rapid screening purposes. However, it has been recommended that molecular results be interpreted cautiously and, when possible, confirmed by additional phenotypic or quantitative methods to enhance diagnostic certainty **(Jang *et al.*, 2025)**.

### 4.1.7. Sequencings study of *Y. enterocolitica*

The PCR-amplified product of the 16S rRNA gene from selected *Yersinia enterocolitica* isolates was subjected to nucleotide sequencing. The resulting sequences were analyzed and compared against known sequences in the NCBI GenBank database using the BLAST (Basic Local Alignment Search Tool) algorithm. The analysis revealed a high degree of similarity (≥99%) with the reference sequence of *Y. enterocolitica*, thereby confirming the molecular identity of the isolates at the species level. The sequence data corresponded to conserved regions of the 16S rRNA gene

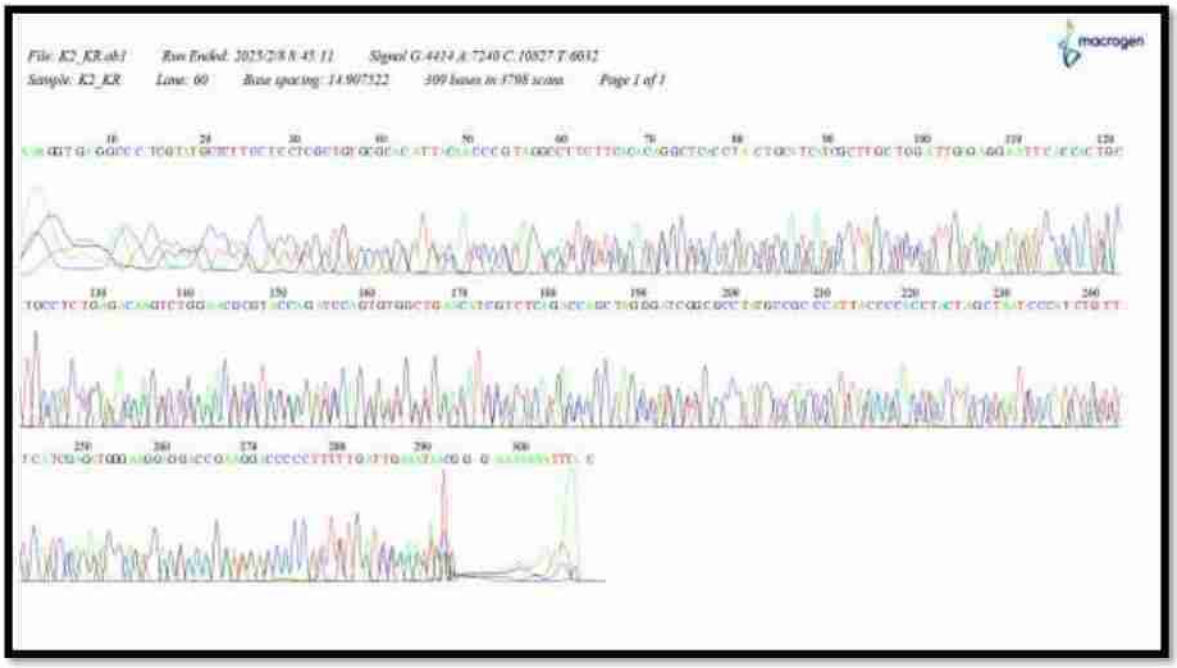


**Figure (4.5) Nucleotides sequences of 16SRNA isolated from *Y. enterocolitica***

The sequencing of the 16S rRNA gene provided definitive molecular confirmation of the identity of the bacterial isolates as *Yersinia enterocolitica*. This approach is widely regarded as a reliable and accurate method for species-level identification of bacterial pathogens, particularly in cases where phenotypic methods yield ambiguous results **(Martinez-**

**Porchas *et al.*, 2020)**. The high sequence similarity with published *Y. enterocolitica* 16S rRNA sequences corroborates the PCR findings and supports the specificity of the primers used in the initial amplification.

The use of 16S rRNA gene sequencing has been extensively validated for taxonomic classification due to the gene's conserved and hypervariable regions, which enable discrimination among closely related bacterial species **(Yarza *et al.*, 2022)**. In the present study, the conserved nature of the sequenced regions further confirms the accuracy of the species assignment. These findings are in agreement with the work of **Rawat *et al.*, (2021)**, who demonstrated that sequencing of 16S rRNA provided a robust framework for identifying *Yersinia* species from environmental and food matrices.

