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Molecular Study of Some Virulence Genes of *Salmonella Typhimurum* Isolated from Pigeons in Thi-Qar Province

A Thesis

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in Veterinary Sciences (**Microbiology**)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{وَمَا مِنْ دَابَّةٍ فِي الْأَرْضِ وَلَا طَائِرٍ يَطِيرُ بِجَنَاحَيْهِ
إِلَّا أُمَّةٌ أَمْثَالُكُمْ ۚ مَا فَرَّطْنَا فِي الْكِتَابِ مِنْ شَيْءٍ ۚ ثُمَّ
إِلَىٰ رَبِّهِمْ يُحْشَرُونَ}

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Dedication

To my dear family; my mother and my father
My wife and my sons
and every one who supported me.

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Summary

One of the most prominent types of zoonotic pathogenic bacteria is *Salmonella* spp; specifically *Salmonella Typhimurium*. Pigeons are reservoirs and carriers of these pathogens; they can defecate the organisms without developing any evident clinical symptoms, thus resulting in higher environmental contamination rates and further spreading of the infection among other avian species and livestock.

As pigeons live both in urban and rural areas, they become very relevant in the epidemiology of salmonellosis. Apart from that, one should note the emergence of the resistant to antimicrobials *Salmonella* strains, which have become a major public health issue, reducing the range of treatment options and promoting horizontal transfer of resistance genes. Therefore, it is necessary to conduct the accurate identification of *Salmonella Typhimurium*, its antibiotic resistance profile, and epidemiological distribution among pigeons.

To begin with, the samples were enriched in Selenite F broth. Following this, the isolates were cultured on selective media. *Salmonella-Shigella* (SS) agar and Xylose Lysine Deoxycholate (XLD) agar. The conventional characteristics of this pathogen included coloration as pale but with black centers due to the production of hydrogen sulfide, with microscopy revealing Gram-negative bacilli.

The isolate were identification by the VITEK 2 Compact system, which gave a high confirmation rate of 98% for *Salmonella* spp. A total of 100 rectal swab samples were collected and examined for bacterial pathogens. The molecular technique that was performed included the use of Polymerase Chain Reaction (PCR) targeting the *invA* gene (284 bp), which was detected in 100% of isolates. Further identification of the *stm* gene (523 bp) revealed

that all the isolates were found to be *Salmonella Typhimurium*. The total prevalence of the pathogen was 5% (5/100). On the other hand, *Escherichia coli* was found to be the predominant isolate at 70%, *Klebsiella spp.* (15%) and *Enterobacter spp.* (10%).

According to age, the presence of 80% was detected in young pigeons with age ranging from 1 month to 2 years, meaning that pigeons were more vulnerable at younger ages. As far as sampling sites are concerned, the results obtained during sampling were statistically significant in all the sampling sites considered ($p = 0.05$). The positive sample were 3 from Nasiriyah, 1 Al-Gharraf and 1 Al-Shatra.

The antimicrobial susceptibility test, it was revealed that the isolates of *Salmonella Typhimurium* exhibited relatively high levels of antibiotic resistance. Complete resistance to these agents, which is 100%. against fosfomicin, ampicillin, cefotaxime, gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin, amoxicillin/clavulanic acid and imipenem. Then, Furthermore resistance levels were relatively high against levofloxacin, streptomycin and ceftazidime, with approximately 80%, of isolate showing resistance, doxycycline was found to be the least resistance antibiotic, where 60% of the isolates were resistant to it.

The molecular analysis, showed that all the isolates, 100%, had the antimicrobial resistance genes *tetA* with fragment size 576 bp related to tetracycline resistance, and *blaCTX-M* of 593 bp. The gene *blaCTX-M* that is strongly associated with β -lactam resistance. Besides, all isolates were multidrug-resistant (MDR), which is 100%, whereas 60% were extensively drug-resistant (XDR). Overall, these results indicate that the high levels of resistant *Salmonella Typhimurium* have been isolated from pigeons and actually, it can be important in public health concern.

In conclusion, it becomes apparent from the current study that there are highly resistant *Salmonella Typhimurium* strains in pigeons which can cause these birds to act as carriers of zoonotic antimicrobial-resistant pathogens. Therefore, continuous epidemiological surveillance together with stringent antimicrobial stewardship practices are strongly recommended.

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Abbreviations

Abbreviation	Meaning
%	Percentage
±	Plus-minus (standard deviation)
°C	Degree Celsius
µg	Microgram
µl	Microliter
aadA	Aminoglycoside resistance gene (adenylyltransferase)
dfrA	Trimethoprim resistance gene (dihydrofolate reductase)
DNA	Deoxyribonucleic acid
DO	Doxycycline
ESBL	Extended-Spectrum Beta-Lactamase
Fig.	Figure
fliC	Flagellin gene (phase 1 antigen)
G	Gram
GM	Gentamicin
HE	Hektoen Enteric agar
hilA	Hyperinvasion locus gene
invA	Invasion gene (conserved <i>Salmonella</i> marker)
ISO	International Organization for Standardization
LPS	Lipopolysaccharide
MDR	Multidrug-Resistant
Mg	Milligram

Abbreviation	Meaning
Min	Minute
ml	Milliliter
MLST	Multilocus Sequence Typing
mm	Millimeter
NTS	Non-typhoidal <i>Salmonella</i>
OIE	World Organisation for Animal Health (Office International des Épizooties)
P	P-value (probability value)
PCR	Polymerase Chain Reaction
R	Correlation coefficient (Pearson r)
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
SD	Standard Deviation
SPI	<i>Salmonella</i> Pathogenicity Island
SS	<i>Salmonella</i> – <i>Shigella</i> agar
ST	Sequence Type
Stm	<i>Salmonella Typhimurium</i> specific gene
Stn	<i>Salmonella</i> enterotoxin gene
strA	Streptomycin resistance gene
sul1	Sulfonamide resistance gene (dihydropteroate synthase)
TBE	Tris/Borate/EDTA buffer
tetA	Tetracycline resistance gene (efflux pump)
tetB	Tetracycline resistance gene (efflux pump)

Abbreviation	Meaning
TSI	Triple Sugar Iron agar
UK	United Kingdom
USA	United States of America
V	Volt
WHO	World Health Organization
WGS	Whole gene sequence
XDR	Extensively Drug-Resistant
XLD	Xylose Lysine Deoxycholate agar
χ^2	Chi-square

Chapter One

Introduction

1. Introduction

Enteric bacteriosis remains quite an important issue both for veterinary and human healthcare, primarily due to their widespread presence, as well as their ability to infect other animals, including humans, as their zoonotic potential increases the scope of the problem. As far as the typical pathogens are concerned, one of the bacteria, known to be significant food and environment-born ones is, without any doubt, *Salmonella* spp. They are known to infect a great number of animals, among which there are birds, domestic animals, and humans (Majowicz *et al.*,2010; Eng *et al.*,2015). Particularly, pigeons can be regarded as significant reservoirs because they live very close to humans and may infect them with some microorganisms by fecal contamination (Haag-Wackernagel *and* Moch, 2004; Abulreesh *et al.*,2019).

