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Investigations of Bacterial contamination with *Salmonella* species in local and Imported Chicken Meat in Kerbala Province

A Thesis

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University of Kerbala as a Partial fulfillment of the Requirement for the
Degree of Master in the Sciences of Veterinary Medicine in Public Health

BY

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بسم الله الرحمن الرحيم

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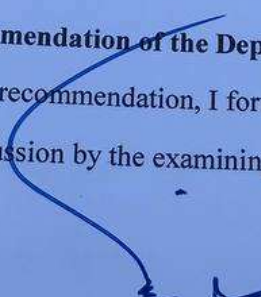


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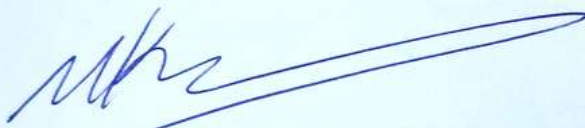
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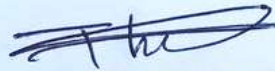
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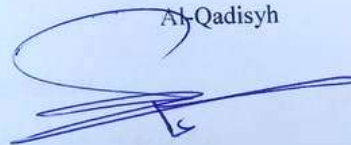


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Dedication

I dedicate this humble work ,first and foremost ,To **Allah** the Generous the Source of Light and Wisdom

To **His Prophet Mohammad**, peace and blessings be upon him, the Seal of the Messengers and Master of Creation, To his pure family, the stars of guidance and the lamps of darkness,

To **the soul of my beloved father** may Allah have mercy on him, who taught me that knowledge has value and hope has life, and I ask Allah to make this work a continuous charity in the balance of his good deeds.

To **my dear mother** the source of compassion and the secret of sincere supplication, who was and still is a warm embrace in all circumstances.

To **my dear husband** whose support and encouragement have been a constant source of motivation for me .i dedicate this work to you, thank you for your unwavering support and love.

To **my lovely daughters** my shining stars I dedicate this work may it be a source of inspiration and knowledge for you and may you always for your dreams.

Rawaa Hussein fakhry

/ / 2025

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I also thank my mother, my family, my brothers, and my sisters for taking care of me and supporting me throughout the study period, so may God reward them with the best reward on my behalf.

Summary

Summary

This study was conducted to investigate the prevalence of *Salmonella* in chicken meat .including two types of chicken meat , locally slaughteres chicken meat and imported chicken meat. The work started from September /2024 until January /2025.

Collection a total of 120 chicken meat samples were collected from various regions in Karbala City, The samples were divided into two categories with 60 samples locally chickens meat (n=30) and imported chicken meat (n=30), From each chicken, thigh and breast portions were aseptically removed using sterile scalpels and placed into individually labeled sterile bags. All samples were transported under refrigerated conditions (4°C) to the laboratory within 24 hours for subsequent analysis. A total 120 sample were analyzed using conventional culture techniques, biochemical identification and confirmation through the VITEK2system molecular identification was achieved using PCR targeting the 16-SRNAregion which yielded specific amplicon of 1400 bp in positive samples

The result of culture on (XLD agar)showed the highest percentage of *salmonella* contamination in locally slaughtered chicken meat (20%) while the lowest percentage of contamination in imported chicken meat(12%)

The bacteriological examination revealed that *Salmonella* isolates obtained from different broiler production sources belonged to four species, distributed as follows: *S. Typhimurium* (11 isolates, 52%), *S. enteritidis* (7 isolates, 33%), *S. Goodwood* (2 isolates, 10%), and *S. senftenberg* (1 isolate, 5)

Biochemical identification tests were also performed. The urease test showed no urea hydrolysis. In addition, *Salmonella* demonstrated gas production and hydrogen sulfide formation in the TSI test, while citrate utilization was confirmed

using Simmons citrate medium. The distribution of positive cases showed that the highest occurrence was recorded in the locally chicken meat more than imported chickens meat.

Molecular analysis yielded highly positive results, as a substantial proportion of the *Salmonella* isolates (16 isolates) carried the targeted genomic region, locally chickens meat was recorded 15% while the imported chicken meat was been 11.5%.

Antibiotic susceptibility testing showed that all isolates (100%) were resistant to ampicillin and tetracycline. A high level of chloramphenicol resistance was also recorded (90.47%). Moderate resistance was observed for ciprofloxacin (32.81%), while doxycycline resistance (52.39%) reflected inconsistent antibiotic use in poultry production. Resistance to nalidixic acid reached 62%, and amikacin resistance was 81%. Among the 21 isolates obtained.

The current study found the PCR assay demonstrated a sensitivity of true positive (TP) was= 16 ,while the false negative=5 .

The finding of this study highlight the widespread presence of *Salmonella enterica* in meat product available in kerbala local market .The bacterium demonstrated resilience to ability to lived at low to moderate temperatures suggesting their ability to survive even under poor food storage or processing condition .Molecular detection via polymerase chain reaction PCR provided rapid and accurate method for identification and the prevalence data underscore the need for improved hygiene and monitoring practices in food production and distribution system to mitigate public health risks.

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List of Abbreviations

Abbreviations	Meaning
AIDS	Immune Deficiency Syndrome
ATP	Adenosine triphosphate
BP	Base pair
FAO	Food and Agriculture Organization of the United Nations
LPS	Lipopolysaccharide
MIC	Minimum inhibitory concentration
µg/mL	microgram / mL
MLNs	the mesenteric lymph nodes
NTS	non-typhoidal Salmonella
PAHO	Pan American Health Organization
OIE	Organisation for concentration
RNA	ribosomal ribonucleic acid
WHO	World Health Organization
MIC	Minimum Inhibitory concentration

Chapter one

Introduction

Introduction:

Salmonella is a genus of Gram-negative, facultatively anaerobic, rod-shaped bacteria belonging to the family *Enterobacteriaceae*. These organisms are of critical concern globally due to their significant role in foodborne illnesses and zoonotic diseases. The genus *Salmonella* comprises two main species, *Salmonella enterica* and *Salmonella bongori*, with *S. enterica* being responsible for the vast majority of human and animal infections **(Billah & Rahman, 2024)**. With the growing incidence of antimicrobial resistance and the high prevalence of *Salmonella*-associated diseases in both developed and developing countries, understanding the biology, transmission, epidemiology, and public health implications of this pathogen has become increasingly important **(Lamichhane et al ., 2024)**.

The genus was named after the American veterinary pathologist Daniel Elmer Salmon, who first isolated the bacterium in the late 19th century, although it was his colleague Theobald Smith who actually discovered it. Since then, *Salmonella* has been extensively studied due to its pathogenic potential, diverse serotypes, and ability to adapt to a wide range of hosts and environments **(Ray, 2025)**. It is estimated that *Salmonella* causes millions of cases of gastroenteritis and tens of thousands of deaths each year, especially in regions with poor sanitation and inadequate food safety practices**(Majowicz et al ., 2010)**.

There are over 2600 known serovars (or serotypes) of *Salmonella*, which are classified based on their O (somatic) and H (flagellar) antigens. Among these, *Salmonella enterica* serovar *Typhi* (*S. Typhi*) and *S. Paratyphi* are responsible for causing typhoid and paratyphoid fever, respectively systemic illnesses that can be fatal without treatment. Other non-typhoidal *Salmonella* (NTS) serovars such as *S.*

Enteritidis, *S. Typhimurium*, and *S. Heidelberg* primarily cause self-limiting gastroenteritis but can lead to severe invasive infections, especially in immunocompromised individuals **(Eng et al ., 2015)**.

The epidemiology of *Salmonella* infection is complex and multifactorial. Humans can acquire the infection through ingestion of contaminated food or water, direct contact with infected animals, or through person-to-person transmission in certain settings. Common sources of *Salmonella* include raw or undercooked poultry, eggs, unpasteurized milk, and contaminated produce. In developing countries, poor hygiene, inadequate sewage disposal, and limited access to clean water contribute significantly to the high burden of typhoidal and non-typhoidal salmonellosis **(Thye et al., 2025)**.

Several studies have reported an increase in antimicrobial resistance in *Salmonella* strains isolated from foods of animal origin. The spread of this antimicrobial resistance has been largely attributed to the indiscriminate use of antibiotics, both in human and animal therapeutics. Some studies have shown an association between the use of antimicrobials in animals and the development of antimicrobial resistance in bacteria isolated from humans **(Junod, 2013)**.

In the livestock industry, antibiotics are used for therapeutic, prophylactic, or growth-promoting purposes in sub-therapeutic doses for long periods in animals intended for human consumption **(Cota, 2014; Errecalde, 2004)**. This generates antibiotic residues in meat, milk, eggs, and other food products and they have the potential to induce the emergence and selection of resistant bacteria that are transmitted through the food chain **(Güerri MLS, 2002)**.

The problem of antibiotic resistance is positioned as one of the key elements for the development of global health, on a par with new pandemics, emerging infections, and global convergence in health climate change. Therefore, its surveillance and control are critical for the proposed major by 2035 (**Galán JC, 2014**)

Aims of the study:

To isolate and identification *salmonella* bacterial contamination and to quantify and compare the levels of bacterial contamination in locally slaughtered and imported chicken meat versus imported chicken meat as well as to identify the specific bacterial species present in chicken meat by the followings:-

- 1-To isolate and identify *Salmonella* from bacterial cultures using standard microbiological techniques.
- 2-To characterize the isolates by biochemical tests.
- 3-To detect *Salmonella* species by the Polymerase Chain Reaction (PCR) method.
- 4-To evaluate the antibiotic susceptibility profiles of the isolates using standard antimicrobial testing methods.
- 5-To analyze the genetic sequences of the isolates for molecular characterization.

Chapter Two

Literatures Review

2. Literatures Review

2.1. *Salmonella* Review

Salmonella was discovered in 1885 by Daniel E. Salmon, an American veterinary surgeon. It is a genus of bacteria included in the *Enterobacteriaceae* family, composed of facultatively anaerobic Gram-negative bacilli. Its size ranges from 0.7 x 2 to 1.5 x 5 microns. *Salmonella* exhibits the general characteristics of Enterobacteriaceae. Among them, they are glucose fermenters, catalase-positive, and oxidase-positive (Su & Chiu, 2007).

Salmonella exhibits the general characteristics of Enterobacteriaceae. Among them, they are glucose fermenters, catalase-positive, oxidase-negative, and are usually motile (using peritrichous flagella); with the exception of *Salmonella Gallinarum* (Knight-Jones *et al.*, 2010).

Salmonella strains are classified into serotypes based on the wide diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H), according to the Kauffmann–White classification. Approximately 2,579 serotypes are currently recognized (Gutema *et al.*, 2019). This number is constantly increasing.

Currently, two species are recognized: *S. enterica* and *S. bongori*; distinguishable from each other by metabolic characteristics (OIE, 2008& Renaloe, 2011), in turn *S. enterica* is subdivided into six subspecies, which are distinguished by some biochemical characteristics (Desai *et al.*, 2013& Pearce *et al.*, 2021) which are:

Classification of S.T.

- Subspecies I: subspecies *enterica*
- Subspecies II: subspecies *salamae*
- Subspecies IIIa: subspecies *arizonae*
- Subspecies IIIb: subspecies *diarizonae*
- Subspecies IV: subspecies *houtenae*
- Subspecies VI: subspecies *indica*. The Roman numeral V is reserved for *S. bongori* serotypes to avoid confusion with the serotype name *S. enterica* subsp. *Enterica* (Park & Andam, 2020).

The most common subspecies is *Salmonella enterica* or subspecies I which was found primarily in mammals. This subspecies is the most common cause of human disease (Sánchez *et al.* , 2011). The other five subspecies of *Salmonella enterica*, as well as *Salmonella bongori*, are found primarily in non-human hosts, although they can also cause disease in humans. Of the 2,500 serotypes, 1,504 belong to *Salmonella enterica* or subspecies I (Su & Chiu, 2007).

2.2. Antimicrobial Resistance Mechanisms in *Salmonella* spp.

They are sensitive to an antimicrobial when the antimicrobial is able to inhibit their multiplication or cause their death, and the infection in a host is controlled. Conversely, bacteria are resistant if they can survive the concentration of antibiotics present in the tissues (García, 2014). There are three main mechanisms by which sensitive bacteria can become resistant to antimicrobials:

1- Enzymatic inactivation of the antibiotic: the bacteria produce enzymes that inactivate the antibiotic. The most important enzymes of this type are beta-

lactamases, and many bacteria are capable of producing them. There are also aminoglycoside-modifying enzymes, and there are enzymes that can inactivate chloramphenicol, tetracyclines, and macrolides (**Konyali, et al ., 2023**).

2- Bacterial modifications that prevent the antibiotic from reaching the target site: Bacteria produce mutations in the cell wall that prevent the entry of certain antibiotics (beta-lactams) or alter transport systems (aminoglycosides in anaerobes). In other cases, they can cause the antibiotic to escape through an active expulsion mechanism (**Dierikx et al ., 2021**).

3- Alteration by bacteria of their target site, preventing or hindering the action of the antibiotic: This includes alterations in deoxyribonucleic acid (DNA) gyrase (which constitutes a common mechanism of resistance to quinolones), 23S ribosomal ribonucleic acid (rRNA) (macrolides), and penicillin-binding protein enzymes necessary for cell wall formation (beta-lactam resistance) (**Hasman et al., 2005; Denagamage et al ., 2019**).

A single bacterium can develop multiple resistance mechanisms against one or many antibiotics. (**Castro et al ., 2020**). Innate resistance refers to permanent, genetically determined mechanisms not associated with increased antibiotic doses. This mechanism is primarily phenotypic, although some genetic factors predispose the microorganism to activate this mechanism (**Souza et al ., 2020**).

2.3. Food poisoning

Food poisoning caused by bacteria of the *Salmonella* genus is one of the most reported diseases worldwide (**Rahman et al ., 2018**), one of the most prevalent zoonoses in developed countries, and one of the main causes of gastrointestinal diseases in humans (**Evangelopoulou et al ., 2010**). The estimated number of human *Salmonella* infections is over 93,800,000 cases, with 155,000 deaths annually

worldwide (**Boyle et al , 2007; Campioni et al , 2012**). In Latin American, Asian, and African countries, prevalence ranges from 200 to 500 cases per 100,000 inhabitants (**Bäumler et al ., 2000**). Cases can vary from mild to severe, and can sometimes be fatal. Fatal cases occur most frequently in vulnerable populations, including infants, the elderly, and immunocompromised individuals (**Gordon, 2008**). *Salmonella* is rarely transmitted from person to person, so food is considered the primary and most likely source of human exposure (**Small et al ., 2022**). It is estimated that 95% of these infections are associated with foods of animal origin (**Miranda et al ., 2009**). Generally, the implicated foods are: chicken, beef, pork, sausages, milk, eggs, and their derivatives (**Meyer et al ., 2010**).

In recent years, several studies have reported an increase in antimicrobial resistance in *Salmonella* strains isolated from foods of animal origin. The spread of this antimicrobial resistance has been largely attributed to the indiscriminate use of antibiotics, both in human and animal therapeutics. Some studies have shown an association between the use of antimicrobials in animals and the development of antimicrobial resistance in bacteria isolated from humans (**Young et al ., 2016**). In human therapeutics, the overuse of antibiotics observed in many parts of the world, especially in cases of minor infections, incorrect use due to lack of access to appropriate treatment, and underuse due to a lack of financial resources to complete treatment, does not yield the expected therapeutic results and is associated with the development of antimicrobial resistance (**Su et al ., 2004**). In the livestock industry, antibiotics are used for therapeutic, prophylactic, or growth-promoting purposes in sub-therapeutic doses for long periods in animals intended for human consumption (**Cota E, 2014; Lee et al ., 1994**). This generates antibiotic residues in meat, milk, eggs, and other food products (**Ehuwa et al ., 2021**), and they have the potential to

induce the emergence and selection of resistant bacteria that are transmitted through the food chain (**Bell & Kyriakides, 2008**).