The absence of mutations or unexpected sequence variations in the amplified region suggests genetic stability in the conserved domain of the 16S rRNA gene among the studied isolates. This observation is consistent with reports indicating that the core regions of the 16S rRNA gene in *Y. enterocolitica* remain highly conserved across strains isolated from different sources ***(Chakraborty et al., 2021)***.

However, it has been noted that while 16S rRNA sequencing is effective for species-level identification, it may lack the discriminatory power needed to distinguish between certain subspecies or biotypes of *Y. enterocolitica* **(Wang *et al.*, 2023)**. For epidemiological studies or pathogenicity assessments, further sequencing of virulence-associated or housekeeping genes (e.g., *ail*, *yst*, or *inv*) may be recommended **(Zheng *et al.*, 2022)**.

## 4.2. Results of Experimental study:

### 4.2.1. UV exposure of bacterial isolates from calf meat, chicken, eggs surfaces


**Figure (4.7) 15 minutes UV exposure to *Yersinia enterocolitica***

Complete inhibition of *Y. enterocolitica* growth was observed following 20 minutes of UV-C exposure. No colonies were detected on any of the CIN agar plates inoculated from calf, chicken, or eggshell samples treated for this duration, indicating full inactivation of the bacteria under these conditions (4.8).

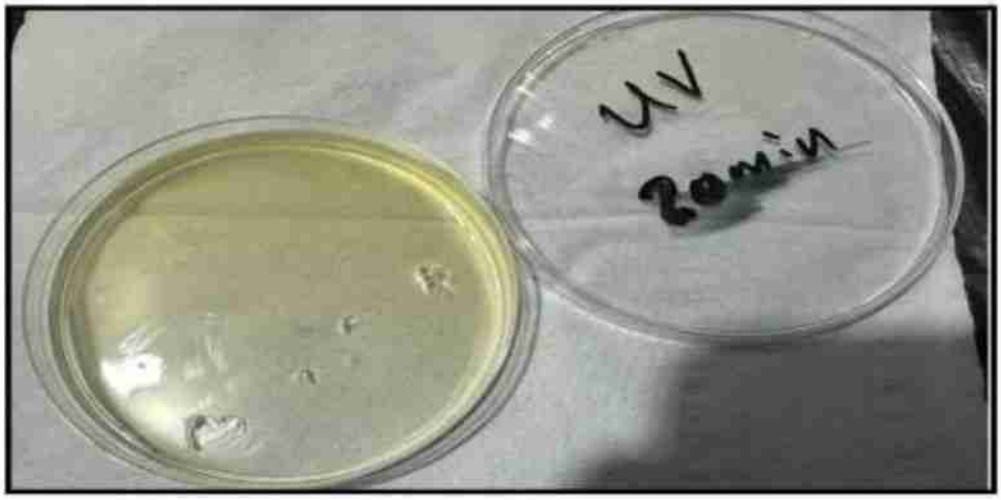


**Figure (4.8) 20 minutes UV exposure to *Yersinia enterocolitica***

The results demonstrated a time-dependent bactericidal effect of UV-C light on *Yersinia enterocolitica* present on calf, chicken, and eggshell surfaces. The persistence of heavy bacterial growth following 10 minutes

of exposure suggests that this duration was insufficient to induce significant DNA damage or membrane disruption in the bacterial cells, which is consistent with earlier findings by **Guerrero-Beltrán and Barbosa-Cánovas (2004)**, who reported that short UV-C exposures often result in only partial microbial inactivation.

A reduction in colony numbers observed after 15 minutes of treatment indicates that longer UV-C exposure exerted a measurable stress on the bacterial cells, likely through cumulative DNA damage and oxidative effects. This partial inactivation is in agreement with studies such as that of **Keklik *et al.*, (2012)**, which demonstrated that UV-C treatment can reduce bacterial load on meat surfaces when applied for moderate durations.

The absence of any viable colonies following 20 minutes of UV-C exposure confirms the effectiveness of prolonged UV-C treatment in completely inactivating *Y. enterocolitica*. It has been suggested that exposure beyond 15 minutes leads to the formation of lethal pyrimidine dimers in bacterial DNA and irreversible cell damage **(Gayán *et al.*, 2015)**. Furthermore, the use of UV-C light at 254 nm has been proven to penetrate thin biofilms and reach exposed bacterial cells, making it an effective non-thermal sanitization method for surface decontamination in food processing environments **(Yaun *et al.*, 2003)**.

These findings emphasize the potential of UV-C treatment as a practical intervention method for reducing or eliminating *Y. enterocolitica* contamination in food products of animal origin, particularly when adequate exposure time is ensured.