Salmonella is classified under the *Enterobacteriaceae* family, and its characteristics include being a gram-negative, non-spore-forming bacterium and having a rod shape (Jajere, 2019). Of the numerous strains, *Salmonella* enterica serovar *Typhimurium* is repeatedly highlighted among some of the most frequently cited non-typhoidal serovars causing infections in both animals and humans (Ferrari *et al.*,2019; Fàbrega *and* Vila, 2013). From an epidemiological perspective, it is significant because it is highly adaptable, persists in the environment, and is capable of causing gastrointestinal infection that may present itself in terms of mild enteritis, although sometimes progressing to severe systemic infection (Coburn *et al.*,2007).

However, traditional bacteriological methods remain crucial for the isolation and initial, rather crude, differentiation of *Salmonella* spp. Such an approach predominantly includes selective enrichment followed by differential culture media, namely Selenite F broth, *Salmonella*-Shigella (SS) agar, and Xylose Lysine Deoxycholate (XLD) agar (ISO 6579-1, 2017;

Andrews *et al.*,2016). A major diagnostic marker involves the ability of these microorganisms to form hydrogen sulfide with black centers in the colonies, making them distinguishable from other enteric bacteria despite similar appearance backgrounds (Cheesbrough, 2006).

In addition to the conventional methods, identification systems such as the VITEK 2 Compact have helped to achieve greater accuracy of bacterial identification, as well as speeded up the process compared to the conventional methods (Funke *et al.*,2019). These rely on biochemical profile patterns that are built into the system, leading to a high level of accuracy, and thus minimizing the chance of human error (Pincus, 2010). Nevertheless, despite all the benefits offered by automation, the molecular methods are increasingly being considered indispensable for obtaining accurate results (O'Hara, 2020).

PCR techniques, for instance, have revolutionized the diagnosis of *Salmonella* infections due to their focus on specific virulence-related and housekeeping genes. *InvA* has been extensively used as a broad target gene to detect *Salmonella* spp, while the *stm* gene is subsequently utilized for identification of *Salmonella Typhimurium* (Malorny *et al.*,2004; Shanmugasundaram *et al.*,2009). However, it should be noted that the role of these molecular diagnostics tools goes beyond "confirmation" of the presence of the pathogen; they can also predict its pathogenic capacity and serve as a predictor of its activity within the host (Elkenany *et al.*,2019).

Antimicrobial resistance among *Salmonella* strains is currently one of the major global health problems. As a matter of fact, this does not come as a surprise. However, misuse and abuse of antibiotics within the veterinary and agriculture industries have led to the development of antimicrobial-resistant strains (Founou *et al.*,, 2021; WHO, 2021). The presence of genes responsible for antimicrobial resistance, such as *tetA* (tetracycline resistance) and *blaCTX-M* (production of extended-spectrum β -lactamase), is significant

since this reduces treatment options and makes the entire treatment strategy challenging (Nguyen *et al.*,2019; Bevan *et al.*,2017).

Moreover, the appearance of MDR (multidrug-resistant) and XDR (extensively drug resistant) *Salmonella* is increasingly being reported worldwide. Such a development poses considerable challenges for infection control and public health efforts (Magiorakos *et al.*,2012). Many times, such pathogens possess multiple genes that allow them to survive after exposure to diverse antimicrobial compounds and in various environments (even harsh conditions) (Prestinaci *et al.*,2015).

The findings obtained from epidemiological investigations tend to indicate that factors such as geographic location, environmental sanitation, and host-related attributes do play a significant role in the prevalence of enteric bacteria infections (Roth *et al.*,2019). Among all these attributes, age is one factor that keeps on resurfacing in connection with prevalence rates, since young animals remain particularly vulnerable due to immature immune responses and regular exposure to contaminated environments (Wigley, 2018; Barrow and Freitas Neto, 2018).

It is important to determine the prevalence of enteric bacteria like *Escherichia coli*, *Klebsiella* spp; *Enterobacter* spp; and *Salmonella* spp. among pigeons in order to determine any potential public health hazards (Podschun and Ullmann, 1998). Although *E. coli* has been found to be the dominant bacteria, the presence of pathogenic and antibiotic-resistant bacteria necessitates regular monitoring and management (Roth *et al.*,2019).

Aim of study

1. To isolate and identify *Salmonella Typhimurium* from pigeons using conventional bacteriological methods, the VITEK® 2 Compact system, and polymerase chain reaction (PCR).
2. To investigate the distribution and prevalence of *Salmonella Typhimurium* isolates among pigeons according to age and sampling location.
3. To detect selected virulence genes (*invA* and *stm*) and antimicrobial resistance genes (*tetA* and *blaCTX-M*) in the identified isolates using PCR.
4. To evaluate the antimicrobial susceptibility profiles of the isolates and determine the occurrence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains

Chapter Two

Literature Review

2. Literature Review

2.1 Overview of *Salmonella Typhimurium*

There is a wide range of investigations that have been carried out by researchers regarding *Salmonella Typhimurium* because this non-typhoidal *Salmonella* serovar causes infections not only in various animals but also transmits diseases in humans and is responsible for severe foodborne outbreaks across the globe. Specifically, this particular serovar causes infection through contact with people who already have an infection or with the animals who have been infected and shows relatively high rates of infection among poultry and livestock and in food products contaminated (Coburn *et al.*,; 2007). There has been increased public health concern due to the ability of this pathogen to survive various environmental conditions and become resistant to antibiotics (Eng *et al.*,, 2015).

As for its genetic makeup, *S. Typhimurium* has complex elements that produce a whole set of virulence traits which let the bacterium invade hosts, and sort of slip past the immune system undetected. The organism also carries certain features that researchers view as useful for studying how pathogens enter and colonize hosts, and how bacteria cause disease in the first place. Understanding its taxonomic classification, and also its biological roles and medical relevance will help people craft more successful strategies for preventing human and veterinary disease (Rabsch *et al.*,; 2022).

2.1.1.Taxonomy and classification

The genus *Salmonella* sits in the family *Enterobacteriaceae*, under the order *Enterobacterales*, and it covers two species *Salmonella enterica* and *Salmonella bongori* (Brenner *et al.*,2000) . In practice, *Salmonella enterica* breaks down into six distinct subspecies with *S. enterica* subsp. *enterica*

considered the dominant driver behind diseases in warm-blooded animals, and in humans (Grimont *and* Weill, 2007).

Now, that subspecies includes more than 2600 different serovars, and researchers usually track those by looking at O and H antigen differences, using the Kaufmann–White–Le Minor scheme (Issenhuth-Jeanjean *et al.*,2014). *Salmonella Typhimurium* is also recognized by the official wording *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* , and it falls within the group called non-typhoidal *Salmonella* (NTS) serovars (Ferrari *et al.*,2019).