An example of the scale of the problem is the antimicrobial resistance developed by *Salmonella* against fluoroquinolones, one of the antimicrobials of choice for treating salmonellosis in adults. In some countries, a significant increase in cases of resistant *Salmonella* in animals, food, and human infections was observed after fluoroquinolones began to be widely used in animals intended for human consumption (**WHO, 2001**).

For the above reasons, and because of the general relevance of the issue to public health, it is essential to understand the situation in Iraq over the last 10 years regarding antimicrobial resistance in *Salmonella* spp. isolated from foods of animal origin, obtaining and analyzing data such as *Salmonella* serotypes and the antibiotics to which they most frequently exhibit resistance, based on a systematic review of epidemiological studies. The above can generate important information for implementing specific measures that can help contain the problem in Iraq.

2.4. Methods for Determining Bacterial Susceptibility

There are different methods for determining the sensitivity of a given microorganism to antimicrobials. These tests classify microorganisms into three categories: sensitive, intermediate, and resistant. The main methods are:

1) Dilution method: This method is based on determining the growth of the microorganism in the presence of increasing concentrations of the antimicrobial, which is diluted in the culture medium (broth or agar). This method includes the following: broth microdilution and agar dilution (**Gupta et al ., 2019 and Iwiński et al., 2022**).

2) Disk diffusion method: This method involves placing sensidiscs on the agar surface of a Petri dish previously inoculated with the microorganism. Test interpretation is based on the correlation between the diameter of the zone of inhibition (mm) and the minimum inhibitory concentration) MIC ($\mu\text{g/mL}$) for each antimicrobial and microorganism. (**Shoaib *et al* ., 2021**).

3) E-test: This is a quantitative plate technique that allows a direct reading of the minimum inhibitory concentration. The reading is performed directly, noting the point of highest antibiotic concentration where growth occurs (**Lenchenko *et al* ., 2020**).

4) Automated methods: These methods generally use broth microdilution systems on microplates and interpret bacterial growth in the different wells using an autoanalyzer or, in the case of simpler systems, by simple optical reading (**Ajulo *et al* ., 2020**).

2.5. Epidemiology of *Salmonella* Infection

Salmonella spp. is associated with the development of diarrheal diseases, which continue to be one of the most important causes of morbidity and mortality, especially in infants, children, and the elderly (**Sun *et al* ., 2020**).

It has been estimated that in Asia, Africa, and Latin America, depending on socioeconomic and nutritional factors, the probability of a child dying from diarrheal disease before the age of 7 is up to 50%. Immunocompromised people and/or those with underlying chronic diseases, such as those with Acquired Immune Deficiency Syndrome (AIDS), contract the disease more easily, making the growing antimicrobial resistance even more important (**Hlashwayo *et al.*, 2023**). As previously mentioned, one of the most important characteristics of human salmonellosis is its foodborne transmission, particularly from animal sources. A total

of 121 *Salmonella* spp. outbreaks occurred in the United States in 2006, causing more than 3,300 cases reported by the Center for Disease Control and Prevention's Information System (Zuo *et al.* , 2025).

During the period 1993-2002 in Argentina, 60 outbreaks of Salmonellosis occurred, resulting in 889 illnesses and 4 deaths. 6.7% of the outbreaks were caused by *Salmonella* serovar Enteritidis, 1.7% by *S. arizonae*, and in 90% of cases the corresponding serovar could not be identified. In these cases, 25% of the causes of the outbreak were attributed to the consumption of contaminated egg products, mayonnaise, and poultry (Shariat *et al.* , 2021). Since the mid-1980s, cases of *Salmonella* resistance to various antibiotics began to be reported. In 1984, Holmberg identified 18 people from four states in the United States who were infected with *Salmonella* Newport after eating beef hamburgers, showing resistance to ampicillin, carbenicillin, and tetracycline (Osaili *et al.* , 2020). conducted a systematic review of studies on the resistance of *Salmonella* spp. isolated from food-producing animals in Southeast Asia (Hao, 2012). Previous study found that resistance to ampicillin, tetracycline, and sulfamethazine was high in countries such as Malaysia, Thailand, and Vietnam, where resistance rates ranged from 22% to 49%, 41% to 92%, and 17% to 68%, respectively. In these countries, *Salmonella* spp. strains were primarily isolated from samples of poultry and pigs. In another study, resistance to sulfamethoxazole and ampicillin in *Salmonella Enteritidis* strains isolated from poultry carcasses in Cambodia was 60% and 90%, respectively (Costa *et al.* , 2014).

In the European Union, nearly 91,034 cases of human salmonellosis were reported in 2012. These isolates showed high resistance to common antibiotics such as ampicillin (27.6%), streptomycin (23.6%), sulfonamides (28.9%), and tetracyclines (30.0%), moderate resistance to nalidixic acid (14.4%), and high levels of multidrug resistance were observed in some countries (28.9% of the total).

Resistance to clinically important antimicrobials such as ciprofloxacin and cefotaxime was relatively low on average (5.1% and 1.1%, respectively). Resistance to quinolones (ciprofloxacin and nalidixic acid) was generally higher in *S. Enteritidis* than in *S. Typhimurium* (**Gordon et al ., 2008**).

In countries such as USA, Bolivia, and Colombia, *Salmonella* resistance to ampicillin can exceed 50%. Specific data, such as country, number of samples resubmitted, percentage of resistance, and year (**Guerrero et al ., 2020**).

Dubai is the country with the lowest resistance rate over the nine years of surveillance. In Iran, no ampicillin resistance was reported in 1996, 23 but the proportion of resistant strains increased systematically to 3%, 5%, 6%, 10%, and 17% for 1998, 2000, 2002, 2004, and 2005, respectively (**Khan et al ., 2010**). In other countries, such as Saudia arabia and Libya, resistance has decreased significantly. Resistance to chloramphenicol and trimethoprim-sulfamethoxazole has similar results. In Colombia, resistance to ampicillin and cotrimoxazole for *Salmonella* spp. has decreased in recent years, from 40% to 10%. Since 1997, the resistance rate of *Salmonella* spp. to cotrimoxazole in Kwait has ranged from 75% to over 90% (**Jamal et al ., 2021**).

In 2010, the Latin American Network for Antibiotic Resistance Surveillance (Relavra) presented its annual report on *Salmonella* spp. cases and the corresponding results of antimicrobial resistance in humans (**Campos et al ., 2012**).

In research conducted by the National Institute of Health in Peru, *Salmonella* spp. resistance rates were found between 1% and 7% for antibiotics such as chloramphenicol, ampicillin, and cotrimoxazole. In countries such as Brazil, Paraguay, Venezuela, Chile, and the Caribbean, the *Salmonella* spp. resistance trend is similar to these same antibiotics (**Guerrero et al ., 2022**).

There were 1013 *Salmonella* spp. isolates in Chile, with the following antimicrobial susceptibility results: ciprofloxacin 0%, nalidixic acid 8%, ampicillin 2%, amoxicillin-clavulanic acid 0%, cefotaxime 0%, ceftazidime 0%, chloramphenicol 3%, trimethoprim-sulfamethoxazole 2%, nitrofurantoin 11%, streptomycin 17%, cefoxitin 0.1%. Reports in Colombia included: *S. Enteritidis* 231 isolates, and the resistance was: ciprofloxacin 0%, nalidixic acid 2%, ampicillin 2%, amoxicillin-clavulanic acid 0%, cefotaxime 0.5%, ceftazidime 0.9%, chloramphenicol 0%. Trimethoprim-sulfamethoxazole 3%. Also, 204 *S. Typhimurium* isolates were reported with resistance to nalidixic acid (17%), ampicillin (32%), amoxicillin-clavulanic acid (1%), ceftazidime (1%), chloramphenicol (10%), and trimethoprim-sulfamethoxazole (27%). *S. Typhi* also reported 87 isolates resistant to chloramphenicol (1%) and trimethoprim-sulfamethoxazole (1%). (**Cabrera et al ., (2006)** reported 3 isolates of *S. Typhi*, 25 of *S. Enteritidis*, and 37 of *S. Typhimurium*. Of all the serotypes isolated, none presented antimicrobial resistance to the 26 antibiotics tested (ciprofloxacin, nalidixic acid, ampicillin, amoxicillin-clavulanic acid, ceftazidime, chloramphenicol).

In Honduras, 60 cases of *Salmonella* spp. were reported with antimicrobial resistance to ciprofloxacin (6%), nalidixic acid (5%), ampicillin (22%), amoxicillin-clavulanic acid (8%), cefoxitin (2%), cefotaxime (15%), ceftazidime (1%), and chloramphenicol (6%) (**Maradiaga et al ., 2015**).

Although there are results and reports of *Salmonella* spp. isolations and antimicrobial resistance, these results come from outbreak studies, so they may not adequately reflect the epidemiology of antibiotic resistance in *Salmonella* spp. isolated from foods of animal origin. Information from studies where the isolates are not associated with these antibiotics is very limited (**Eng et al ., 2015**).

2.6. Public Health Implications

Salmonella infections were monitored before the 1970s. This zoonotic species is responsible not only for foodborne illnesses but also for hospital-acquired infections. Following the Swann Committee report, concern grew about resistance to antibiotics that were used for human treatment and at the same time were used as growth promoters in farm animals, prompting public health authorities to consider the consequences of shared use (O'Bryan *et al.* , 2022).

According to the World Health Organization (WHO) and the World Economic Forum, antibiotic resistance is one of the greatest global public health problems because: 1) it impedes the ability to control infectious diseases, increasing morbidity and mortality; 2) by reducing therapeutic efficacy, it threatens progress and leads to consideration of a dramatic regression of modern medicine to the pre-antibiotic era; 3) it keeps patients infectious, facilitating disease transmission to other individuals; 4) it increases health care costs; and 5) it threatens health security and harms trade and the economy. When pathogens become resistant to antimicrobial agents, they can be more dangerous to human health due to potential treatment failure (WHO , 2014). This is because many diseases have stopped responding to commonly used antibiotics (WHO, 2005), both due to overuse and the lack of new agents on the market. Furthermore, antibiotics are the only drugs that, if used inappropriately, can generate resistance. Therefore, urgent public attention and action are needed; otherwise, antimicrobial resistance threatens to take the world back to the era of no effective treatment (Pires *et al.* , 2014). Generally, gastroenteritis caused by *Salmonella* in humans should not be treated with antimicrobials because they can prolong the excretion of the microorganism as well as the carrier state. However, in some cases, *Salmonella* causes a systemic infection that requires antibiotic treatment. Between 1 and 2% of *Salmonella Typhimurium*

strains require antimicrobial therapy due to the mortality it can cause (**Arboite de Oliveira F, 2006**). Such is the importance of the antimicrobial resistance developed by *Salmonella* that in a study of more than 1 million cases of *Salmonella* infections, Furthermore, in recent years, *Salmonella* resistance to the most commonly used antibiotics for its treatment has increased, including amoxicillin-clavulanic acid, third-generation cephalosporins, and fluoroquinolones Giraud, *et al* ., 2006). In addition, as one of the actions taken by the WHO to contribute to the containment of antimicrobial resistance, a survey was conducted among ordinary people in different countries. The results of this survey demonstrate the urgent need to improve understanding of the problem of antibiotic resistance. This study revealed confusion among people regarding this serious threat to public health and a lack of understanding of how to prevent it, highlighting the need to improve communication and understanding of the problem (**Bula-Rudas *et al* ., 2015**). Therefore, it is urgent to propose and implement appropriate measures at the global level. To control the problem of antimicrobial resistance as soon as possible, otherwise the emergence of resistance could increase, making it more difficult to control **Mkangara, 2023**).

2.7. Economic and Social Implications

Salmonellosis represents a massive cost to society in many countries. Data on the cost of foodborne illnesses are scarce and come from only a few studies (**Chen *et al* ., 2004**). Antimicrobial resistance is associated with an additional US\$20 billion in direct medical costs in the United States annually (**Rönnqvist *et al* ., 2018**. When human infections with resistant pathogenic bacteria occur, treatment is often inadequate or prolonged.

In Denmark, the estimated annual cost of human salmonellosis is US\$15.5 million. Data on the cost of foodborne illnesses are generally not calculated or

reported in developing countries (**Barbour *et al* ., 2015**). The costs of salmonellosis treatment are increasing with the development of antimicrobial resistance, as it dangerously compromises medical treatment for this disease and lengthens hospital stays, leading to increased expenditures for health systems. This jeopardizes the effectiveness of health care programs, likewise, the economic, social, and emotional costs for patients are significant, but have not been measured(**Galán *et al* ., 2023**).

Regarding the association of antibiotic use in animals with the emergence of resistant strains in samples of human origin, this has also led to economic consequences and even social alarm. For example, in Russia, the rejection of imported chickens from the United States has been reported due to the presence of antibiotic residues in their meat. This has led many producers in this country to respond by abandoning the use of quinolones to treat sick animals, therefore, antimicrobial resistance is a public health problem as well as an economic burden (**Asperilla *et al* ., 1997**)

Justification for conducting this study, antimicrobial resistance developed by bacteria of the *Salmonella* genus is important and concerning. Some studies suggest that resistance is increasing, which has significant health, social, and economic implications (**Yada *et al* ., 2023**). The problem of antibiotic resistance is positioned as one of the key elements for the development of global health, on a par with new pandemics, emerging infections, and global climate change. Therefore, its surveillance and control are critical for the proposed major convergence in health by 2023 (**Galán *et al* ., 2023**). Experts on this topic from the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), and the WHO have concluded that foodborne antimicrobial-resistant microorganisms, such as *Salmonella*, constitute a danger to food safety and, consequently, to human health Although the problem of antimicrobial drug

resistance in Latin America appears to be increasing (**Reyes *et al.* , 2025**). systematic reviews that include studies conducted in the region that allow for an accurate assessment of the situation are lacking.

Considering that since 1998, PAHO has recognized the need to monitor antimicrobial resistance and use the information generated to formulate policies, regulation, and therapeutics in order to make relevant decisions on the issue (**Abdulrahman, 2022**), some of the measures and policies that can be implemented

Policies for the surveillance of antimicrobial resistance in *Salmonella* spp. and other bacteria of public health importance. Surveillance systems should cover both the human and animal health sectors to collect and compare data.

Policies for the appropriate and rational use of antibiotics in animals intended for human consumption and in human clinical settings. To this end, the distribution and sale of antimicrobials must be controlled, mandatory prescriptions for their use must be established, and the appropriate use of antimicrobials in animals and humans must be monitored. Implement measures to prevent the use of antimicrobials as growth promoters in animals intended for human consumption, and promote the creation of regulations and standards in each of the Latin American countries regarding the prudent use of antimicrobials in both human and veterinary medicine, with the appropriate sanctions, if applicable, if regulations are not complied with. develop more efficient policies to control and prevent the transmission of *Salmonella* spp. to humans through the consumption of food of animal origin and guide and support producers and stakeholders in food value chains on good practices in animal husbandry, health, biosecurity, management, food safety, and hygiene (**Wang *et al.* , 2023**), thereby preventing diseases and reducing the need for antimicrobial use as well as the implement communication and education measures to prevent foodborne diseases such as salmonellosis, emphasizing hygiene and proper food handling. In

general, analyzing data obtained from different studies on antimicrobial resistance in pathogens that affect food safety is essential, as it is a source of information for understanding and improving food safety, in order to preserve public health (O'Bryan *et al* ., 2021).