### 4.2.2. Thermal methods of bacterial isolates from meat (calf and chicken), eggs shells

Following thermal treatment of contaminated calf, chicken, and eggshell samples, differences in bacterial viability were observed based on

temperature and exposure time. In samples treated at 60°C for 30 minutes, growth of *Yersinia enterocolitica* was still observed. Characteristic bull's-eye colonies developed on CIN agar, indicating the survival of viable bacterial cells across all tested food matrices figure (4.9).


**Figure (4.9) Heat exposure to *Yersinia enterocolitica* colonies in 60 $^{O}$C for 30 minutes**

Conversely, in samples treated at 70°C for 15 minutes, *Y. enterocolitica* was completely inactivated. No growth was detected on any of the inoculated plates for calf, chicken, or eggshells. The absence of colonies confirmed that this treatment condition was sufficient to eliminate viable bacterial cells under the experimental settings used figure (4.10).

The thermal resistance of *Yersinia enterocolitica* observed at 60°C highlights the organism's ability to withstand sublethal heat stress, particularly when embedded in protective food matrices such as meat and eggshells. It has been reported that this pathogen can survive moderate heat treatments due to the presence of stress response systems, including heat-shock proteins and membrane adaptation mechanisms that are activated at temperatures below its critical threshold **(Kastner *et al.*, 2021)**.

The complete absence of growth following exposure to 70°C for 15 minutes suggests that this temperature is sufficient to induce irreversible cellular damage and protein denaturation, resulting in bacterial death. Similar results were reported by **Barba *et al.*, (2022)**, who demonstrated that heating at temperatures above 65°C effectively disrupts the integrity of bacterial membranes and nucleic acids, especially in gram-negative pathogens such as *Yersinia*.

The disparity between the two treatments underscores the importance of using precise thermal parameters in food safety practices. Sublethal heating, such as at 60°C, may reduce but not fully eliminate bacterial populations, potentially allowing for post-treatment regrowth if storage conditions are favorable **(Milani *et al.*, 2020).** In contrast, the lethal effect observed at 70°C supports the current recommendations for proper cooking and pasteurization standards aimed at eliminating enteric pathogens in animal-derived foods **(Nowak *et al.*, 2023).**

Moreover, the food matrix itself may play a protective role by insulating bacterial cells from direct heat exposure. Fat and protein content in meat, for instance, can influence thermal conductivity and reduce heat penetration, thereby requiring more rigorous heating conditions to ensure full bacterial inactivation **(Zhao *et al.*, 2021).**

The results of this study confirm that while 60°C for 30 minutes may reduce bacterial load, a treatment of 70°C for 15 minutes is required to

ensure complete decontamination of *Yersinia enterocolitica* in calf, chicken meat, and eggshells.

# Chapter Five
# Conclusions and Recommendations

# 5. Conclusions and Recommendations

## 5.1. Conclusions

1. *Yersinia enterocolitica* was successfully isolated from artificially contaminated calf, chicken, and eggshells, demonstrating its ability to persist across various animal-derived food matrices.
2. The highest isolation rate of Yersinia enterocolitica was recorded in raw milk production rather than calf, chicken, and eggshells.
3. Exposure to UV-C light for 10 minutes was found to be insufficient, as all tested samples exhibited heavy bacterial growth, indicating limited inactivation at this duration.
4. Heat treatment at 60°C for 30 minutes failed to eliminate the pathogen from all tested samples, suggesting that this temperature is inadequate for complete microbial inactivation.
5. No bacterial growth was observed following thermal exposure at 70°C for 15 minutes, indicating that this condition is sufficient to achieve full destruction of *Y. enterocolitica* in meat and eggshell surfaces.
6. The complete susceptibility to FosBAC Plus highlights its potential as an effective therapeutic option against *Y. enterocolitica* in foodborne cases.

## 5.2. Recommendations

1- It is recommended that thermal treatment at 70°C for at least 15 minutes be implemented during the processing of animal-derived food products to ensure complete inactivation of *Yersinia enterocolitica*.

2-Short-duration UV-C exposure (10–15 minutes) should not be solely relied upon for microbial control, especially in foods with uneven surfaces or higher organic content, as partial bacterial survival has been observed, and to ensure maximum efficacy, UV-C exposure should be extended to at least 20 minutes, particularly when applied to high-risk food items such as raw meat and eggshells.

4-Regular microbiological monitoring should be incorporated into food safety programs to detect and control *Y. enterocolitica* contamination, especially in raw milk, meat, and poultry products.

5-it is recommended to identify *Y. enterocolitica* contamination in another food product.