For classification, molecular typing methods work pretty well, for example multilocus sequence typing (MLST) plus whole-genome sequencing. With these tools, people can place *Salmonella* into taxonomic clusters based on phylogenetic ties, which in turn go beyond the limits of plain serotyping (Achtman *et al.*,2012). Genomic work has also highlighted *S. Typhimurium* lineages across the world, like sequence type ST19 as well as the invasive ST313 lineage, so the evolutionary trajectory, and the public health relevance of this serovar becomes clearer (Okoro *et al.*,2012).

Table 2.1 Taxonomy of the *Salmonella*

Taxonomic Rank	Classification
Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	<i>Enterobacterales</i>
Family	<i>Enterobacteriaceae</i>
Genus	<i>Salmonella</i>
Species	<i>Salmonella enterica</i>
Subspecies	<i>S. enterica</i> subsp. <i>Enterica</i>
Serovar	<i>Typhimurium</i>

2.1.2 General characteristics of *Salmonella* spp.

The *Salmonella* is a Gram negative rod, growing and surviving well facultative anaerobic. *Salmonella* motile by peritrichous flagella. Unlike other bacteria that form spores to withstand harsh environments, the bacterium is non-spore forming bacterium. This feature ensures that the bacterium can maintain its physiological functions while surviving difficult conditions such as low moisture and temperature changes, allowing them to remain viable in food sources as well as the agriculture field environment (Fàbrega *and* Vila, 2013).

There is also the biochemistry characteristic of *Salmonella* spp; which is mainly utilized in laboratories for identification since it has the ability to convert nitrates, produce hydrogen sulfide, and ferment glucose; however, the species cannot ferment lactose (Jajere, 2019). In addition, the bacteria use the outer membrane of LPS, as main constituents that have the ability to induce an immune response, as well as virulence factors (Rietschel *et al.*,2003).

When analyzing the genome of the *Salmonella* strain *Typhimurium*, there are several virulence factors, such as type three secretion systems. The latter is located within *Salmonella* pathogenicity islands (SPI-1, SPI-2). Moreover, the presence of type three secretion systems plays an important role in the process of penetrating epithelial cells, but also in surviving in macrophages, meaning intracellular persistence (Hansen-Wester *and* Hensel, 2001). There are additional ecological advantages for the pathogen due to its ability to form biofilms, which will help in protecting it from disinfectants, resulting in epidemiological importance (Steenackers *et al.*,2012).

2.1.3 Importance in human and veterinary medicine

Salmonella Typhimurium is the causative agent of gastroenteritis infections in humans, infecting millions annually throughout the world, mostly in developing nations (Majowicz *et al.*,2010). This occurs because

humans consume contaminated meat, dairy products, poultry products, resulting in abdominal pains, fever, and vomiting (Scallan *et al.*,2011).

Some patients who are immuno-compromised and young children as well as the elderly might suffer from invasive salmonellosis even though not all infections by this strain lead to complications and are treated (Feasey *et al.*,2012). There are some strains of *Salmonella Typhimurium* that have shown an increase in their pathogenicity and are resistant to antibiotics, making their treatment more complicated (Parisi *et al.*,2018).

S. typhimurium is the primary pathogen in veterinary science that infects poultry, cattle, swine, and domesticated animals, causing enteritis, septicemia, and reproductive issues (Uzzau *et al.*,2000). The infected animals act as carriers who transmit these pathogens by contaminating the environment to infect the entire food chain (Van Pelt *et al.*,2018).

The emergence of multidrug-resistant *S. Typhimurium* strains among food-producing animals poses a critical One Health risk in that their resistant genes may be transferred to human pathogens, thereby reducing treatment opportunities (Michael *et al.*,2015).

2.2. *Salmonella typhimurium*

One of the most adaptable and clinically significant Typhi and para-Typhi *Salmonella* strains appears to be *S. Typhimurium*, largely due to its elaborate biology, wide host specificity and relatively large clinical impact on both humans and animals. This particular pathogenic success can be attributed to the complicated yet rather straightforward interaction between its molecular virulence strategies, ability to persist in the environment and exploit multiple routes of infection simultaneously. In order to successfully evaluate the epidemiology of this strain and understand its pathogenesis, it is critical to understand the biological and molecular aspects of this organism, as

well as its wide host specificity and clinical significance (McClelland *et al.*, 2001; Baumler *et al.*, 2011).

2.2.1.Biological and molecular characteristics

From an biological perspective, the bacteria can be described as being a facultative intracellular pathogen in that it thrives both extracellularly in the external environment and as an intracellular pathogen residing within host cells' immune systems. The genome of *S. Typhimurium* has a length of about 5 Mb and it has numerous genes associated with metabolic activities, stress resistance, and virulence traits. This allows for the adaptation of the pathogen in varied ecological environments (McClelland *et al.*,2001).

Molecularly speaking, the disease-causing capacity of *Salmonella* is mostly attributed to the *Salmonella* Pathogenicity Islands such as SPI-1 and SPI-2. These Pathogenicity Islands contain the Type III Secretion Systems and these are necessary for tissue invasion as well as intracellular growth. Through this secretory apparatus, effector molecules can be secreted into the host cells to manipulate the cytoskeletal rearrangements, inflammation pathways as well as the vesicle trafficking properties of the host cell (LaRock *et al.*,2015; Lou *et al.*,2019). Other than these factors, there are also virulence factors encoded by plasmids as well as regulatory mechanisms that regulate gene transcription and expression based on their environment (Rychlik *et al.*,2018).

Yes, indeed, since the role of LPS, outer membrane proteins, and fimbriae is to assist in immune evasion and increase colonizing abilities. This means that, in general, it becomes easier to understand how these biological molecular properties give *Salmonella Typhimurium* a distinctively pathogenic phenotype (Zhou *et al.*,2020).

2.2.2.Host range and zoonotic potential

An important characteristic of *Salmonella Typhimurium* is that it has an extensive host range, meaning it is capable of infecting both people and a variety of hosts, including animals that live on farms and those kept as pets in a completely nonchalant manner. The thing that sets *S. Typhimurium* apart from other host-restricted serovars is that it exhibits this impressive adaptation to the intestinal environment, which helps it colonize new environments and transmit across species (Baumler *et al.*,2011).

In the case of livestock, particularly in poultry, cattle, and pigs, they usually act as the main reservoirs, and they may be infected by the pathogen without showing any signs but still shed it into their environment. This carriage makes it easier for the pathogens to contaminate food products and water supplies (Foley *et al.*,2011; Antunes *et al.*,2016). Additionally, environmental persistence in the farms aids in the maintenance of the transmission chain, as it virtually never stops (Callaway *et al.*,2014).

Factors that make it easy to develop a zoonotic disease include intensive rearing of animals along with global movement of food. Therefore, integrated One Health approaches must be taken in order to consider human health, animal health, and environmental health together and not separately (Destoumieux-Garzón *et al.*,2018).