2.8. Implicancias economics and sociales

The salmonellosis represents a large cost to the society and many countries, the resistance antimicrobial activity is available with US\$20,000 million dollars in direct medical costs and annual estimates (Brown *et al* ., 2021). It produces infections among human beings with persistent pathogenic bacteria, and it is very clear that the results that result from this disease are more common. In Dinamarca, the annual cost of salmonellosis in humans is 15.5 million dollars, Losing costs for salmonellosis infections caused by antimicrobial resistance, which compromising on the health care provided by the hospital and in the hospital, produced in the hospital(Zakir *et al* ., 2021).

The respect to the use of antimicrobial agents in animals and the persistent deceives in human origin, this prevents economic consumption and includes social alarm. For the project, in Russia, it has been communicated and received the import of salmonellosis infections. United for the presence of antibacterial residues in the meat. It also happens that a large part of the products produced by this country have responded by abandoning the use of quinolones to treat sick animals (Millanao *et al* ., 2021).

2.9. Pathogenicity of Salmonellosis

Pathogenesis of spp. is a multi-factorial phenomenon and varies with the serovars, dose, age, and immune status of the host, as the infection is almost always

acquired by ingestion of microorganisms, usually as contamination of food or water (**Bhat *et al* ., 2022**).

The disease is sometimes endemic in farms, stables, and clinics, its incidence often high, but the occurrence of clinical infection is low, Stresses resulting from transportation, overcrowding, pregnancy, parturition, water deprivation, oral antimicrobial therapy, surgery, depressed immunity systemic viral, and chronic diseases may precipitate clinical disease in human (**Telli *et al* ., 2022**). The portal of entry for *Salmonella* spp. infection in the mouth, usually through ingestion of fecally contaminated water or food, infection occurs in a susceptible human host, the incubation period shortens and the risk for infection and disease increases with the ingested dose (**Khan & Shamim, 2022**).

Gastric acid provides an important barrier to *Salmonella* spp. accessing the small intestinal mucosa, and in turn, the reticuloendothelial system, natural and vaccine-induced immunity provide partial protection against typhoid fever (**Kakooza *et al.*, 2021**).

Salmonella species are facultative intracellular pathogens , It can invade different cell types, including epithelial cells, M cells, macrophages, and dendritic cells, as a facultative anaerobic organism, *Salmonella* uses oxygen to make ATP in an aerobic environment (when oxygen is available), however, in an anaerobic environment (when oxygen is not available) *Salmonella* produces ATP by fermentation by substituting one or more of four less efficient electron acceptors than oxygen at the end of the electron transport chain, sulfate, nitrate, sulfur, or fumarate (**Eng *et al* ., 2015**).

Enteric fever has been suggested to recognize infection of the oral route and systemic invasion that happens across the gastrointestinal tract, once ingested, the

organisms multiply in the small intestine over the period of 1-3 weeks, breach the intestinal wall, and spread to other organ systems and tissues (Figure 2-1) (Salieb-Beugelaar & Hunziker, 2015).

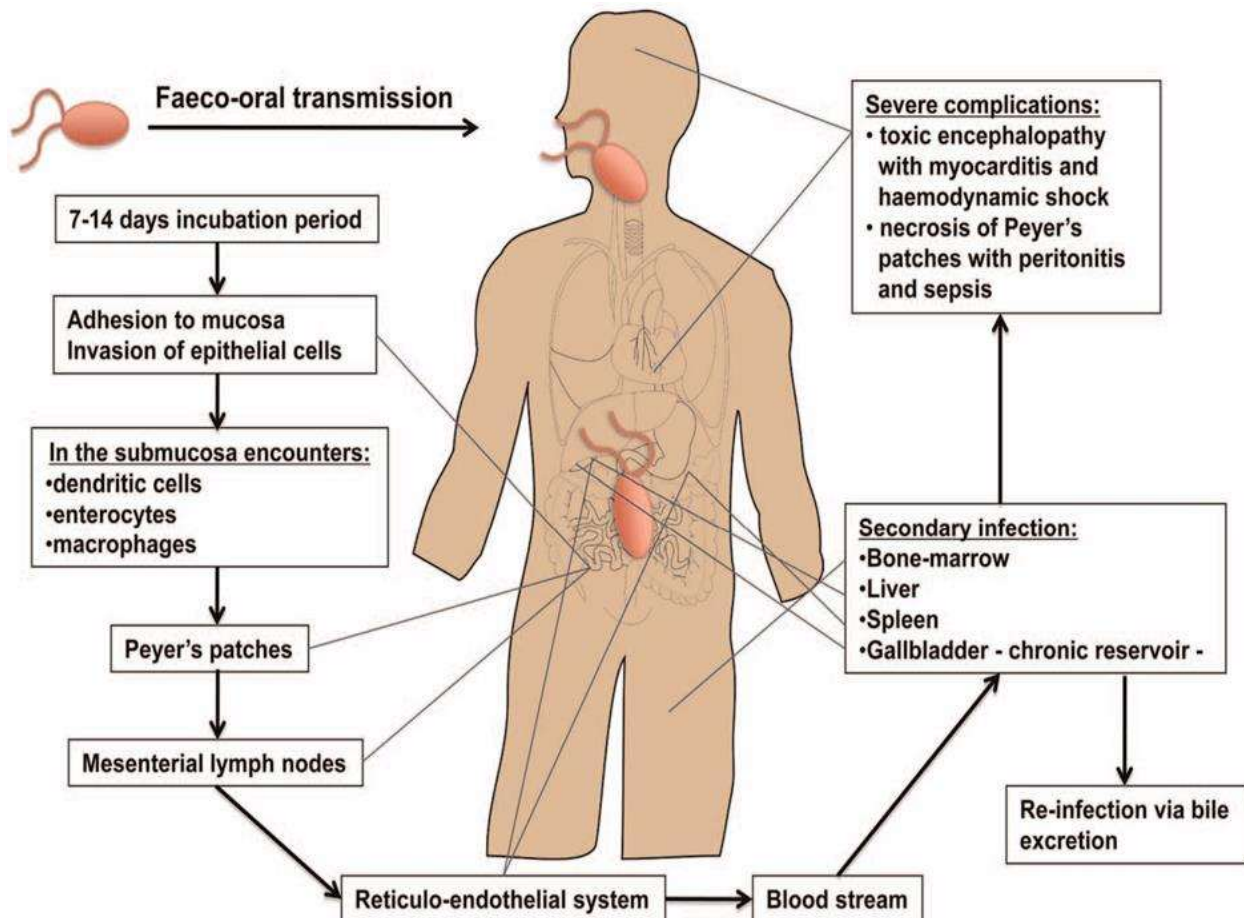


Figure 2-1: Dissemination of *Salmonella* spp. during systemic infection (Salieb-Beugelaar & Hunziker, 2015)

Salmonella interactions with non-phagocytic cells and phagocytic cells, the primary site of invasion of *Salmonella* strains is thought to be the M cells in the Peyer's patches. Starting in the late 1990, *Salmonella* flagella was highlighted in host early innate immunity against *Salmonella* because the flagella were identified by several independent groups as the mediator that causes intestinal epithelial or

macrophage inflammation following infection, reported that *Salmonella* enterica serovar Typhi flagella induced cytokine release from human monocytes, and impaired antigen presentation by human macrophages (**Boyle et al ., 2007**).

The stages of *Salmonella* pathogenicity are, initially, the bacteria enter the gastrointestinal tract, after ingestion of contaminated food or water, and survive the gastric acid, because of the ability of these bacteria to avoiding and penetrating the acidic barrier of the stomach, which is an effective chemical and protective barrier against many pathogenic organisms, which is an essential step in the pathogenesis of these bacteria. in the small intestine, they adhere to and invade the mucosal epithelial cells, preferably the microfold (M) cell on Peyer's patches (layer of lymphoid nodules) (**Harrell et al., 2021**).

This is followed by inflammation, and phagocytosis of bacteria by neutrophils and macrophages and recruitment of T and B cells, the bacteria translocate to the lymphoid follicles, the mesenteric lymph nodes, and to reticuloendothelial cells in the spleen, and liver via the bloodstream (transient primary bacteremia) (**Santos et al ., 2003**). Because *Salmonella* may target specific types of host cells, such as dendritic cells, and or macrophages that favor dissemination through the lymphatics, and bloodstream to the mesenteric lymph nodes (MLNs), and to deeper tissues during this phase the bacteria encounter macrophages, and become phagocytosed, however, they can form *Salmonella*-containing vacuoles, and survive and replicate within the macrophages (**Guiney & Fierer, 2021**).

After the incubation period (7-14 days) the bacteria then re-enter the bloodstream , But in a low concentration (<1colony-forming unit/ml blood), when this secondary bacteremia is reached the clinical symptoms with fever and general

discomfort usually start to appear, secondary infections can also appear in the liver, spleen, bone marrow, and gallbladder (**Nayak & Dasgupta, 2022**)

Chapter Three

Methodology

3. Materials and Method

3.1. Materials

3.1.1. Equipment and Instruments

Table 3-1: The equipment's and Instruments used in this study

Type of Instruments	Manufacturer/State
Autoclave	Humanlab (DAC-60)(Korea)
Benzen burner	Amal (Turkye)
Cool box	India
Deep freezer	GFL (Germany)
Digital camera	Canon (Japan)
Distillator	BOECO (Germany)
Freezer and Refrigerator	CONCORD (Malaysia)
Centrifuge	Hettich\Germany
Incubater	Memmert (Germany)
Iron loops	Oxoide (England)
Laboratory centrifuge	Memmert (Germany)
Laminar Cabinate (HOOD)	VISION (Korea)
Light microscope	NOVEX (Holland)
Micropipette tips (different sizes)	China
Microscope	Novel XSZ-N107T
Microscope cover slides	Sail Brand (China)
Microscope slides	Sail Brand (China)
Millipore filter 0.45mm	Difico (USA)
VITEK2 system	Biomerieux France
Plastic petri dishes	Afco (Jordon)
Plastic test tube clear	Afco (Jordon)
Plastic test tube turbid	Afco (Jordon)
Pyrex bakens and flasks (different sizes)	Falcon (USA)
Specimen container 60 ml	China
Sterile syringes	Medeco (Belgum)
Sterile transport media swap	Amies (China)
Swab	China
Uv transmitter	Biobase\China
Vortex Mixer	Belgium
Water bath	Memmert (Germany)

3.1.2. Chemicals and Reagents

Table 3-2: Chemical materials.

Type of Chemicals	Manufacturer's name
Absolute Ethanol	Merk (UK)
Absolute Formalin	Chemanol (Saudi Arabia)
Agarose	Merck (Germany)
Api-20E strip	Bio-merieux (France)
Brilliance <i>Salmonella</i> agar base supplement	Oxoid (UK)
Crystals iodide	(Jordon)
Glycerol	Himedia (India)
Gram stain solutions	BDH (UK)
Kovac's reagent	BDH (UK)
Loading dya	Kapa bio systems (USA)
Mineral oil	Bio-merieux (France)
NaCl	BDH (UK)
Normal saline	PSI (Saudi Arabia)
Oxidase reagent	BDH (UK)
Potassium iodide	(Jordon)
Povidon 10% a\h	Poviofix (Turkey)
Urea solution	Himedia (India)

3.1.3. Culture Media

Table 3-3: The culture media used in current study

Medium	Manufacture company/State
Brain heart infusion agar	Oxoid (UK)
Blood agar base	Mast diagnostic (UK)
Brain heart infusion broth	Oxoid (UK)
Brilliance <i>Salmonella</i> agar base	Oxoid (UK)
MacConkey agar	Himedia (India)
Nutrient agar	Oxoid (UK)
Nutrient broth	Himedia (India)
Peptone water	Oxoid (UK)
Simmons citrate agar	Himedia (India)
Soybean casein digest agar	Himedia (India)
SS agar (<i>Salmonella Shigella</i> agar)	Oxoid (UK)
Tetrathionate broth base	Himedia (India)
Triple sugar iron agar	Himedia (India)
Urea Agar Base, Christensen	Himedia (India)
Xylose- Lysine Deoxycholate (XLD) agar	Himedia (India)

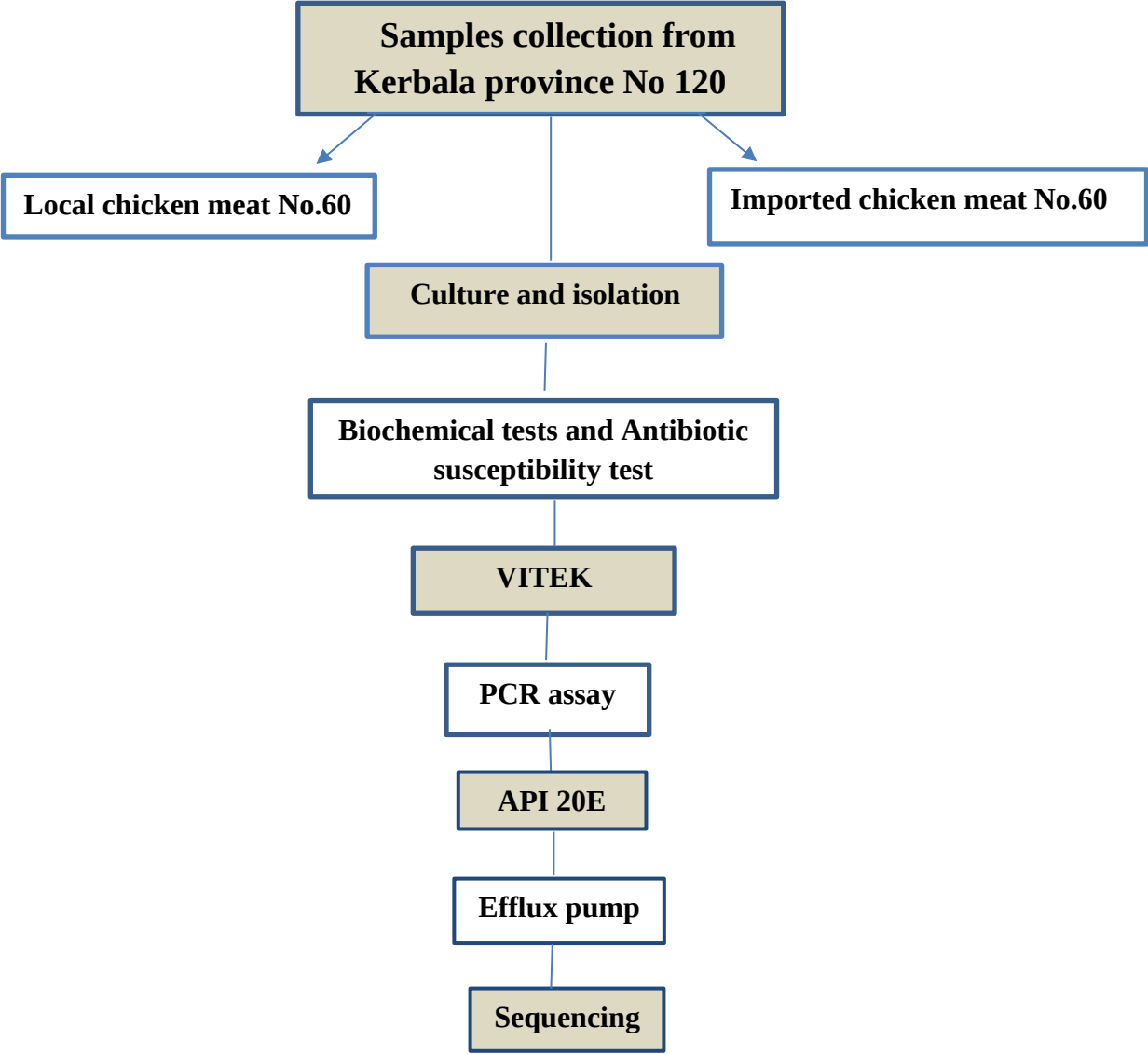


Figure (3-1) experiment design

3.1.4.collection of samples

A total of 120 fresh chicken meat samples were collected from different regions in Kerbala City, Iraq. It was obtained from unregulated places in the city of Karbala during the period from (September 2024-January 2025). The samples were categorized into two groups contain 60 samples collection from locally slaughtered chickens meat and imported chicken meat form different region of Karbala province, From each chicken, thigh and breast cuts were aseptically excised using sterile scalpels and placed in individually labeled sterile bags. Samples were transported under refrigeration (4°C) to the laboratory within 24 hours for further analysis

3.1.4.1.Culturing of specimens

Take out 1 g chicken from each sample for all species with a sterile wood spatula, put it into an Erlenmeyer flask etc. and add 9 ml buffered peptone water, Mix and Incubate at 37°C overnight (18-24 hours) then Transfer 1 ml of the pre-enrichment with a pipette to 10 ml Tetrathionate broth (Müller-Kauffmann). Incubate at 37° C overnight (18-24 hours) then Spread a 10 µl loop full from the inoculated and incubated Tetrathionate broth on XLD and on SS agar plates and incubate at 37°C overnight (18-24 hours) and read the XLD plates and SS plates *Salmonella* suspect colonies on XLD (**WHO, 2010 and Ezat et al., 2016**).