6-It is advised that combined or hurdle approaches, such as UV-C treatment followed by mild heat or antimicrobial rinses, be explored to enhance decontamination efficiency without compromising food quality.

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## الخلاصة

أُجريت دراسة حديثة للكشف عن انتشار بكتيريا *Yersinia enterocolitica* في الحليب الخام، ولحوم العجول، ولحوم الدجاج، والبيض في أسواق محافظة كربلاء. جُمعت 200 عينة، 50 عينة من كل عينة، بطريقة معقمة، وأُرسلت إلى المختبر لعزلها وتحديد نوعها. أظهرت نتائج زراعة العينات على وسط CIN وجود مستعمرة مميزة لبكتيريا يرسينيا إنتيروكوليتيكا، وتبين من خلال صبغة غرام أن البكتيريا سلبية الغرام، مرتبة في أزواج أو سلاسل بأطوال مختلفة. أما نتائج الاختبارات البيوكيميائية لبكتيريا يرسينيا إنتيروكوليتيكا، فكانت إيجابية لإنزيمات الكاتالاز وهيدروكسيد البوتاسيوم، بينما سلبية لأوكسيديز تخمير اللاكتوز. تم توحيد مزرعة بكتيريا *Yersinia enterocolitica* لمدة ٢٤ ساعة عند درجة عكارة ٠.٥ ماكفارلاند. قطّعت عينات لحم البقر والدجاج بطريقة معقمة وغُمرت في المعلق البكتيري، بينما تم تلقيح قشور البيض الكاملة سطحيًا بالغمس لضمان التلوث. ثم قُسّمت العينات الملقحة إلى مجموعات تجريبية للتدخل المضاد للميكروبات. عولجت حراريًا باستخدام حمام مائي، مع تعريض العينات لدرجة حرارة ٦٠ درجة مئوية لمدة ٣٠ دقيقة و٧٠ درجة مئوية لمدة ١٥ دقيقة. تعرّضت مجموعات منفصلة لأشعة فوق البنفسجية-ج بطول موجي ٢٥٤ نانومتر لفترات تعريض ١٠ و١٥ و٢٠ دقيقة. بعد كل معالجة، جُنّست جميع العينات في محلول مُعادل، وخُفّفت بشكل متسلسل، ثم وُضعت على أجار سيفسولودين-إرجاسان-نوفوبيوسين (CIN). حُضّنت الأطباق عند درجة حرارة ٣٤ درجة مئوية لمدة ٤٨ ساعة. تم تقييم قابلية البكتيريا للحياة من خلال تعداد مستعمرات عين الثور المميزة، مع تسجيل النتائج كوجود أو غياب للنمو.

أظهرت النتائج أن أعلى معدلات مقاومة لوحظت ضد الأموكسيسيلين والبنسلين ج. كما لوحظت مقاومة كبيرة ضد سبيراميسين، ولينكومايسين، ونيتروفورانتوين. ولوحظ مستوى مقاومة متوسط لسيبروفلوكساسين، وسيفاليكسين، وجنتاميسين، وستربتوميسين، وتوبراميسين، وإريثروميسين. أظهرت النتائج أن 34 عينة تم تأكيدها إيجابية من خلال تحليل تفاعل البوليميراز المتسلسل (PCR)، مما يمثل معدل كشف بنسبة 17%. استند هذا التحديد إلى تضخيم جين S rRNA16، والذي استُخدم لتحديد البكتيريا على نطاق واسع. من ناحية أخرى، أظهرت النتائج أن قابلية البكتيريا للحياة لوحظت في جميع مصفوفات الأغذية بعد التعرض للأشعة فوق البنفسجية-C لمدة 10 دقائق، مع تكوين مستعمرات كبيرة مما يشير إلى انخفاض طفيف في الحمل البكتيري. تم تحقيق انخفاض جزئي في عدد الميكروبات بعد التعرض المطول لمدة 15 دقيقة، كما يتضح من الانخفاض الملحوظ في عدد المستعمرات؛ ومع ذلك، لم يتم تحقيق القضاء التام. لم يتم إثبات

جمهورية العراق

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جامعة كربلاء –كلية الطب البيطري

فرع الصحة العامة البيطرية

# الكشف عن وجود يرسينيا القولون في بعض المنتجات الغذائية في محافظة كربلاء وتقييم تأثير الطرق الحرارية والاشعة فوق البنفسجية على حيويتها

رسالة مقدمة الى
مجلس كلية الطب البيطري – جامعة كربلاء كجزء من
متطلبات نيل درجة الماجستير في الطب البيطري / فرع
الصحة العامة

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