2.2.3.Clinical significance

In terms of clinical practice, *S. Typhimurium* can be thought of as an important causative agent of non-typhoidal salmonellosis and can often present itself with acute gastroenteritis, more or less. The common presentation is comprised of diarrhea, fever, abdominal cramps, and also vomiting, with severity depending on the balance of the immune system in the particular host (Havelaar *et al.*,2015). In most instances, the infection will

resolve itself, not requiring any antimicrobial treatments but for certain vulnerable patients, it might be more complicated than that (Ao *et al.*,2015).

Going beyond these common intestinal manifestations, there are now reports about such invasive diseases as bacteremia, meningitis, or focal infections of organs becoming more common, particularly for individuals who are immunocompromised. Certain genetic lineages appear to have greater virulence together with higher levels of multi-drug resistance (Gordon *et al.*,2018; Kariuki *et al.*,2020).

As far as animals are concerned, *S. Typhimurium* is known to be responsible for enteritis, septicemia, as well as reproductive problems, and this poses serious economic challenges. On the other hand, *S. Typhimurium* continues being a persistent threat regarding food safety. It is important because it affects human and animal health, and it becomes regarded as one of the priority pathogens (Barrow *et al.*,2012).

2.3. Epidemiology of *Salmonella* infections

The epidemiology of *Salmonella* is more or less an example of how the biological characteristics of the pathogens, the host's vulnerability, environmental stresses, and the entire food production chain interact. *Salmonella Typhimurium* continues being one of the most commonly isolated serovars across the globe owing to its wide host range and the ability to survive in diverse ecological niches. When considering the creation of preventive and control measures that will actually be effective, it is important to understand the prevalence of *Salmonella*, the major routes of transmission, and the reservoirs involved in its propagation (Kirk *et al.*,2015; Ferrari *et al.*,2021).

2.3.1.Global prevalence of *Salmonella*

In the world, salmonellosis infections have been reported as one of the common sources of infection in foodborne diseases, with *S. Typhimurium* being the most prevalent serovar in animals and human infections. The problem is mainly evident in situations where the safety of food is lacking, while the levels of sanitation are minimal (Kirk *et al.*,2015). Nevertheless, in highly industrialized countries, there are also many high numbers of cases recorded due to intensive farming and complex food supply systems (Pires *et al.*,2021).

The distribution pattern of serovars within various parts of the world is influenced by several factors such as diet and agricultural practices and surveillance. Despite this variation, it appears that *S. Typhimurium* continues to remain relevant throughout the world owing to its adaptable nature as well as efficient transmission (Ferrari *et al.*,2021). The more recent genotyping also suggested that there was a widespread prevalence of the closely related clones in various continents, including the repeated identification of similar clone strains (Baker *et al.*,2018).

2.3.2.Transmission routes

The mode of infection for *Salmonella Typhimurium* infection occurs mainly through the fecal-oral route, meaning that one is likely to pick it up via contact with feces and subsequently ingest it orally. Normally, infection is associated with the consumption of infected foods and contaminated water. However, the source of infection can also be a bit more complicated. For instance, poultry meat, eggs, prepared meats, as well as non-pasteurized milk, are cited frequently as potential sources of the disease (Painter *et al.*,2013). Of course, poor handling and cooking practices would increase the chances of transmission of the disease significantly (Carrasco *et al.*,2012).

However, there is also environmental exposure, such as water, soil, and even farming equipment that can serve as sources of transmission, indicating indirect modes. Furthermore, the pathogen can also be transmitted directly between people in households or institutions that do not observe proper hygiene practices (Winfield *and* Groisman, 2003). The wide array of potential sources makes it difficult to manage the problem, hence the need to address multiple factors simultaneously in any effort aimed at controlling the pathogen.

2.3.3.Role of animals as reservoirs

The role of animals is to be the main carriers of the pathogen and maintain its presence within the ecosystem while acting as a bridge for *S. Typhimurium* infection among people due to the spillover effect; poultry farms play the most important role as asymptomatic animals may spread the bacteria and contaminate meat and eggs products (Gast *et al.*,2017).

Cattle and pigs play an important role because they release the *Salmonella* bacterium through their waste, thus polluting surrounding areas as well as water bodies. Some wildlife species may play secondary reservoir roles, as well as being mechanical vectors (Vittecoq *et al.*,2016).

In general, persistence of *S. Typhimurium* among such animal hosts reflects poorly on farm-level biosecurity, regular monitoring, and management of antimicrobial treatment (Van Boeckel *et al.*,2015).

2.4. Pigeons as reservoirs of *Salmonella Typhimurium*

Pigeon (*Columba livia*) is an example of fairly widely distributed synanthropic bird species which is able to adjust to urban, peri-urban, and agricultural habitats. Since pigeons live very close to humans, domestic livestock, and food production facilities, there are frequent interactions between the pigeon and humans and domesticated animals. The source of

their food comes from human sources such as organic wastes, animal food or any agricultural refuse. Moreover, pigeons utilize structures built by man including buildings, barns, bridges, etc. to build nests and roost in them (Haag-Wackernagel, 2004; Sol and Senar, 1995; Carlen *et al.*,2021).

Because of these behavioral and ecological characteristics, there may be accumulation and consequent distribution of their feces in urban and farming settings increasing the risk of contamination. For all of the above reasons, there has been increased attention given to the possible carriage of enteric pathogens, particularly those capable of zoonosis, in pigeons since they are highly mobile and adaptable, and remain in urban ecosystems for an extensive period of time (Chaka *et al.*,2020; Haag-Wackernagel *and* Moch, 2004). Classification of pigeon is described below in Table 2.2.

Table 2.2. Taxonomic classification of the pigeon (*Columba livia*)

Taxonomic Rank	Classification
Domain	Eukaryota
Kingdom	Animalia
Phylum	Chordata
Class	Aves
Order	Columbiformes
Family	Columbidae
Genus	<i>Columba</i>
Species	<i>Columba livia</i>

Pigeons play a big role as reservoirs for *Salmonella enterica* serovar *Typhimurium*, and this is a major zoonotic pathogen tied to foodborne illness in people and also in animals. A lot of studies show that pigeons can carry *S. Typhimurium* without showing any obvious symptoms, then shed it off and on, or sometimes pretty much all the time, through their feces, which ends up contaminating water sources soil animal feed, and even those food preparation places (Nolan *et al.*,; 2021).

What makes this persistence stronger is that the bacterium can keep going even under harsh conditions , and also because pigeons are around other animals a lot including poultry. Plus when researchers detect antimicrobial resistant *S. Typhimurium* strains in pigeons, it really underlines why pigeons matter for the epidemiology of *salmonellosis* , and it suggests they may help spread multidrug resistant bacteria around the environment, sort of within a One Health, point of view (Chaka *et al.*,; 2020; Steele and Dietrich , 2024).