3.1.4.2.Bacterial identification

The growing isolates were identified according to their colonial morphology, pattern of gram staining, biochemical reactions and on basis of molecular detection

3.1.4.3.Bacterial isolation

The bacteria isolated from animal sources were taken from an overnight culture of transport media and streaked on XLD agar after that isolates suspected to be of interest were subculture S.S agar to ensure growth.

3.1.4.4. Microscopic examination

The suspected colony was transferred to a glass microscopic slide, fixed and stained with gram's stain. Their gram reaction, shape of cells and their arrangements were observed under light microscope at (X40) (**Quinn *et al.* ., 2002**).

3.1.4.5. Biochemical tests

According to single isolated colonies from plate showing typical salmonella-like growth were picked and subjected to primary biochemical screening tests. (**Markey *et al.*, 2014**).

3.1.4.6. Catalase test

This reagent was prepared by mixing 3 ml of hydrogen peroxide solution 22% with 97 ml D.W It was made by spreading a single colony of bacteria from nutrient agar on a clean slide, and then few drops of Hydrogen peroxide (H_2O_2) were added. The immediate production of O_2 bubbles indicated a positive reaction (**Collee *et al.*, 1996**)

3.1.4.7 Oxidase test

This reagent was prepared by dissolving 1 gm (tetramthy-paraphenylene –diamine-dihydrochloride) in 100 D..W It was made by spreading a single colony of bacteria from nutrient agar on a filter paper which soaked previously with oxidase reagent. The conversion of the color of colony to dark purple during 2-10 seconds indicates a positive reaction. (**De Vries *et al.*, 1996**)

3.1.4.8. Indole test

This test was done by inoculated with 5 ml Peptone water, with the bacterial incubation at 37°C for 24-28 hours, and addition of 0.5 ml of Kovac's reagent mixed thoroughly and examined. The result apositive is indicated by the development of ared color in the reagent layer while a negative result is confirmed by the absent of any red coloration (**Mukhtaruzzaman and Atiquzzaman, 2020**)

3.1.4.9. Urease test

This media was prepared added 10 ml of 20% urea solution to an autoclave urea agar base. The total volume was adjusted to 100 ml with distilled water, then the media was cooled to 50 to 55°C, mixed thoroughly, then 4 to 5 ml distributed per sterile tube, which was slanted until the media was solidified. After streaking the surface of a urea agar slant was streaked with a young bacterial culture and incubated at 37°C. Result was read Changing the color of medium from yellow to pink indicated a positive result that was indicated to produce urease enzyme (McFadden, 2000).

3.1.4.10. Simmons citrate test

Simmons citrate slant was inoculated with a young bacterial culture and incubated at 37°C for 48-72 hr. Changing of the color from green to blue is an indication of a positive test (McFadden, 2000).

3.1.4.11. Triple Sugar Iron (TSI) test

This medium was prepared by dissolving 63.6 grams of TSI agar in one liter of distilled water, followed by heating to complete dissolution the medium was then dispensed unto test and autoclaved at 121 °C for 15 minutes. A small amount of a colony was harvested with a sterile (1uL) needle, stabbed into the butt of the tube and streaked over the surface of the slant. Slants were incubated at 37°C for 24 hr. with caps loosened. Results were recorded (MacFaddin, (2000). Table(3-4).

Table (3-4) TSI positive result

Slant Reaction/color	Butt Reaction/color	CO ₂ gas	H ₂ S
Alkaline /Red	Acid / Yellow	Gas bubbles +	black precipitate +

3.1.4.6 Motility test:

Semi-solid Mannitol medium was prepared as in 3.2.1, and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility was examined after 35-37°C for 24 hour. Motility was indicated by the presence of diffuse growth (appearing as colouring of the medium) away from the line of inoculation. With exception of *Salmonella Pullorum-Gallinarum*, all *Salmonella* species are motile (Quinn, 2002).

3.1.4.7. Antibiotic susceptibility Test (Disc-diffusion method):

Antibiotic susceptibility of *Salmonella* isolates was assessed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Briefly, isolated colonies were emulsified in sterile saline to achieve a turbidity equivalent to the 0.5 McFarland standard. The standardized inoculum was then evenly spread onto Mueller–Hinton agar plates using a sterile cotton swab. Commercial antibiotic-impregnated disks were carefully placed on the surface of the inoculated medium, and plates were incubated at 37°C for 18–24 hours. After incubation, the diameters of the inhibition zones surrounding each disk were measured and interpreted as susceptible, intermediate, or resistant based on CLSI interpretive criteria. This procedure provides an accurate phenotypic evaluation of the antimicrobial resistance patterns of *Salmonella* isolates. Table (3-5)

Table(3-5):Antibiotic used in susceptibility test

No	Antibiotics	Disc symbol	Concentration	Company \origin
1	Ampicillin	AMP	25/5mcg	Bioanalyse/Turkiye
2	Amikacine	AK	10 mcg	Bioanalyse/Turkiye
3	Choramphenicol	CHL	30mcg	Bioanalyse/Turkiye
4	Ciprofloxacin	CIP	10mcg	Bioanalyse/Turkiye

5	Doxycycline	DC	30mcg	Bioanalyse/Turkiye
6	Nalidixic acid	NA	30mcg	Bioanalyse/Turkiye
7	Tetracycline	TE	10mcg	Bioanalyse/Turkiye
8	Erythromycin	NA	15mcg	Bioanalyse/Turkiye
9	Gentamicin	TE	10mcg	Bioanalyse/Turkiye
10	Sulfamethoxazole	TE	10mcg	Bioanalyse/Turkiye

3.1.5 Molecular Study

3.1.5.1. Material and Kits

Table (3-6) Molecular Material and Kits

Material and Kits	Company/ Origin
Absolute Ethanol, Isopropanol	ROMIL pure chemistry, (UK)
Addbio DNA Extraction kits	Addbio, (Korea)
Agarose, Ethidium Bromide Solution (10mg/ml), GoTag Green Master Mix, Nuclease Free Water, TAE 40X, Quantiflor dsDNA System.	Intron, (Korea)QA
Primers	Macrogen, (Korea)
Ladder(100bp)	Promega, (USA)

3.1.6.1. Instrument

Table 3-7: Molecular instrument

Instrument	Company/ Origin
Biopette Variable Volume 2-20 ul	Germany
Centrifuge	Fisher Scientific, (USA)
Gel Imaging System	Major Science, (Taiwan)
Micro spin Centrifuge	My Fugene, (China)

Microwave Oven	GOSONIC, (China)
OWL Electrophoresis System	Thermo, (USA)
Quantus Florometer	Promega,(USA)
Thermal Cycler	BioRad,(USA)
Vortex	Quality Lab System, (England)

3.1.7.1 Specific primers

In this study, Primers was used for molecular identification are lyophilized and was investigated by Macrogen, Korea. Three primers were used for three genes **16SRNA** primer sequences are described below.

Table (3-8) Primers used for the amplification of selected virulence genes that use in study.

Gene	Primer sequence (5' to 3')	Product size/bp	Reference
16SRNA	F : TTG TTC ACT TTT TAC CCC TGAA R : CCC TGA CAG CCG TTA GAT ATT	1400 bp	(Barghouthi <i>et al</i> ., 2011)

3.2 .Methods:

3.2.7 .Preparation of culture media

All media were prepared and sterilized according to the instructions of the manufacturer company, some media autoclaved at 121 °C, 15 pound/Inch² for 15 minutes, and then all media were distributed into sterile Petri- dishes or test tubes. The media was incubated at 37°C for 24 hrs. to ensure they are contamination free. Some media required special additives.

3.2.7.1 Peptone water medium

This medium was prepared by dissolving 15 gm of the medium into (1L) of distal water (DW) and then heated to boiling with frequent agitation to

dissolve its components completely. After that, it was poured into sterile test tubes and sterilized by autoclaving 121°C at 15 pound/Inch² for 15 minutes (Collee *et al.* , 2022)

3.2.7.2 Tetra thionat broth:

This is an enrichment medium for isolation of *Salmonella species* from faeces, urine or other pathological materials; it was prepared by suspending 77.40 gm from powder with 980 ml of purified DW Heat just to boiling. Cool less than 45-50°C and aseptically add 20 ml solution of iodine. Mix well and dispense in 10 ml quantities in sterile tubes. This complete medium should be used on the day of preparation. Use the medium immediately after addition of iodine (Goodswen *et al.*, 2021).

3.2.7.3 Nutrient media

This medium was prepared by dissolving 28 gm of the medium into (1L) of DW and then heated to boiling with frequent agitation to dissolve its components completely. sterilized by autoclaving 121°C at 15 pound/Inch² for 15 minutes, after that allow it to cool but not solidify and pour the media into petri dish and leave plates on sterile surface until the agar has solidified and media was incubated at 37°C for 24 hrs. to ensure was contamination free (Dubey & Maheshwari, 2023).

3.2.7.4 Nutrient broth

Suspend 13.0 gm in 1000 ml of DW Heat if necessary, to smelting the medium completely. Dispense into tubes or flasks as desired. Sterilized by autoclaving at 15 pound/Inch² for 15 minutes (Goodswen *et al.* , 2021).

3.2.7.5 MacConkey agar:

This medium was prepared by dissolving 52g in (1L) of DW and then boiled to dissolve its components completely, after that sterilized by autoclaving at 121°C, 15 pound/Inch² for 15 minutes, and media was incubated at 37°C for 24 hrs to ensure they are contamination free (Dubey & Maheshwari, 2023).

3.2.7.6 Brain heart infusion agar (BHIA):

This medium was prepared like (MacConkey agar) but the amount of the medium was (47 gm/1000 ml).

3.2.7.7 Brain heart infusion broth (BHIB):

This medium was used to activate, grow and as stock culture medium for *Salmonella* isolates. It was prepared by dissolving (37 gm) of medium in (1000 ml) DW, and adding 15 % glycerol, pouring into sterile test tubes and then sterilized by autoclaving. (MaFadden, 2000)

3.2.7.8 Xylose-Lysine Deoxycholate agar (XLD)

This medium was used to isolate and identify *Salmonella spp* from stool samples, prepared by dissolving 56.68 gm of powder in 1000 ml of sterile DW, heated to boiling with frequent agitation to completely dissolve the medium, then cooled to 45-50°C in water bath and poured into petridishes, then incubated in an incubator at 37°C for 24 hours to remove any contaminated plates (Dubey & Maheshwari, 2023).

3.2.7.9 S.S.agar (Salmonella-Shigella agar)

A differential, selective media used to isolate and identify *Salmonella spp* from stool samples, prepared by dissolving 63 gm of powder in 1000 ml of sterile DW, heated to boiling with frequent agitation to completely dissolve the medium, then cooled to 45-50°C in water bath and poured into petridishes, then incubated in an incubator at 37°C for 24 hours to remove any contaminated plates (Ray, B., & Bhunia, 2025).

3.2.7.10 Brilliance *Salmonella* Agar Base with supplement

Chromogenic media prepared by suspend 27 gm of powder in 500 ml of sterile DW, add the contents of 1 vial of *Salmonella* selective supplement, resuspended as directed. Mix well and sterilize by bringing to the boil with frequent agitation to completely dissolve the medium, then cooled 50°C mix well and poured into petri dishes, then incubated in an incubator at 37°C for 24

hours to remove any contaminated plates (**Dubey & Maheshwari, 2023**).

3.2.7.11 Soyabean Casein Digest Agar

Prepared by suspend 40.0 gm of powder in 1000 ml of sterile DW, heated to boiling with frequent agitation to completely dissolve the medium, after that sterilized by autoclaving at 121°C, 15 pound/Inch² for 15 minutes, and media was incubated at 37°C for 24 hrs. to ensure they are contamination free. this media used as maintenance media (**Marinea, 2021**).

3.2.7.12 Blood agar base

Prepared by used 37 gm of powder blood agar base and dissolving it into (1L) of DW following the company's instructions boiling mixture to dissolve all components completely and sterilization by autoclaving at 121°C, 15 pound/Inch² for 15 minutes. The solution was cooled to approximately 50°C; 5 ml of sterile sheep blood was added aseptically for each 95 ml of medium and mixed well gently by shaking to minimize air bubbles formation. Finally, the media was poured into sterile Petri-dishes and left to solidify at 25°C, and after solidification, the plates were treated as above to ignore the contaminated plates and were kept at 4°C till use (**Collee et al., 2022**).

3.2.7.13 Urea agar

This medium was used to examine the ability of *Salmonella spp* to produce urease enzyme, prepared by dissolving (24 gm) urea agar base in (950 ml) DW, the pH was adjusted to 6.8, the medium then sterilized by autoclaving at 15 lbs/inch², 121°C for 15 minutes.

Fifty ml of sterile 40 % urea solution was added to the sterilized medium and mixed well. The Urea agar medium was distributed (under aseptic condition) in 5 ml amount as slant in sterile tubes, and allowed to solidify in a slop form (**Collee, et al ., 2022**).

3.2.7.14 Triple Sugar Iron (TSI)

This medium was used for identification of *Salmonella spp*, prepared by dissolving (64 gm) of medium in (1000 ml) DW and after autoclaving, the medium was distributed in 5 ml amount as slant in sterile tubes, and laid in a slant position till the medium solidified (**Barghouthi et al ., 2021**).

3.2.7.15. Simmon - citrate medium

This medium was used to examine the ability of *Salmonella* to utilize of sodium citrate as the carbon source, prepared by dissolving (24.28 gm) of medium in (1000 ml) DW and after autoclaving, the medium was distributed in 5 ml amount as slant in sterile tubes, and kept in slant position until solidification of the medium (**Barghouthi, 2021**).

3.2.7.16. Muller Hinton agar

Suspend 38.0 gm in 1000 ml distilled water .Heat to boiling to dissolve the medium completely sterilized cool to 45-50 c mix well and pour into sterile petri plates used for sensitivity test (**Hadi and medicine, 2020**).

3.2.2 . Preparation of solution:

3.2.2.1. Normal saline solution

About 8.5 gms of NaCl was dissolved in one liter of DW and then filtered and sterilized by autoclaving at 121°C, 15 lb/inch for 15 minutes, then cooled and stored in refrigerator in 4°C (**Collee et al ., 1996**).

3.2.2.2 .Iodine solution

Six gm of iodine crystals was mixed with 5 gms of potassium iodide and dissolved in 20 ml of DW and put in sterile container, then cooled and stored in refrigerator in 4°C (**Collee et al., 1996**).

3.2.2.3. Urea solution

It was prepared by dissolving 40 gms of urea crystals in one liter of distilled water, then sterilized by the Millipore filter (0.45 micrometer), cooled and stored in refrigerator in 4°C.