2.4.1. Ecology of pigeons (*Columba livia*)

Due to the fact that they live in dense flocks and often breed on building roofs, barns, bridges, and utility sites, high accumulation of feces occurs, which later contaminates soils and even water bodies (Hughes *et al.*,2021). Additionally, interactions between pigeons, other species of wildlife and rodents may promote spread of infections via complex transmission pathways (Levisohn and Weisman, 1980).

Overall, the nature of pigeon ecology whereby these birds have relatively long life spans and often disperse along with flocking behavior promotes survival and dissemination of pathogens such as *Salmonella*. This is especially true in areas where sanitation and wildlife management practices are lacking (Friend *et al.*,2019).

2.4.2 *Salmonella* carriage in pigeons

Pigeons have been found to be able to shed the pathogen without showing any symptoms themselves. The shedding causes fecal contamination of the environment with the subsequent possibility of infection in other animals or even humans (Chaka *et al.*,2020). Molecular analysis has provided evidence of the occurrence of *Salmonella Typhimurium* in free-living and domesticated pigeons. Genetic characteristics of these pathogens appear to be

almost identical to those of *Salmonella* occurring in livestock and people, thus posing concerns about potential cross-species transmission (Lopez *et al.*,2022).

Several factors can induce shedding of *S. Typhimurium* in higher levels and increase chances of intra-flock as well as inter-flock transmission. These include such factors as migration, lack of sufficient nutrition resources, overcrowding and changes in seasons (Nolan *et al.*,2021). Pigeons in areas of animal markets, farms and food processing facilities are considered the high-risk population due to potential fecal contamination of water and agricultural products (Gast and Porter, 2020).

Some studies have also described the occurrence of antimicrobial-resistant strains of *Salmonella* obtained from pigeon droppings, raising concerns about the possibility of pigeons being pathogenic reservoirs of antimicrobial resistance for further use in human and animal pathogens Rukambile *et al.*,2019).

2.4.3 Public health implications

The public health significance of the carriage of *Salmonella* by pigeons is sort of linked to the fact that these organisms will be able to deposit their infected droppings into food resources, water supplies, and animal feed as well as urban environments generally.

Exposure to humans can occur either through direct contact with these contaminated areas, eating the contaminated foodstuff, or indirect consumption after exposure of livestock to infected droppings (Carvalho *et al.*,2023).

In cases where pigeons are in large numbers within the city environment, there have been observations of increased bacteria contamination on public bench tops, in playgrounds, and even facades of

buildings (Fernandes *et al.*,2021). In cases involving individuals whose immunity system has been weakened, children, as well as older adults, exposure to *Salmonella* through the environment may cause illness, and it may even increase the burden of healthcare (Majowicz *et al.*,2010).

In addition, the occurrence of antibiotic resistance among the strains of *Salmonella* from pigeons has added further difficulty to the issue since there is a risk of therapy failure when there is a transfer from animals to humans (Dolejska *et al.*,2010). In summary, pigeons can be classified as a somewhat overlooked link within the transmission chain of *Salmonella*.

2.5. Isolation and identification of *Salmonella Typhimurium*

Detection and characterization of *Salmonella Typhimurium* can be regarded as being rather critical for epidemiological surveillance, outbreak investigations, and clinical diagnosis purposes. Despite the use of molecular methods being on the increase in recent times, classical microbiological approaches will always form the basis of such testing since they allow obtaining live isolates and confirming the serovar identity while evaluating its antimicrobial resistance. Specifically, sample collection followed by selective cultivation and characterization are the actual key to successful detection (Jajere, 2019; Olaimat *and* Holley, 2020).

2.5.1. Conventional culture methods

As the first step towards separating *Salmonella* from samples from clinical cases, the environment, and from foods, a pre-enrichment in a nonselective broth needs to be carried out. This is because this early-stage procedure allows for the retrieval of stressed or low-level bacteria. Therefore, it is able to boost the sensitivity of the entire isolation process slightly (Andrews *et al.*,2018). The pre-enriched culture is then transferred to selective enrichment broths, including Rappaport-Vassiliadis or tetrathionate

broth, to reduce competing flora and promote the growth of the organism (ISO 6579-1:2017).

Following the enrichment stage, the culture samples are streaked on selective and differential agars, where the identification can be made based on the characteristic colonies. Of course, this process may seem to require too much time (24-72+ hours), but it is necessary, particularly if there is a need for the separation of pure cultures (Jajere , 2019).

2.5.2.Selective and differential media

Selective and differential media are somewhat used to visually identify presumptive *Salmonella* colonies from other bacteria. Among these include the agar Xylose Lysine Deoxycholate (XLD), Hektoen Enteric (HE), Brilliant Green (BG), and *Salmonella*–Shigella (SS) agar, all which indicate carbohydrate fermentation, as well as hydrogen sulfide production (Olaimat and Holley, 2020).

Typically on the XLD agar, the *S. Typhimurium* species forms red colonies with black centers due to lysine decarboxylation and hydrogen sulfide production, but produces dark green colonies to black on the HE agar (Andrews *et al.*,2018). In this case, the contrasting colony colors are important phenotypic hints although not completely definitive, since further testing is required to eliminate atypical species (Olaimat and Holley, 2020).

2.5.3.Biochemical identification

A presumptive isolate of *Salmonella* goes through a series of biochemical tests to ensure that the genus and species have been correctly determined. Standardized tests include TSI media, urease, indole, citrate tests, and motility test results (MacFaddin, 2000). As for the case of the typical *S. Typhimurium*, its biochemical tests would be characterized by having an

alkaline slant and acid butt in the TSI media, as well as the presence of hydrogen sulfide, negativity to urease test, indole test, positivity to citrate test, and motility (Jajere, 2019).

With regard to the automated identification systems like API 20E, VITEK 2, and Biolog, there is standardization of biochemical profiles, increased speed, and also accuracy as compared to manual testing methods. Probability scores in species determination are obtained using these devices, which are common in clinical and laboratory settings (Olaimat *and* Holley, 2020).

2.5.4.Serological confirmation

Final identification of *Salmonella Typhimurium* depends mostly on serotyping using somatic, (O), and flagellar, (H), antigens based on the Kaufmann-White-Le Minor method. Slide agglutination tests using appropriate antisera will allow you to detect an antigen formula specific for *Salmonella Typhimurium* (Grimont *and* Weill, 2007; Issenhuth-Jeanjean *et al.*, 2014).

It is very important for epidemiological associations, outbreak investigations, and source attribution, especially when combined with other molecular techniques like PCR or WGS. If the serological identification is performed correctly, it will enable distinguishing close serovar relatives that can hardly be distinguished by any biochemical technique alone (Achtman *et al.*, 2012).

2.6. Molecular identification of *Salmonella Typhimurium*

Technological improvements in molecular biology have actually changed the way we detect as well as characterize bacterial pathogens. Regarding *SalmonellaTyphimurium*, the use of molecular methods, such as Polymerase Chain Reaction (PCR) and gene sequencing, makes detection

more rapid and specific compared to the common culture-based techniques. Such methods are especially important in obtaining accurate diagnosis, along with surveillance and outbreak investigations, especially when rapid results are required or the isolates have atypical characteristics (Chin *et al.*,2022; Hu *et al.*,2021; Wu *et al.*,2020).

2.6.1.PCR-based detection

One of the most popular molecular techniques to detect *Salmonella* bacteria is called polymerase chain reaction, or PCR for short. The main principle of PCR allows amplifying some selected DNA sequences, thus, even in case of low numbers of bacteria or their viable but non-culturable state, PCR will still be able to detect them, as the test itself doesn't depend on living microorganisms. In short, PCR will be able to find out pathogens, which would otherwise remain undetected by culture technique, hence PCR becomes a valuable tool for screening (Malorny *et al.*,2020).

There are several PCR-based tests, based on different conserved sequences, related to *Salmonella* genus and its serovars. The obtained amplicons serve as the proof of pathogen presence. Further development of such technique is the real time quantitative PCR, also called qPCR, which can quantitatively detect the number of copies of amplified genes, providing some semi-quantitative information on the bacterial concentration. That is why PCR tests have become so popular among diagnostics centers and public health laboratories due to high sensitivity, specificity, and rapidness (Shi *et al.*,2019).

2.6.2.Target genes (*invA*, *fliC*, etc.)

It should be emphasized that selecting proper genetic targets is quite important for molecular identification accuracy. For example, the *invA* gene represents one of the most common PCR targets for the genus *Salmonella*

since it codes an important protein involved in the invasion process of the host cell and, at the same time, is highly conserved. For this reason, amplification of the *invA* gene is almost considered as gold-standard procedure of identifying this species since it is presented in numerous reference protocols for PCR (Rahn *et al.*,2020).

Next, there are serovar specific targets such as the *fliC* gene encoding the phase 1 flagellin, used for differentiation among the *Salmonella* serotypes, including *S. Typhimurium* (Alzwghaibi *et al.*,2018). The gene is responsible for coding the antigenic fraction of the flagella and, thus, allows differentiating *Salmonella* serotypes (in particular, the *S. Typhimurium*) from other serotypes if other targets are added. Moreover, multiplex PCR protocols also include other targets such as *hlyA* (regulatory gene within pathogenicity island 1) and *stx* (enterotoxin gene), mainly because of providing extra reliability of the test results (Balasubramanian *et al.*,2021).

2.6.3. 16S rRNA gene sequencing

However, sequencing the 16S rRNA gene is an accurate and dependable way of bacterial identification. This is because it utilizes phylogenetic conservation, as well as the variations that exist between different sequences. There are conserved areas of the gene, which have the ability to bind the universal primers. On the other hand, there are areas that vary, and they are used for distinguishing the different bacteria species. The use of 16S rRNA sequencing is essential for confirming genus and species identification when the biochemical and PCR results appear ambiguous (Clarridge, 2019).

While it is evident that 16S rRNA sequencing cannot distinguish very similar serovars efficiently, it can be relied on when combined with PCR tests and biochemical methods. High-throughput platforms for 16S sequencing are

now able to accelerate and make the process cheaper. This has made it possible to routinely perform these tests in reference labs and even in research institutions. This kind of workflow also contributes immensely to phylogenetic studies by determining the evolutionary linkages between *Salmonella* and the surrounding genera (Chang *et al.*,2021).

2.7Antimicrobial resistance in *Salmonella*

Salmonella antimicrobial resistance (AMR) has been quite an enormous threat to global health due to its negative effects on the successful treatment of infections in humans and other living organisms. This is caused by both genetic and biochemical methods that limit antibiotics' ability to inhibit the growth of bacteria, as it seems they fail. AMR is also fueled by the utilization of antimicrobials in the fields of healthcare, agriculture, and livestock farming, understanding resistance mechanisms, as well as the distinction between intrinsic resistance and acquired resistance, and the implication of multidrug-resistant strains, is very significant in regard to surveillance and management (Mohammed *et al.*,2022; Zhou *et al.*,2023).

2.7.1 Mechanisms of antibiotic resistance

Resistance to *Salmonella* bacteria to antibiotic is driven by different mechanisms that tend to affect the efficacy of medications. For example, there is an enzymatic breakdown of antimicrobials (e.g; *beta-lactamases*), target *al.*,terations at the level of mutations that alter the binding properties of fluoroquinolones to DNA gyrase, and reduced permeability of the outer membrane. Active efflux systems play a critical role in removing antibiotic molecules from the bacterial cell. Genetically speaking, mobile genetic elements, including plasmids, transposons, and integrons are responsible for resistance genes transmission among *Salmonella* and different bacteria populations (Li *et al.*,2021).

In detail, the prevalence of Extended-Spectrum Beta-Lactamases (ESBLs) confers resistance to cephalosporins. At the same time, point mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* are responsible for decreased susceptibility to fluoroquinolones, which are used to treat complicated cases of salmonellosis (Deng *et al.*,2023).

2.7.2 Intrinsic vs. acquired resistance

Thus, intrinsic resistance refers to those cases where the microorganisms have a genetic capacity to fend off specific types of antibiotics even without ever being exposed to them. In other words, it represents the natural resilience of these cells that they acquire at birth and do not obtain throughout their life. For example, multiple serotypes of *Salmonella* spp. show natural resistance to various antibiotics due to such characteristics as low outer membrane permeability and efflux pumps' activity (Munita and Arias, 2016).

However, acquired resistance appears following genetic modifications, which may either occur by mutation or by means of horizontal gene transfer. Here is where “resistance determinants” tend to appear, and the acquisition process may often depend on plasmids or integrative genetic elements which provide new genes for the recipient bacteria. New genes can be responsible for producing enzymes that detoxify the antibiotic; modify the targets of the antibiotic action, or increase the number of efflux systems in the recipient cell. The acquired form of resistance, however, allows a very rapid response under selective pressure from antibiotic action, hence leading to multiple-drug resistant *Salmonella* strains (Zhou *et al.*,2023; Robertson *et al.*,2023; Punchihewage-Don *et al.*,2024).

2.7.3 Public health concern of multidrug-resistant *Salmonella*

Multidrug-resistant *Salmonella* poses a significant threat to public health mostly due to the lack of treatment options and a high probability of treatment failure. Multidrug-resistant *Salmonella* strains resistant to at least three antibiotic classes have been reported in humans, animals, foods, and the environment, meaning that multidrug-resistance appears to spread quite widely (Islam *et al.*,2023).

If infection is caused by the MDR strain of the bacteria, it results in increased hospitalizations, additional costs of care, and poor treatment outcomes, especially when it comes to children and immunocompromised patients. However, it does not happen in isolation as the global emergence of multidrug-resistant strains of the pathogen is promoted by inappropriate use of antibiotics in hospitals and agriculture. Therefore, it is crucial to implement appropriate antibiotic stewardship together with surveillance and infection prevention and control efforts (Hoelzer *et al.*,2011).