3.2.3. Reagents

3.2.3.1 Oxidase reagent

This reagent was prepared by dissolving 1g of tetramethyl p-phenylene diamin dihydrochloride in 100 ml of DW and kept in a dark bottle at 4 °C (**AL-Joda & Jasim, 2021**).

3.2.3.2 Kovac's reagent

It was prepared by dissolving 5gm of p-dimethlamine-benzaldehyde in 75ml of isoamyl alcohol, then 25 ml of HCl (6 %) was added, and stored in a glass - Stoppard bottle in refrigerator. It was used to detect the Indole production (**McFadden, 2000**)

3.2.3.3 Catalase reagent

It was prepared by adding 1 ml of 30% H₂O₂ to 9 ml of DW to obtain final concentration of 3%, , stored in a dark bottle (**López *et al* ., 2024**).

3.3. Identification by (Api 20E) system:

The bacterial isolates were further identified by using analytical profile index (API 20E) system according to the procedure suggested by the manufactured company (Bio- Merieux). This system is designed for the performance of 20 standard biochemical tests Lysine decarboxylase (LDC), Ornithine decarboxylase(ODC), citrate utilization(CIT), H₂S production, glucose(GLU), Mannitol (MAN), Inositol (INO), Sorbitol (SOR), Rhaminose (RHA), Melibiose(MEL) and Arabinose(ARA), β -galactosidase,(ONPG), Arginine dehydrolase (ADH), Urease(URE), Tryptophane deaminase(TDA), Indol production(IND), Acrtoin production(VP), Gelatinase(GEL), Sucrose(SAC), Amygdaline(AMY) and Cytochrome-oxidase(OX). The bacterial suspension was prepared for all the isolates from purified isolated colonies by using API suspension medium, and the turbidity was adjusted to 0.5 McFarland tube (1x10⁸ CFU/ml). By using a sterile Pasteur pipette, then bacterial suspension was transferred to the 20 microtubes and inoculated according to the manufactures' instructions. A period of incubation at 37°C for 24 hrs, the isolates were identified by using the API system (Numerical coding) for confirmatory identification at species levels (**MacFaddin, 2000**).

3.4. Maintenance of bacterial isolates

3.4.1.Short term storage

The isolates after definitive diagnosis were cultured on slants of brain heart infusion agar or Soyabean Casein Digest Agar incubated 37°C for 24 hours, then preserved at 4°C, they may be re-cultured monthly to maintain their viability and activity (for short term maintenance).

3.4.2.Long term storage

For long term maintenance, the definitive isolates were cultured on BHIB with glycerol 15% and incubated at 37°C for 24 hours, then after turbidity occurred, then maintained frozen for several months (**Sanderson and Zeigler, 1991**). Frozen stock cultures stored at -20°C were sub-cultured on fresh BHIA, incubated in aerobic condition at 37°C for 24 hours

3.5. VITEK for Confirmatory identification:

VITEK-2 system (Biomérieux, France) with a planned identification card was working in this study for confirmation. The 64-well card contains 43 colorimetric substrates for phenotypic identification species of bacterial, such as *S. Typhimurium* after purified onto selective media like XLD and incubated at 37°C for 24 hr. then, a suspension of the bacteria was prepared in saline (0.45% to 0.50% NaCl) put in a polystyrene tube to a density equivalent to a McFarland tube number 0.5. The density was determined using Vitek2 DensiChek spectrophotometer then, the tube and card were inserted into the Vitek2 cassette, and the card was auto-inoculated within the Vitek 2 instrument by a method depended on vacuum-release. Wells in the card are easily noticed, scanned and read each 15 minute, with a total number of incubation time which about 8 hours (**Gardner and Altman, 1995**) the result are listed in appendi

3.6. Conventional PCR Method detection (Molecular identification)

3.6.1. Preparing the primers

The primers were suspended depending on manufacturer's instructions by dissolving the lyophilized primers with nuclease-free water to form stock

solution with concentration of 100 μM (pmol/ μl) after spinning down. A concentration of 10 μM were use in work getting by add 10 μl from stock solution to 90 μl of nuclease-free water, Stored at 4°C. Primer working solution was prepared by diluting the stock solution with nuclease-free water, using the equation $C_1V_1 = C_2V_2$ to get final working solution for each primer pairs.

3.6.1.1. Culture isolates:

16 isolates divided to in each region 1 samples from local slaughter and one samples from imported chicken meat were grown in 5 ml BHIB broth with incubation for 18-24 h at 37°C.

3.6.1.2. DNA extraction from bacteria

3.6.1.3. DNA Extraction

1. For pellet calls, 1ml of overnight culture for 2min at 13000 rpm. Supernatant then discarded
2. The cell pellet was re-suspended completely in 200 μl of Buffer CL.
3. For protein digestion and cell lysis, 20 μl of Proteinase K solution (20 mg/ml) and 200 μl of Buffer CL was added to cell pellet, then the tube mixed vigorously using vortex and Incubated at 56°C for 30 min.
4. After incubation, 200 μl of Buffer BL was added to sample then the tube mixed vigorously using vortex and Incubated at 70°C for 30 min.
5. From absolute ethanol 200 μl was added to the sample, pulse-vortex to mix the sample thoroughly.
6. All of the mixtures were transferred to the mini-column carefully, then centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and the collection tube was replace with a new one.
7. From Buffer BW 600 μl was Added to the mini-column, then centrifuge

for 1 min at 6,000 x g above (>8,000 rpm) and the collection tube was replaced with a new one.

8. From Buffer TW 700 µl was applied. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). The pass-through was discarded and the mini-column was reinserted back into the collection tube.

9. The mini-column was Centrifuge at full speed (>13,000 x g) for 1 min to remove residual wash buffer, then the mini-column was placed into a fresh 1.5 ml tube.

10. Elute the genomic DNA by centrifugation at 13,000 rpm

3.6.1.4. Quantitation of DNA:

Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted Quantifluor Dye was mixed. After 5 min incubation at room temperature, DNA concentration values were detected.

3.6.1.5. Reaction Setup and Thermal Cycling Protocol

Reaction Setup was described in table (3-8) for each three targeting genes.

Table (3-9) showed Reaction Setup for three gene, *spy*, *spvb* and *hila*

No. of Reaction	11	rxn
Reaction Volume /run	20	µl
Safety Margin	5	%

3.6.1.6. Master mix preparation

The preparation of master mix was done according to manufacturing company that was describing in table (3-9)

Table (3-10) showed the volume of each components of master mix solution.

Master components	mix	Stock	Unit	Final	Unit	Volume	
						1 Sample	10.05 Samples
Master Mix		2	X	1	X	10	101.000
Forward primer		10	μM	1	μM	1	10.1
Reverse primer		10	μM	1	μM	1	10.1
Nuclease Free Water						5	50.5
DNA		10	ng/μl	10	ng/μl	3	
Total volume						20	
Aliquot per single rxn	17 μl of Master mix per tube and add 3 μl of Template						

3.7.1.7. PCR Program

The program steps of PCR include six steps; each step needs specific temperatures (thermo cycling) and specific time that was describing in table (3-11).

Table (3-11) showed the steps of PCR program and thermo cycling

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing C°	16SRNA	00:30	
	60		
Extension	72	01:00	
Final extension	72	07:00	
Hold	10	10:00	1

3.6.1.7. Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted

3.6.1.7.1. Solutions of PCR program

Ethidium bromid stain The final concentration of stain(5mg/ml)was prepared by dissolving 0.05 g of stain in 10 ml of distilled water then storing it in an opaque bottle until use (**Green and sambrook , 2012**)

3.6.1.8.2.Preparation of agarose

Preparation of agarose was done according to the protocol of the manufacture and according to the following steps:

1. 100 ml of 1X TAE was taken in a beaker.
2. 1 gm (for 1%) agarose was added to the buffer.
3. The solution was heated to boiling (using Micro Wave) until all the gel particles were dissolved.
4. 1µl of Ethidium Bromide (10mg/ml) was added to the agarose.
5. The agarose was stirred in order to get mixed and to avoid bubbles.
6. The solution was allowed to cool down at 50-60C°.

3.6.1.8.3. Casting of the horizontal agarose gel

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 minutes. The comb was carefully removed and the gel was placed in the gel tray. The tray was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel

3.6.1.8.4. DNA loading

PCR products were loaded directly For PCR product, 5µl was directly loaded to well. Electrical power was turned on at 100v/mAmp for 75min. DNA moves from Cathode to plus Anode poles. The Ethidium bromide stained bands in gel

were visualized using Gel imaging system.

3.6.1.8.5. Summary of Data Production

3.6.1.8.5.1. DNA Concentration (ng/μl)

DNA concentrations are explained in table (3-12) for each sample was used in molecular study.

Table (3-12) Explained Concentrations of DNA for each sample

Species	Sample	Conc./μl
Chicken thigh	01	20
Chicken thigh	02	15
Chicken thigh	03	22
Chicken thigh	07	12
Chicken breast	09	20
Chicken breast	12	20
Chicken breast	14	24
Chicken breast	17	15
Chicken breast	90	20
Chicken breast	101	21

3.7. DNA Sequencing and Bioinformatics Analysis

The PCR products of the 16S rRNA gene were purified using (eddbioprepar DNA Purification Kit) and sent to (Macrogen Inc., South Korea) for Sanger sequencing via DHL shipment. Bidirectional sequencing was performed using the same primers as the PCR assay.

Raw nucleotide sequences were received in ABI format and analyzed using BioEdit software (version 8.1). Sequences were trimmed to remove low-quality bases, and forward/reverse reads were assembled into consensus sequences. The consensus sequences were compared against the NCBI GenBank database using BLASTN to confirm taxonomic identity.

3.8. Gel Electrophoresis to analyze DNA

The method of (**Sambrooke *et al.*, 1989**) was adopted to prepare agarose gel, as shown in the steps below

1. Agarose powder (1 g) was dissolved in 100 ml of buffer 1, heated to boiling, and left until the mixture became clear.
2. After the mixture temperature reached 45-40°C, 5 milliliters of ethidium bromide dye was added to it.
3. Pour the liquid mixture into an agarose gel tray containing a comb at one end to create wells within the gel layer. Then, leave it to solidify at room temperature.
4. After the gel layer solidified, the comb was carefully removed and the tray was replaced in the sequencing apparatus. TBE (Tris boric acid EDTA buffer) was then added to the sequencing tank until it completely covered the gel layer to a height of approximately 1 cm.
5. 10 milliliters of previously prepared double-stranded DNA were added to each well in the gel layer. 5 milliliters of DNA ladder were then added to the well on the left side of the added samples to determine the sizes of the double-stranded DNA
6. The electrophoresis process was carried out by connecting the electrodes of the power supply to an electric current and operating at 150 milliamps for an hour.
7. When the blue loading solution reached approximately 2 cm before the end of the gel, the electrophoresis was stopped.
8. Using an ultraviolet transillumination device at a wavelength of 260 nm, the agarose gel layer was examined to identify DNA bands (PCR product nm and estimate their molecular size by comparing them with size indicator.

3.9 Statistical Analysis

The Statistical Analysis using the Chi –square test ,and the statistical significance was determined by the p-value,with a significance thershod set at <0.05.statistical analyses were perform using spss software version (Wagner,2019)

Chapter four

Results and Discussion

4. Results and Discussion

4.1. Occurrence of *Salmonella* spp. by using culture assay

According to the chi-squared analysis, the techniques used to detect *Salmonella* spp. in chicken showed a significant difference ($p < 0.001$). Table 4-1 shows the distribution of *Salmonella* spp. isolates by chicken type and isolation method used; the conventional method revealed a total of 21/120 positive cases for *Salmonella* spp. *Salmonella* bacteria were isolated from chicken thigh and breast samples collected from different areas in Karbala city using selective culture media. The results showed variations in isolation rates between the different areas and .The result of culture on(XLD agar) was showed the highest percentage of salmonella contamination in locally slaughtered chicken meat(20%) while the lowest percentage of contamination in imported chicken meat(12%). Bacteria was found on XLD agar with small red colony with black center. figure (4-1)



Figure (4-1): Isolated *Salmonella* on XLD agar

The highest isolation rate was Statistical analysis revealed statistically significant differences between the isolation rates in the different areas and chicken species studied. The identity of the bacterial isolates was confirmed through morphological and biochemical characteristics(Table 4-1) Figure (4-2).

Table1 (4-1): Occurrence of *Salmonella* spp. in local, imported chicken meat by culture assay

Location	Local Chicken No. 60		Imported chicken No. 60		Total No. 120	
Culture	No. of positive	No. of negative	No. of positive	No. of negative	No. of positive	No. of negative
Karbala city	12	48	9	51	21	79
Percentage	(20%)	(80%)	(12%)	(88%)	(17.5%)	(82.5%)

P value and statistical significance

Test

Chi-square

Chi-square

0.3649

DF

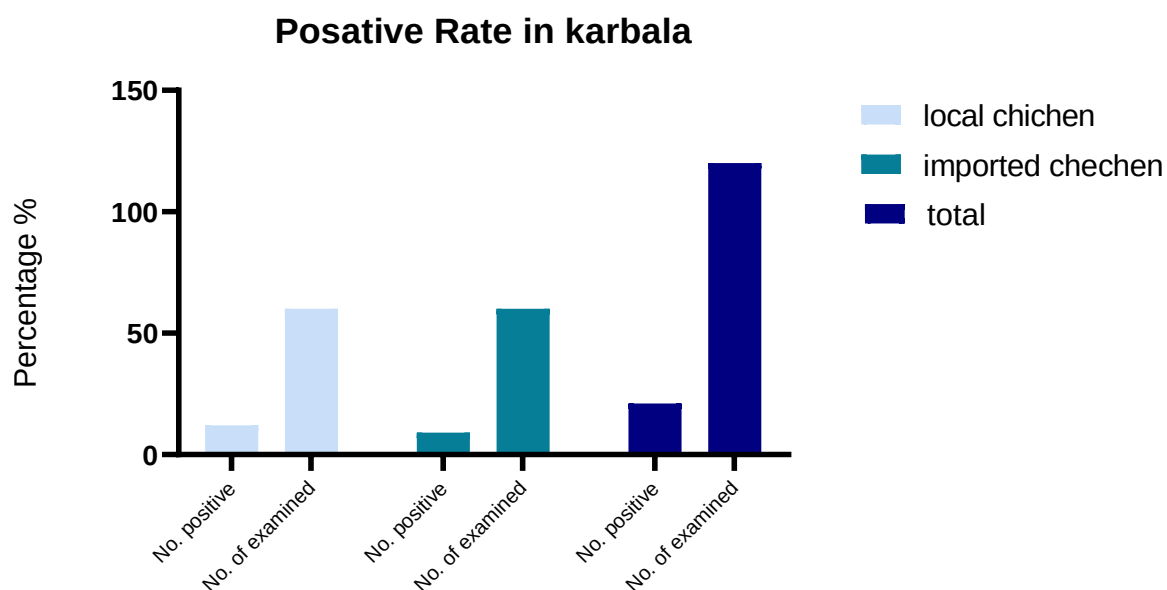
2

P value

0.8332

P value summary

ns

**Figure (4-2): Occurrence of *Salmonella* spp. in local, imported chicken meat by culture assay**

This determination of *Salmonella* spp. in chicken products using molecular biology techniques, such as PCR, is the first field study conducted in an area such as districts of Karbala, which is the main supplier of chicken in Iraq. The chicken production industry is an important sector in the country (**Ahmed et al ., 2020**).

The importance of producing safe products lies in the ability to market them with a certain degree of certainty about their origin and sanitary quality, which translates into a reasonable level of consumer confidence in the products they purchase. Furthermore, it increases the likelihood of successfully accessing increasingly competitive and demanding markets (**Rortana et al ., 2021**). The presence of pathogenic microorganisms in chicken and the diseases they cause is one of the essential and growing problems in public health, due to their increasing frequency, the emergence of new forms of transmission, the emergence of vulnerable population groups, and the socioeconomic impact they cause (**Al-Shafee et al ., 2024**).

When comparing the results obtained in this study using the conventional method with other studies conducted in kerbala and other Iraqi countries that used the same methodology, they were found to be similar to those reported by (**Alfarras et al .2022**), who isolated *Salmonella* spp. in 8 (7.07%) of raw milk from the Maysan Iraq. However, they are lower than those of (**Al-Shafee et al . 2024**), who isolated *Salmonella* spp. in 8.66% of chicken product in the Wasit region, and those of in Baghdad, who isolated *Salmonella* spp. in 10 % of Baghdad market. Additionally, this study with agreement of (**Mohammed & Habib, 2025**) who reported that 26% of fresh retail chicken meat samples and 39% of raw chicken carcasses were contaminated with *Salmonella* spp. In contrast, in Mexico(**Bello et al . 2023**) isolated *Salmonella* spp. in 32.44% of raw meat samples, and (**Hernández et al . 2022**) isolated *Salmonella* spp. in 11% of bovine carcass samples.

4.2. Occurrence of *Salmonella* spp. by using PCR assay

According to the chi-squared analysis, the techniques used to detect *Salmonella* spp. in chicken showed non-significant difference ($p > 0.001$). Table 2 shows the distribution of *Salmonella* spp. isolates by chicken type and isolation method used; the PCR assay revealed a total of 16/120 positive cases for *Salmonella* spp. *Salmonella* bacteria were detected in chicken thigh and breast samples collected from different areas in Karbala using PCR technique. The results showed that the total number of positive isolates was 16 out of 120 samples tested (13.3%). The highest positivity rate was recorded in locally chicken meat (15% from 9 isolated) while in imported chicken meat recorded (11.5% from 7 isolated). Statistical analysis using the chi-square test showed no statistically significant differences between the different areas (P value = 0.8955). It is worth noting that all positive isolates were confirmed through culture and biochemical testing for the *Salmonella* spp.

Table(4- 2)(figure 4-2)

Table 4-2: Occurrence of *Salmonella* spp. in local, imported chicken meat by PCR assay

Location	Local Chicken No. 60	Imported chicken No. 60	Total No. 120
Region	No. of positive	No. of positive	No. of positive
Karbala city	9	7	16
Percentage	(15%)	(11.5%)	(13.3%)

P value and statistical significance

Test	Chi-square
Chi-square	0.2206
DF	2
P value summary	ns
P value	0.8955

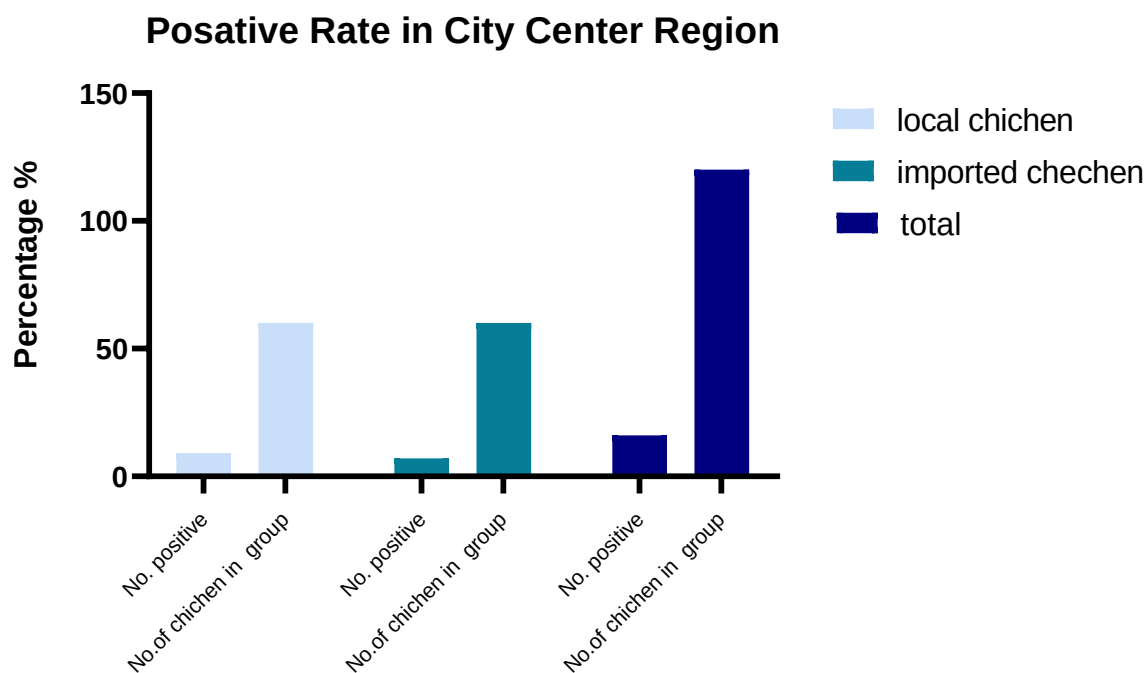


Figure (4-3): Occurrence of *Salmonella* spp. in local, imported chicken meat by PCR assay

Regarding the use of PCR for the rapid detection of *Salmonella* spp. directly from food, a comparison of the results obtained with the RT-PCR technique with other studies conducted worldwide found that they were lower than those reported by (Malorny *et al* . 2004), We *et al* ., 2021) and (He *et al* . 2023), who isolated *Salmonella* spp. in 25.5%, 11%, and 30.9% of food samples from the United States and Canada, respectively. However, they are very similar to the results obtained by (Sasaki *et al* . 2021), who isolated *Salmonella* spp. in 10% of raw meat samples in Japan.

4.3. Isolated *Salmonella* Species

The *Salmonella* species isolated from various aspects of the broiler breeder project environment were distributed among four species, with the following percentages: *S. Typhimurium*: 11 isolates (52%), *S. Enterditis*: 7 isolates (33%), *S. Goodwood*: 2 isolates (10%), *S. Senftenberg*: 1 isolate (5%) as shown in table (4-2) and figure (4-4)

Species	<i>S. Typhimurium</i>	<i>S. Enterditis</i>	<i>S. Goodwood</i>	<i>S. Senftenberg</i>	No. of examined
No.	11	7	2	1	20
Percentages	52%	33%	10%	5%	100%

Table (4-3) *salmonella* species

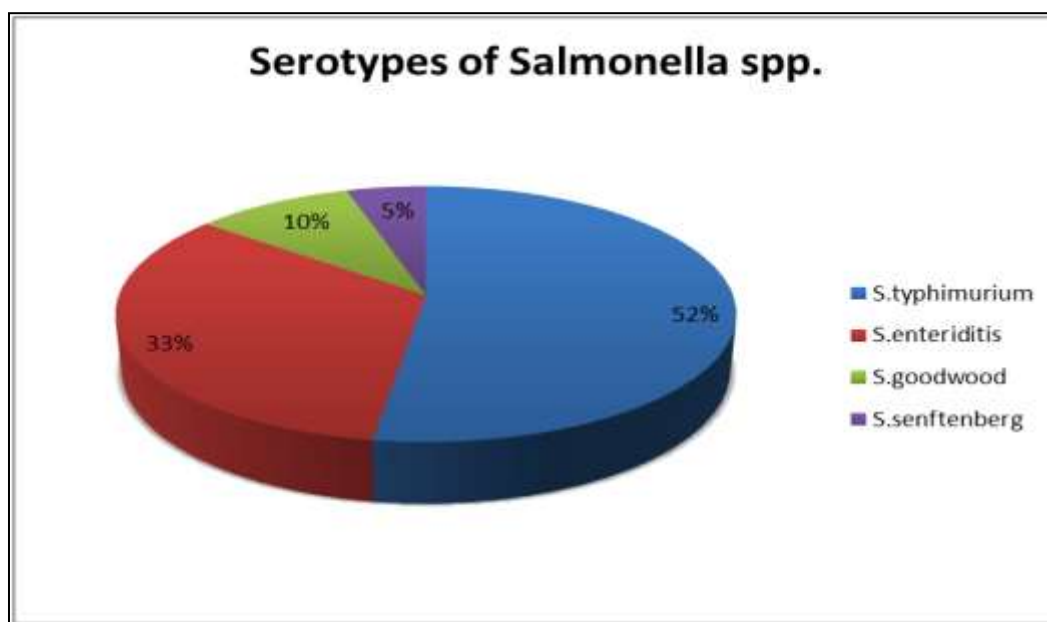


Figure (4-4) shows the numbers and percentages of *Salmonella* species isolated from the broiler breeder project environment.

The predominance of *Salmonella Typhimurium* and *Enteritidis* among isolates from poultry sources is concordant with widespread reports globally that identify these serovars as leading causes of human salmonellosis linked to poultry consumption (**Khan et al ., 2018**). The observed frequency of *S. Typhimurium* ($\approx 52\%$) parallels findings from other regional studies in developing countries, where *S. Typhimurium* often accounts for a large proportion of isolates, sometimes close to half of the total isolates recovered. Similarly, the isolation of *S. Enteritidis* at about one-third of isolates is notably consistent with epidemiological data in poultry meat, being often the second most common serovar in retail chicken especially in settings with less stringent control measures.

Detection of *S. Goodwood* and *S. Senftenberg*, though infrequent in this small dataset, highlights the diversity of *Salmonella* serotypes circulating in the supply chain. Occurrence of less common serovars has been reported previously even at low prevalence in retail and production settings (**Goldblum et al ., 2024**).

This suggests potential environmental or cross-contamination sources warranting further investigation, the relative proportions of serotypes in this local study from Karbala are consistent with patterns seen in other Iraqi poultry investigations, in which *S. Typhimurium* and *Enteritidis* have been commonly described as dominant serovars in poultry farms and slaughterhouses (**Mokgophi et al ., 2021**)

The presence of multiple serovars in a small number of samples implies that control measures such as feed and water hygiene, slaughter sanitation, and breeder stock screening could benefit from enhancement in the local context, in conclusion, the results indicate that *S. Typhimurium* and *S. Enteritidis* are the dominant serovars recovered from the sampled chicken sources in Karbala, consistent with

international and regional patterns of poultry-associated *Salmonella* contamination. The occasional recovery of rarer serovars suggests that surveillance should remain broad in scope. These findings highlight the need for targeted interventions along the poultry production and supply chain to reduce the risk of zoonotic transmission (Kanaan, 2023).

The results indicate that *S. Typhimurium* and *S. Enteritidis* are the dominant serovars recovered from the sampled chicken sources in Karbala, consistent with international and regional patterns of poultry-associated *Salmonella* contamination. The occasional recovery of rarer serovars suggests that surveillance should remain broad in scope. These findings highlight the need for targeted interventions along the poultry production and supply chain to reduce the risk of zoonotic transmission (Shi *et al.* , 2023).

4.4. Isolation, cultivation and characterization of *Salmonella* by

The *Salmonella-Shigella* agar was inoculated with all the samples, and the colonies were of circular with smooth convex and pale color and a black center. Next of all sub-cultured with selective media such as Xylose-Lysine-Deoxycholate (XLD) agar to confirm the xylose fermentation, lysine decarboxylation and the production of hydrogen sulfide whilst this bacterium was found on XLD agar with a small red colony and a black center Figure (4-4). Thereafter Biochemical tests were applied like the Urease test that did not give *Salmonella* the ability to have urea hydrolysis. Besides that, *Salmonella* gas fermenting capability and capability of producing the hydrogen sulfide gaseous element was also determined through the use of the TSI test. The potential that *Salmonella* had to metabolize citrate was a scertained through Simmons-Citrate test Figure (4-5).table (4-4)

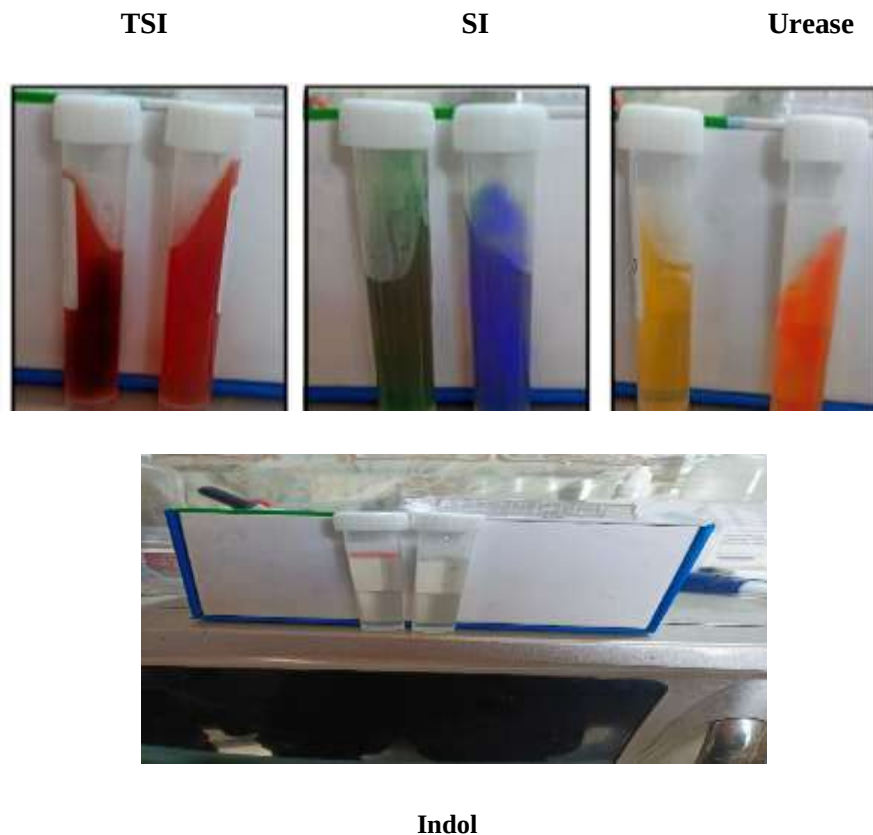


Figure (4-5) results of biochemical tests

Test	Result
Indol	-ve
Urease	-ve
TSI	+ve
Simmo-Citrat	+ve

Table(4-4) identification of *salmonella* Spp by biochemical tests

4.5. Molecular identification of *Salmonella*

4.5. 1. Polymerase chain reaction results of *Salmonella* isolates

All the 21 isolates were subjected to the PCR by using specific primers with amplicon size 1400 bp. The results from this molecular technique were promising due to the extraordinarily high rate of *Salmonella enterica* isolates (16 isolates) containing this genomic region. The electrophoresis results are demonstrated in Figure (4-5).