2.8 Antibiotic resistance genes in *Salmonella Typhimurium*

Salmonella Typhimurium is a relatively common foodborne bacteria, which in recent years has gained resistance against multiple categories of antibiotics. The main reason for such a multidrug resistance (MDR) of the microorganism is some specific resistance genes, which may easily get transferred through plasmids, transposons, and integrons, and thus spread fast in various bacterial populations, creating a rather straightforward process. Understanding the mechanisms of such resistance formation is critical not only from the point of epidemiological monitoring but also when establishing efficient control measures (Muhammad *and* Ahmed, 2025).

2.8.1 β -lactam resistance genes (*bla*TEM, *bla*SHV, *bla*CTX-M)

Beta-Lactams such as penicillin and cephalosporins are relatively common in the treatment of *Salmonella* infections. The main source of resistance is due to *bla*TEM, *bla*SHV, and *bla*CTX-M genes encoding β -lactamases capable of breaking the β -lactam ring rendering the antibiotics ineffective (Antunes *et al.*,2005). As we know, these genes are usually transferred horizontally via plasmids among *Enterobacteriaceae*. There are quite a few recent studies showing rather high prevalence of bla genes in *S. Typhimurium* isolated from poultry, wild birds, and the environment, and so it really emphasizes mobile genetic elements in the development of antimicrobial resistance (Napoli and Wong, 2023).

2.8.2 Tetracycline resistance genes (*tetA*, *tetB*)

Resistance to tetracycline in *S. Typhimurium* mainly results from active efflux mechanisms via *tetA* and *tetB* genes. Such efflux pumps actively pump tetracycline out of the bacteria in order to reduce the level of tetracycline below the inhibitory level inside the bacterial cells, ensuring bacterial survival (Chopra and Roberts, 2001; Roberts and Schwarz, 2016). These mechanisms of resistance are commonly found in association with integron Class 1, transposons, and plasmids that help in the spread of these resistance genes through horizontal gene transfer (Gillings, 2014; Partridge *et al.*,2018). Extensive use of tetracycline in veterinary medicine and animal production has put considerable selection pressure on *tet* genes for maintaining their prevalence among *S. Typhimurium* as well as other species of *Enterobacteriaceae* (Economou and Gousia, 2015; Roth *et al.*,2019).

2.8.3 Aminoglycoside resistance genes (*aadA*, *strA*)

The aminoglycosides including streptomycin and gentamicin are inactivated via a modification mechanism in *Salmonella* using the *aadA* and

strA genes. In essence, this involves adenylation, phosphorylation, and sometimes acetylation of the antibiotic molecule, thereby rendering the antibiotics unable to bind effectively to its intended target within the bacterial cells. Consequently, it is common to observe aminoglycoside resistance occurring alongside other resistance genes, which increases the capacity of bacteria to withstand the effects of antibiotics (Napoli *and* Wong, 2023).

2.8.4 Sulfonamide and trimethoprim resistance genes (*sul1*, *dfrA*)

Resistance to sulfonamide and trimethoprim, on the other hand, has been commonly associated with *sul1* *and* *dfrA*. These two genes encode, respectively, a mutant form of dihydropteroate synthase and a mutant form of dihydrofolate reductase. The overall effect is reduced drug binding affinity that allows bacteria to continue thriving despite exposure (Antunes *et al.*,2005). Moreover, the *sul1* gene is commonly found embedded within the integrons of Class 1, which facilitates multidrug resistance through constant exchange processes (Muhammad *and* Ahmed, 2025).

2.9 Antibiotic resistance in *Salmonella* isolated from birds

However, pigeons, namely *Columba livia*, alongside a wide variety of wild bird species, are now considered carriers of strains of *Salmonella Typhimurium* resistant to several antibiotics and capable of transferring multiple resistance genes (Martin-Maldonado *et al.*,2024; Muteeb *et al.*,2024). Thus, the resistance determinants leading to development of the multidrug-resistant strain include such factors as *blaTEM*, *blaCTX-M*, *tetA*, *tetB*, *sul1*, *dfrA*, *aadA*, *and* *strA*. Having acquired them, MDR *Salmonella* is able to serve as a mobile vector of pathogens, moving from place to place, including agriculture lands, urban areas, or natural environments (Sodagari *et al.*,2023).

Concerning the risk of zoonotic transfer of MDR *Salmonella* for pigeons, one should note that the risk becomes quite elevated, because they spend time next to people in cities. Pigeons defecate in public spaces, near food production facilities and sources of potable water; thus, indirect infection becomes possible. The transmission of MDR *Salmonella* is possible not only among domesticated birds but among migratory birds as well: the latter carry resistant strains picked at farms, landfills, or sewage areas, and after that migrate to new locations (Muhammad *and* Ahmed, 2025).

The whole role of birds, in how resistance genes move around the environment, is sort of magnified when they bounce between several ecological niches. Like, for example, wild birds might peck through agricultural waste, eat carcasses, or rummage around human refuse, and that can set up chances for horizontal gene transfer between different bacterial groups (Angulo *and* McEwen, 2024).

Moreover, if these resistant strains are present in birds' feces for a significant amount of time, there is a possibility of contamination in water, soil, as well as crops, hence increasing the complexity of this story within the One Health approach. Taking into consideration all the aforementioned factors, it appears crucial to examine the epidemiology of MDR *Salmonella* in an avian reservoir (Martin-Maldonado *et al.*,2025).

2.9.1 Studies on pigeons and wild birds

Recent studies on the subject of interest have led to the discovery of some MDR strains of *Salmonella* in both captive and wild pigeons, sort of, and these include the resistance genes, such as *blaTEM*, *tetA*, *sul1* and *aadA*. This goes to show that birds are significant carriers of AMR, as well as the spreading capability of the organisms. In addition to this, migratory birds could acquire some of these resistant strains from a contaminated

environment, such as chicken farms, and then move them to other niches where they never existed before. (Martin-Maldonado *et al.*,2025).

2.9.2 Comparison with poultry isolates

However, while the pattern in terms of the resistant genes found in the wild birds has some similarities with those found in poultry, there is bound to be variations in terms of the number present and their combination. The possible reason for the occurrence of this phenomenon could be the environment around and the selection pressure due to antibiotics. In addition, the similarity in the marker genes including *tetA* and *blaCTX-M* could lead to the conclusion of interspecies transfer of such genes, whether by direct contact among the birds or through contamination of feed, water, or soil (Wu *et al.*,2018; Begum *et al.*,2024; Zhou *et al.*,2023).