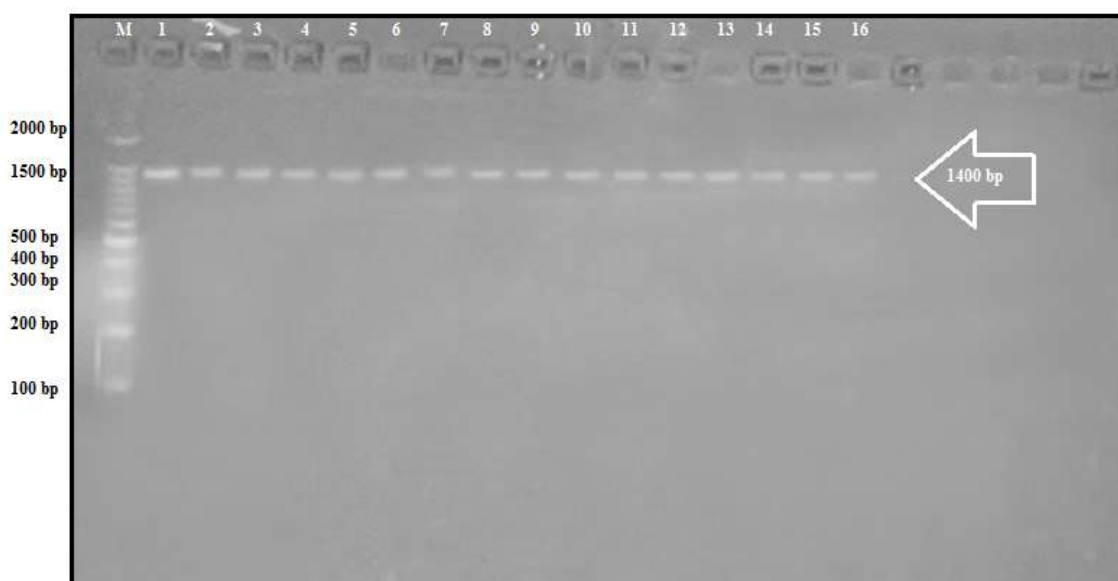


Figure 4-6: Gel electrophoresis of the ISRs region among *Salmonella* isolated from poultry samples. Lane (M) DNA Marker (1500 bp), lane 1 to 16 16sRNA gene positive

The 16S ribosomal RNA (16S rRNA) gene is one of the sequences of the rRNA that are critically important in the taxonomy and identification of bacteria, as even *salmonella* bacteria exhibit an extremely conserved nature that makes the 16S rRNA gene very important in the identifications. The 16 S rRNA gene is common and has served as a molecular marker to prosecute and analyze phylogeny of *Salmonella*.

Sequencing and amplification of this gene have been used to selectively differentiate *Salmonella* even to its closest members of the family of Enterobacteriaceae. It has been regarded as a gold standard of bacterial identification, especially that has been commonly used in food safety and clinical microbiology laboratories (**Fadlalla et al., 2021**).

It has been proved that 16S rRNA gene has conserved and variable regions that enable the design of universal primer and species-level discrimination respectively. polymerase chain reaction (PCR) has targeted the gene to facilitate the rapid detection of *Salmonella* spp. in different sample including food, water, clinical, and environmental sources. As in the case of poultry meat *Salmonella enterica* was detected using PCR amplifying the 16S rRNA gene, with a high degree of sensitivity indicated as compared to traditional culture-based methods (**Singh et al ., 2021**).

The same findings were agreed upon in a research in which PCR was used to identify the presence of *Salmonella* isolates in food samples using 16S rRNA sequencing, as the latter approach proved to be effective and sufficiently specific (**Ahmed et al ., 2020**). In a further study, the phylogenetic assessment was possible using 16S rRNA sequences and prospective identification between the *Salmonella enterica* subspecies, thus contributing to the epidemiological interest in the gene (**Zhang et al., 2022**).

Besides, the superiority of 16S rRNA sequencing was ascertained to be more accurate than biochemical tests, particularly in the identification of both slow-growing and atypical *Salmonella* strains. It is also using this molecular method that scientists have been able to provide evidence of genetic variations within the *Salmonella* serotypes, which could help in studying evolutionary changes and help in identifying outbreaks (**Khan et al ., 2020**).

4.6. Sequencing and sequence analysis of *Salmonella* isolates

Among the 16 positive isolates of the *Salmonella* genus, only one serotypes were identified by using DNA based sequence search. the sequence of the isolates were submitted to the BLASTn search tool of NCBI (www.ncbi.nlm.nih.gov). *Salmonella enterica* was identified with accession number (PV355886.1). as shown in following

LOCUS PV355886 458 bp DNA linear BCT 26-MAR-2025
 DEFINITION *Salmonella enterica* strain IQ1 16S ribosomal RNA gene, partial
 sequence.
 ACCESSION PV355886
 VERSION PV355886.1
 KEYWORDS .
 SOURCE *Salmonella enterica*
 ORGANISM *Salmonella enterica*
 Bacteria; Pseudomonadati; Pseudomonadota; Gammaproteobacteria;
 Enterobacterales; Enterobacteriaceae; *Salmonella*.
 REFERENCE 1 (bases 1 to 458)
 AUTHORS Rawaa,H.F., Ali,R.A. and Hayder,M.A.
 TITLE Direct Submission
 JOURNAL Submitted (21-MAR-2025) Department of parasitology, College of
 veterinary medicine, Karbala, Iraq, karbala 50061, Iraq
 COMMENT Sequences were screened
 for chimeras by the submitter using
 SnapGene Viewer 8.0.

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##FEATURES Location/Qualifiers source 1..458

/organism="Salmonella enterica"

/mol_type="genomic DNA"

/strain="IQ1"

/isolation_source="meat"

/host="Gallus gallus"

/db_xref="taxon:28901"

/geo_loc_name="Iraq: Karbala"

/collection_date="Jan-2025"

/collected_by="Rawaa and Ali" rRNA <1..>458

/product="16S ribosomal RNA"ORIGIN

1 TCCCTAGCTG GTCTGAGAGG ATGACCAGCC ACACTGGAAC TGAGACACGG TCCAGACTCC
 61 TACGGGAGGC AGCAGTGGGG AATATTGCAC AATGGGCGCA AGCCTGATGC AGCCATGCCG
 121 CGTGTATGAA GAAGGCCTTC GGGTTGTAAG GTACTTTCAG CGGGGAGGAA GGTGTTGTGG
 181 TTAATAACCG CAGCAATTGA CGTTACCCGC AGAAGAAGCA CCGGCTAACT CCGTGCCAGC
 241 AGCCGCGGTA ATACGGAGGG TGCAAGCGTT AATCGGAATT ACTGGGCGTA AAGCGCACGC
 301 AGGCGGTCTG TCAAGTCGGA TGTGAAATCC CCGGGCTCAA CCTGGGAAC GCATTCGAAA
 361 CTGGCAGGCT TGAGTCTTGT AGAGGGGGGT AGAATTCCAG GTGTAGCGGT GAAATGCGTA
 421 GAGATCTGGA GGAATACCGG TGGCGAAGGC GGCCCCCT

4.7. Evaluation of antibiotic susceptibility test

This study was also conducted to highlight the current antibiotic- resistant profile of 21 *Salmonella* enterica isolates in order to screen the prevalence of antibiotic resistance in the *Salmonella* population in the chicken, and the effect of this on public health. All 21 isolates were tested for their susceptibility to 8 antimicrobial drugs and classified as resistant, and susceptible, Figure (4-7) table (4-6).

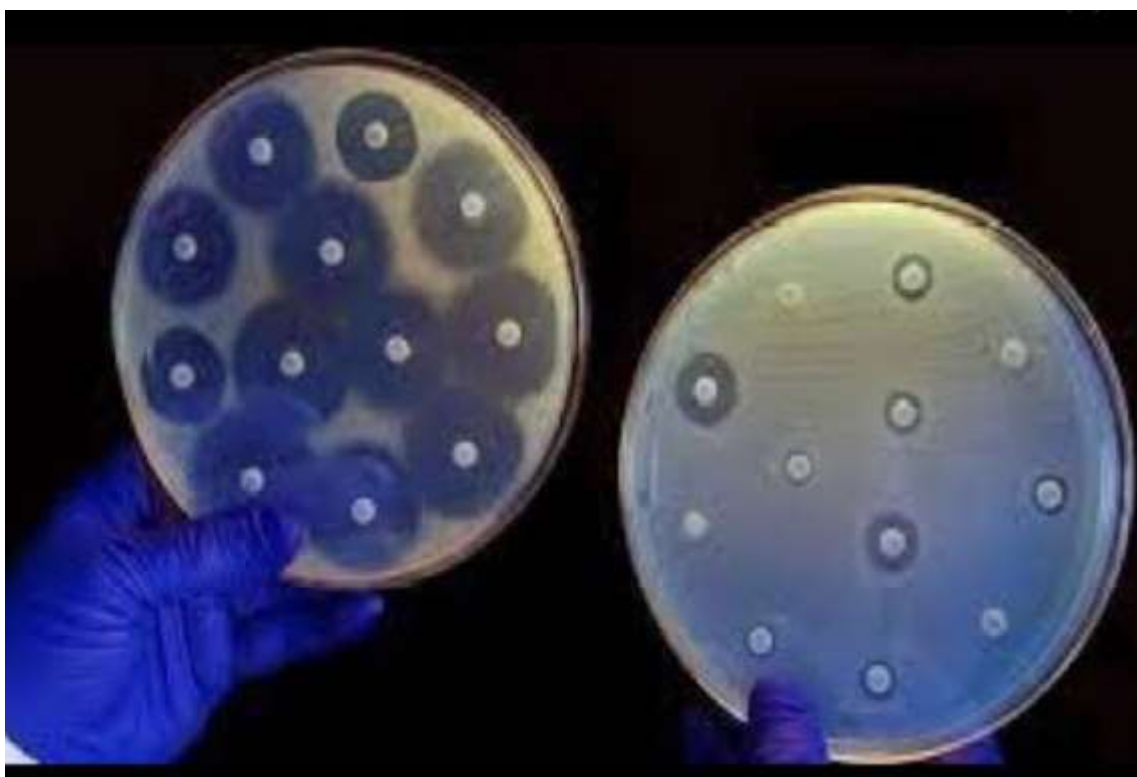


Figure 4-7: This figure demonstrates the extreme resistance of some *Salmonella* isolates according to the antibiotic susceptibility test

The pattern of resistance that was seen through the *Salmonella* isolates in chicken meat of Karbala city in terms of antimicrobial resistance needs serious concerns in food pathogens. The fact that all the tested bacteria were resistant (100%) to both ampicillin and tetracycline is warning and is consistent with other

studies, in which these antibiotics have been accompanied by very high resistance among the *Salmonella* due to the common misuse of these antibiotics in poultry production (**Mir et al ., 2016**) (**Afema et al ., 2018**). The resistant-high level of chloramphenicol (90.47%) is compared to the others where its resistance has been reported by some regions, it is possible that chloramphenicol is restricted in poultry in Iraq, whereas its usage in veterinary has many disputes because of its human health-related expected outcomes (**Thung et al ., 2018**).

A moderate rate of resistance to ciprofloxacin (32.81%) is a cause of concern since the fluoroquinolones play an important role in the treatment of salmonellosis in humans. This could be in terms of the resistance recorded towards the use of enrofloxacin that is cross-resistant to ciprofloxacin in poultry (**Eng et al ., 2015**). It was not surprising to discover the high rate of resistance to erythromycin (76.19%), because *Salmonella* intrinsically has less susceptibility to macrolides, and their application against *Salmonella* in poultry is severely restricted (**Garcia et al ., 2014**). The gentamicin (71.43%) and sulfamethoxazole (71.43%) resistance shows the same tendency as was seen in other studies perhaps due to their frequent application in the poultry farms (**Zaidi et al ., 2008**).

Mixed characteristics of the resistance to doxycycline (52.39%) indicate inconsistent application in poultry, whereas nalidixic acid resistance (62%) can be a reminiscent of quinolone usage in the past (**Parvin et al ., 2020**). The percentage of resistance to amikacin (81%) is in line with the limited application of this drug in poultry and the preservation of its effectiveness (**Hassan et al ., 2021**). Such results demonstrate the necessity to place more restrictions on the use of antibiotics in poultry farming and resistance patterns monitoring to minimize the potential health hazards. Table (4-6)

Table 4-5: Evaluation of antibiotic resistance among *Salmonella* isolated from broiler samples.

Antibiotic	Sensitive(S)	F%	Resistant (R)	F%
Ampicillin	0	(0%)	21	(100%)
Amikacin	4	(19%)	17	(81%)
Chloramphenicol	19	(90.47%)	2	(9.53%)
Ciprofloxacin	16	(76.19%)	5	(32.81%)
Doxycycline	10	(47.61%)	1	(52.39%)
Erythromycin	5	(32.81%)	16	(76.19%)
Gentamicin	6	(28.57%)	15	(71.43%)
Nalidixic acid	8	(38%)	13	(62%)
Sulfamethoxazole	6	(28.57%)	15	(71.43%)
Tetracycline	0	(0%)	21	(100%)
	P<0.01			

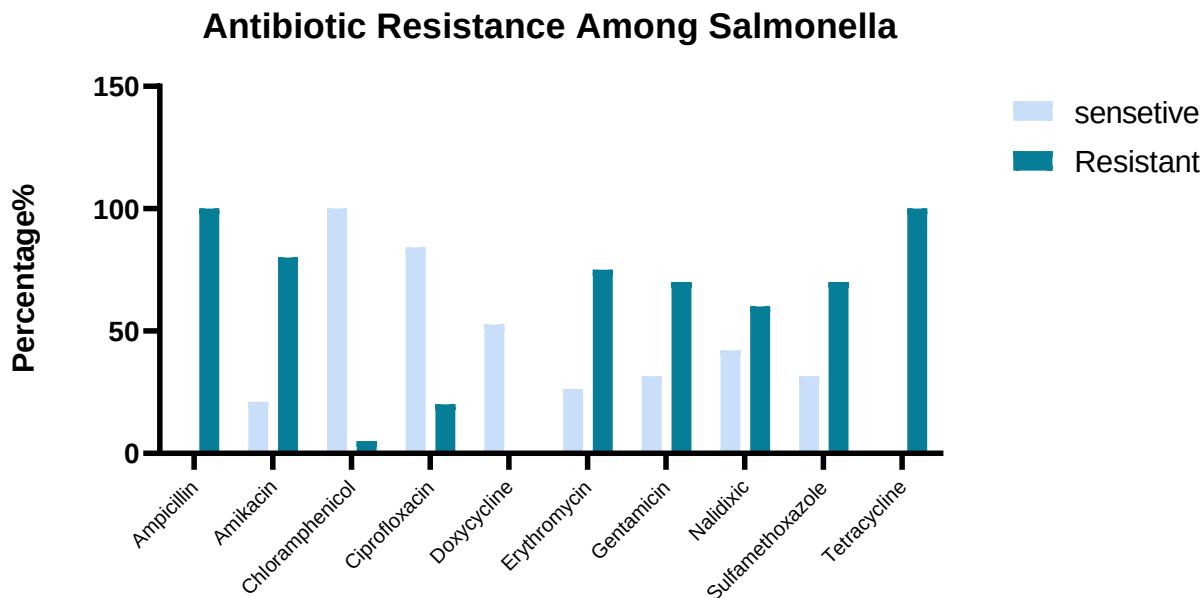


Figure 4-8: Evaluation of antibiotic resistance among *Salmonella* spp isolated from chicken meat samples.

4.8. Phenotypic detection of Efflux pumps

Efflux pumps were detected in 21 *Salmonella* isolates using the ethidium bromide–agar cartwheel method (EtBr CW) using ethidium bromide stain as a phenotypic marker. Thus, the efflux pump activity against ethidium bromide was detected in 16 (of 21) *Salmonella* isolates, which implies the efflux related resistance mechanisms existing highly prevalent in the strains. This observation implies that efflux pumps could play a role in causing antimicrobial resistance in *Salmonella* of chicken meats and this could be the basis of resistances among various drugs namely; tetracycline, erythromycin and ciprofloxacin (Alenazy *et al.* , 2023).

Efflux pumps have been found to extrude many different antimicrobial compounds, dyes like the ethidium bromide being a common model of a substrate

used to characterize multidrug resistance (**Piddock, 2006**). The prevalence of efflux-positive isolates (76.19%) is compared with other studies which have described the part played by efflux systems, including AcrAB-TolC, in having the ability to resist antibiotic and biocide in *Salmonella* (**Webber et al ., 2013**). Ethidium bromide efflux was also detected, which implies the participation of the resistance-nodulation-division (RND) family pumps prevalent in Gram-negative bacteria multidrug resistance (**Blair et al ., 2015**).

The overall meaning of the findings is important, because not only are the efflux pumps associated with developed intrinsic resistance, but also permit higher-level resistance in combination with other mechanisms in cooperation, like target mutation or enzymatic drug deactivation (**Sun et al ., 2014**). The prevalence of efflux in this study can be attributed to the resistance profile to unrelated antibiotics thus the importance of efflux pump inhibitors may serve as an adjuvant treatment to enhance the activity of the administrated antibiotics (**Stavri et al ., 2007**).

Additional studies are suggested to describe the individual efflux systems at work and determine their relative role in clinical resistance. With regards to overcoming resistance among *Salmonella* isolates in poultry, it is possible to research the use of combination therapy of the efflux pump inhibitors to counter resistance to treatment (figure 4-7).

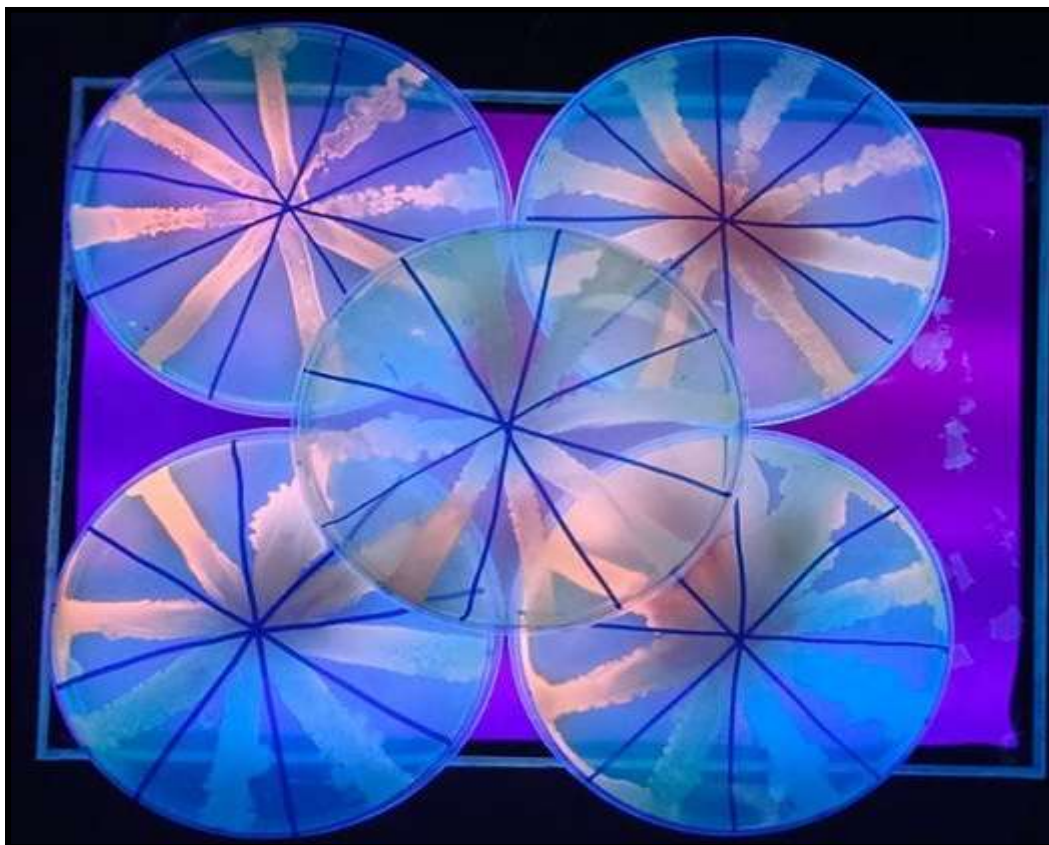


Figure (4-9) Efflux pupm of *Salmonella enteriace* against EtBr

4.9 Specificity and Sensitivity test of *Salmonella*

detection by culture and PCR assay. The results showed that the PCR test had a sensitivity of 76.19% (95% confidence interval, CI, 52.83% to 91.78%), indicating its ability to detect 76.19% of true positive samples confirmed by bacterial culture. The test's specificity was 100% (95% confidence interval, CI, 96.34% to 100%), confirming the absence of any false positives. The positive predictive value was 100% (95% confidence interval, CI, 79.41% to 100%), indicating that all positive PCR results were true. The negative predictive value was 95.19% (95% confidence interval, CI, 90.21% to 97.70%), indicating that the test was accurate in ruling out negative cases.

Table (4-6): Specificity and sensitivity of PCR and Culturing of *Salmonella entericae*

Test Results	<i>Salmonella</i> Present (Culture)	<i>Salmonella</i> Absent (Culture)	Total
Positive (PCR)	TP = 16	FP = 0	16
Negative (PCR)	FN= 5	TN= 99	104
Total	21	99	120

The advantages of PCR in the detection and identification of bacteria that cause foodborne diseases lie in its sensitivity, specificity, and ability to process large quantities of samples in a short time (**Rodríguez-Lázaro *et al.*, 2003**). In this regard, this study demonstrated high specificity and excellent speed due to the short time required for the detection of *Salmonella* spp., which is important information for the animal processing industry that could enter the international market. The results obtained regarding the time variable are similar to those reported by (**Alcazar *et al.*, 2018**), who used the PCR technique to detect *Salmonella* spp. in cheeses within 28 hours. However, (**Gaines *et al.*, 2022**) reported, using the same technique in just 3 hours, the detection of this pathogen directly from chicken carcasses, without pre-enrichment. Although the RT-PCR technique is the most specific and sensitive among those currently available, there are significant microecological differences between the animal processing industry and street foods, such as ground beef, potatoes with meat, and salted beef. Although dilutions of these foods were performed to rule out inhibitions, *Salmonella* spp. was detected more frequently using the conventional method than RT-PCR. This could be due to the presence of substances in the food that have an inhibitory effect on PCR.

Among the many compounds identified as having a negative effect on PCR are some polysaccharides, fats, metal ions, and proteins. Several authors have reported the existence of such inhibitors in food samples, which can act at different levels during the nucleic acid extraction and amplification process and eventually lead to false negatives (**Deng *et al.* ., 2023**).

Therefore, precautions must be taken to prevent or limit their effect on the reaction, primarily in the DNA extraction process (**Kasturi & Drgon, 2017**). In conclusion, the results are promising for the implementation of the molecular technique PCR as a valuable alternative for the detection of *Salmonella* spp. in chicken, due to its specificity and speed, especially when distributing safe products to the consumer market. Finally, regardless of the techniques, the high frequency of *Salmonella* spp. in food sold without sanitary controls in Montería is worrying and constitutes a risk to public health (**Malorny *et al.* ., 2004**).

Chapter five

Conclusion and recommendation

5-Conclusion and recommendation:

5-1Conclusion

Through the current study conclude the following:

1-*salmonella* entericea was in locally and imported chicken meat its widespread distribution in local market of Karbala

2. The result of culture on(XLD agar)showed the highest percentage of *salmonella* contamination in locally slaughtered chicken meat(20%) while the lowest percentage of contamination in imported chicken meat(12%)

3-The bacteriological examination revealed that *Salmonella* isolates obtained from different broiler production sources belonged to four species, distributed as follows: *S. typhimurium* (11 isolates, 52%), *S. enteritidis* (7 isolates, 33%), *S. Goodwood* (2 isolates, 10%), and *S. senftenberg* (1 isolate, 5%). On XLD agar, the colonies

4-Antibiotic susceptibility testing showed that all isolates (100%) were resistant to ampicillin and tetracycline. And low resistant to chloramphenicol (9.53%)

5-The current study found the PCR assay demonstrated a sensitivity of true positive (TP) was= 16 ,while the false negative=5 .therefore molecular detection of *salmonella* entericea proved to be an efficient and reliable method for accurate identification compared to conventional culture techniques

6-The high contamination levels observed emphasize the potential puplic health risk particularly in Iraq where local market and meat slaughterhouses often lack strict regulatory monitoring for local products.

5-2 Recommendations

Through the current study recommend the following:

1-Since was appearance the highest isolation rates in locally slaughtered chicken meat, priority monitoring and intervention programs in different regions of karbala.

2-Strengthen hygiene practices in slaughterhouses and markets in high-risk areas to reduce contamination.

3-Educate poultry handlers, sellers, and consumers in high-prevalence regions on safe handling and cooking practices.

4- Screening and certification processes at entry points to ensure imported poultry meets safety standards.

5-Regulate informal slaughtering practices or encourage their integration into supervised facilities to reduce uncontrolled contamination.

6 -Encourage or mandate vaccination of poultry flocks against *S. Typhimurium* and *S. Enteritidis*, since these are the most prevalent and epidemiologically important serovars.

7- Establish a regional AMR monitoring system to continuously assess resistance patterns in poultry pathogens.

8- Since PCR showed high specificity and reasonable sensitivity, it should be used alongside culture methods for faster and more accurate detection.

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Appendix

Appendix XCII

Result of using VITEK®2 for Confirmatory identification

bioMérieux Customer:		Public Health and Food Safety Lab Microbiology Chart Report		Printed March 25, 2025 3:17:27 PM AST	
Patient Name: Rawaa, Hussein				Patient ID: 23320254	
Location:				Physician:	
Lab ID: 23320254				Isolate Number: 1	
Organism Quantity:					
Selected Organism : Salmonella spp					
Source: ch				Collected:	
Comments:					
Identification Information		Analysis Time: 5.80 hours		Status: Final	
Selected Organism		98% Probability		Salmonella spp	
ID Analysis Messages		Bionumber:		0015610445566210	
Biochemical Details					
2	APPA	-	3	ADO	-
10	H2S	+	11	BNAG	-
17	BGLU	-	18	dMAL	+
23	ProA	-	26	LIP	-
33	SAC	-	34	dTAG	-
40	ILATk	+	41	AGLU	-
46	GlyA	-	47	ODC	+
58	O129R	+	59	GGAA	-
4	PyrA	-	5	IARL	-
12	AGLTp	-	13	dGLU	+
19	dMAN	+	20	dMNE	+
27	PLE	-	29	TyrA	-
35	dTRE	+	36	CIT	+
42	SUCT	+	43	NAGA	-
48	LDC	+	53	IHISa	-
61	IMLTa	-	62	ELLM	-
7	dCEL	-	9	BGAL	-
14	GGT	-	15	OFF	+
21	BXYL	-	22	BAlap	-
31	URE	-	32	dSOR	+
37	MNT	-	39	5KG	+
44	AGAL	+	45	PHOS	+
56	CMT	+	57	BGUR	-

Result of (API 20)



API® 20 E

CE 87223 C

REF: _____

Origine / Source / Herkunft /
 Origen / Origen / Προέλευση /
 Ursprung / Oprindelse / Pochodzenie: *Salmonella spp.*

BIO-MERIEUX

1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ONPG	ABT	LDC	ODC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC	LDC
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	

Autres tests / Other tests / Andere Tests /
 Otras pruebas / Altri test / Outros testes /
 Άλλες εξετάσεις / Andra tester /
 Andre tests / Inni testar:

Ident. / Ταυτοποίηση: *7 704552*

الخلاصة

الخلاصة

أجريت هذه الدراسة لتحقيق في انتشار بكتريا السالمونيلا في لحوم الدجاج. بما في ذلك لحوم الدجاج المذبوحة محليا ولحوم الدجاج المستوردة. بدأ العمل من سبتمبر 2024 لغاية يناير 2025. تم جمع 120 عينة من لحوم الدجاج من مناطق مختلفة في مدينة كربلاء ، العراق. تم تقسيم العينات إلى فئتين، 60 عينة لكل نوع: تم جمع لحوم الدجاج المذبوح محليا (ع=30) ولحوم الدجاج المستوردة (ع=30) ان أجزاء الدجاج التي تم عزلها هي (الفخذ والصدر) تمت إزالتها بطريقة معقمة باستخدام مشروط معقم ووضعها في أكياس معقمة تحمل علامات فردية. تم نقل جميع العينات في ظروف مبردة (4°C) إلى المختبر في غضون 24 ساعة لتحليلها لاحقا . تم تحليل 120 عينة باستخدام طرق الزرع التقليدية والاختبارات الكيميائية، ثم تأكيدها بجهاز VITEK2 في حين جرى التشخيص الجزيئي بواسطة تقنية PCR مستهدفه منطقة 16S-23S حيث ظهر حزم تضخيم بحجم 1400 زوج قاعدي في العينات الموجبة .

أظهرت نتيجة الزرع اعلى نسبه تلوث في لحوم الدجاج المذبوحه محليا بنسبة 20% بينما اقل نسبة تلوث في لحوم الدجاج المستوردة بنسبة 12%. كشف الفحص البكتريولوجي أن عزلات السالمونيلا التي تم الحصول عليها من مصادر إنتاج اللحم المختلفة تنتمي إلى أربعة أنواع ، موزعة على النحو التالي *S.Typhmuri* (11 عزلة ، 52%) ، *S.Enteriditis* (7 عزلات ، 33%) ، *S.Goodwood* (2 عزلات ، 10%) *S.Senftengberg* (1 عزل ، 5%). على ميديا ال (XLD)، ظهرت المستعمرات كمستعمرات حمراء صغيرة مع مراكز سوداء.

بالنسبة لنتائج اختبارات الكيمياء الحيوية. حيث ان اختبار اليورياز السالمونيلا تكون سالبه لهذا الاختبار أي لا تنتج انزيم اليورياز . وبالإضافة إلى ذلك اظهرت السالمونيلا إنتاج كبريتيد الهيدروجين في اختبار (TSI). وأظهر توزيع الحالات الإيجابية أن أعلى نسبة سجلت في لحوم الدجاج المحلية أكثر من لحوم الدجاج المستوردة.

سجلت النسب في التحليل الجزيئي (PCR) عن نتائج إيجابية للغاية ، حيث كانت عزلات السالمونيلا 16 عزلة، وتم تسجيل نسبة اللحوم المذبوحة محليا 15 ٪ بينما كانت نسبة لحم الدجاج المستورد 11.5 ٪. سجلت نتائج اختبار الحساسية للمضادات الحيوية أن جميع العزلات كانت مقاومة Ampicillin- Tetracycline بنسبة 100 ٪ . كما تم تسجيل اقل مقاومة chloramphenicol بنسبة (9.53) ٪ .

ووجدت الدراسة الحالية ان فحص الحساسية والخصوصية للسالمونيلا في PCR إيجابية حقيقية TP = 16

عينة، في حين أن السلبية الكاذبة كانت=5 عينات .

تشير نتائج هذه الدراسة على الانتشار الواسع لبكتريا السالمونيلا المعوية في منتجات اللحوم المتوفرة في سوق كربلاء وقد أظهرت البكتريا قدرة التكيف مع درجات الحرارة المنخفضة مما يشير الى قدرتها على البقاء على قيد الحياة في ظل الظروف تخزين او معالجه غير مثاليه للأغذية. وقد وفر الكشف الجزيئي بتقنية PCR وسيلة دقيقة وسريعة للكشف عنها. فيما تؤكد بيانات الانتشار على ضرورة تعزيز إجراءات النظافة والرقابة في منظومات انتاج وتوزيع الغذاء للحد من المخاطر الصحية العامة.



جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة كربلاء /كلية الطب البيطري
فرع الصحة العامة البيطرية

التحري عن التلوث البكتيري بانواع السالمونيلا في لحوم الدجاج المحلية والمستوردة في
محافظة كربلاء

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات درجة الماجستير في الطب
البيطري / الصحة العامة

اعداد الطالبة

رواء حسين فخري

بأشراف

أ.د / حيدر علي محمد

أ.م.د / علي رضا عبد

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