2.9.3 Geographic variation in resistance patterns

Topographical variations are essential in the development of resistance phenotypes because of where the isolates are obtained from. For example, European isolates normally have high prevalence rates of both *blaCTX-M* and *tetB*, whereas isolates from the Middle East and Africa could have high carriage rates of *sul1* and *aadA*. This, therefore, illustrates how antimicrobials are prescribed, as well as how contaminants disseminate within the environment, based on the prevailing biosecurity measures in the locale (Steele *and* Dietrich, 2024).

2.10Public health and one health perspective

Overall, it can be said that the emergence of multidrug-resistant (MDR) *Salmonella* among animal, bird, and environmental populations is quite a severe threat to public health, making the concept of One Health particularly relevant. The circulation of MDR strains within domesticated poultry livestock or even wild animals could eventually lead to their acquisition by

humans through direct exposure, consumption of contaminated food products, and exposure to the reservoirs in the environment such as water, soil, and all the city spots (Angulo *and* McEwen., 2024; WHO, 2023).

In addition, it should be mentioned that birds, including pigeons and migratory species, may play an important role in spreading resistance genes further since they shed bacteria in public locations, water reservoirs, and agriculture (Martin-Maldonado *et al.*,2025; Steele *and* Dietrich, 2024).

Overall, one health concept involves the relationship between human, animal, and environmental health concerning the challenge of antimicrobial resistance (AMR). Integration in surveillance efforts targeting MDR strains of *Salmonella* contributes towards the early identification of emerging MDR clones, risk assessment, and eventually interventions that are based on evidence rather than intuition (Muhammad *and* Ahmed, 2025). Environmental contamination with resistant pathogens and their genes can act as a reservoir where these remain and facilitate gene transfer through lateral gene transfer among various bacterial groups leading to greater persistence and prevalence of AMR in natural as well as artificial environments (Sodagari *et al.*,2023).

In addition, improper usage of antibiotics within the field of veterinary practices and agriculture, as well as poor waste management processes, accelerate resistance development and spreading. Therefore, the implementation of One Health strategies and approaches, including proper use of antimicrobial agents, biosecurity measures, environmental decontamination, and public awareness campaigns, becomes critical if one desires to curb global dissemination of MDR *Salmonella* and safeguard both human and animal populations (Angulo *and* McEwen., 2024; WHO, 2023).

2.10.1 Zoonotic transmission risks

Salmonella infections can be acquired by individuals through consumption of contaminated food, through direct contact with animals, and even through exposure to contaminated water and soils in the environment. In view of the presence of resistance genes among isolates from poultry and domestic animals, it becomes somewhat obvious why there would be concern regarding zoonotic transmission (WHO, 2023).

2.10.2 Environmental contamination

The introduction of MDR *Salmonella* to the environment is largely because the excretions of animals are introduced into soil, water, and even the more developed urban environments. Such a type of contamination leads to the development of resistance genes via horizontal gene transfer, as well as ensuring their persistence in the environment (Rodríguez-Rojas *et al.*,2018; Shah *et al.*,2024).

2.10.3 one health approach to antimicrobial resistance

In essence, the One Health model is somewhat of an integration of human, animal, and environmental health to address AMR. Coordinated surveillance, proper antimicrobial use, biosecurity measures, and community education definitely come in handy here. And then monitoring resistance genes in wild animals, chickens, and in the environment gives you that evidence needed for risk assessment and policy making and thus, could actually result in the reduction of MDR *Salmonella* dissemination across the world (Angulo and McEwen, 2024).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Equipments and instruments:

Table (3.1): All the equipment and instruments used in this study:

EQUIPMENT:	Manufacture company/State
1- Biosafety cabinet level (2).	US.
2- Autoclave.	Hiray AMA (Japan).
3- Sensitive balance.	KERN (Germany).
4-Rack.	China.
5- Loop.	Hettich (Germany).
6- Bunsen burner.	(USA).
7- Gel documentation system.	Analytik Jena (Germany).
8- Eppendorf Centrifuge.	Wise spin (Korea).
9- Conventional PCR.	Analytik Jena (Germany).
10- UV PCR cabinet.	Analytik Jena (Germany).
11- PCR tube.	Promega.
12- Eppendorf tubes	Ultracruz biotechnology.
13- Plane tube.	China.
14- Micropipette sets from 1µl to 1000µl	CYAN (Belgium).
15- Micropipette tips (different sizes)	Citotest (china).
16- Gel electrophoresis	Bioneer (Korea).
17- Sterile syringes	China.
18- Vortex mixer	CYAN-Cypress diagnostic (Belgium).
19- Incubator	Memmert (Germany).
20- Refrigerator: $5 \pm 3^{\circ}\text{C}$	Kelon (JAPAN).
21- Water Still	LabTech.

22- Water bath	Binder GmbH (Germany).
23- Gloves	Mobic (Oman).
24- Sterile swap	China.
25- Deep freeze	Samsung (JAPAN).

3.2. Culture media

Table (3.2) Culture media used during the study

Culture media	Manufacture company/State
1- Selenite F broth	HIMEDIA.
2- <i>Salmonella</i> Shigella agar (SS)	HIMEDIA.
3- Xylose Lysine Deoxycholate gar(XLD)	Oxoid (UK).
4- Mueller Hinton Agar	Oxoid

3.3 Biological and Chemical materials

Table (3.3) Biological and Chemical materials

Biological and chemical materials
1- Ethanol (99%)
2- Ethidium bromide
3- Glycerol
4- McFarland's Standard solution No. 0.5
5- TBE buffer 10X
6- PCR grade water
7- Agarose
8- Master mix
9- Gram staining
10- Brain Heart Infusion (BHI) broth

3.4. Study design

In serial cross-sectional study, one hundred samples in total were obtained, as represented in Table (3-7). They are the resistance genes identified among the isolates. *Salmonella* infection was found in pigeons with various age groups and genders. All samples were obtained from rectal swabs throughout the period between October 2025 and February 2026.

Samples were selected based on the pigeon distribution scheme, which had been sanctioned by the veterinary authority and through coordination with the nine veterinary clinics scattered throughout the governorate according to their administrative divisions. Subsequently, isolation of *Salmonella* infection was done following the World Organisation for Animal Health (WOAH) 2018 protocol but with slight modification. Following that, the process continued through the initial isolation of the bacteria on *Salmonella* specific selective culture media, SS and XLD agar, and then the subsequent subculture and multiplication (Rajapaksha *et al.*, 2019).

For the identification of isolates, we used the VITEK 2 system, and we tried to keep everything accurate and reliable. In the end, these combined phenotypic and genotypic methods helped raise the detection sensitivity, lowered the risk of false-negative results, and generally improved the dependability of the microbiological work.

After that, the bacterial nuclear parts were extracted, so that a particular genomic region of the *Salmonella* genome could be amplified (*Stm* and *invA*). The DNA was obtained from the isolated bacteria, then the PCR outputs were run on gel electrophoresis, and finally the DNA bands were visualized and illuminated using a gel documentation system (Kipper *et al.*, 2019). This sequence should be explained in Figure (3-1).

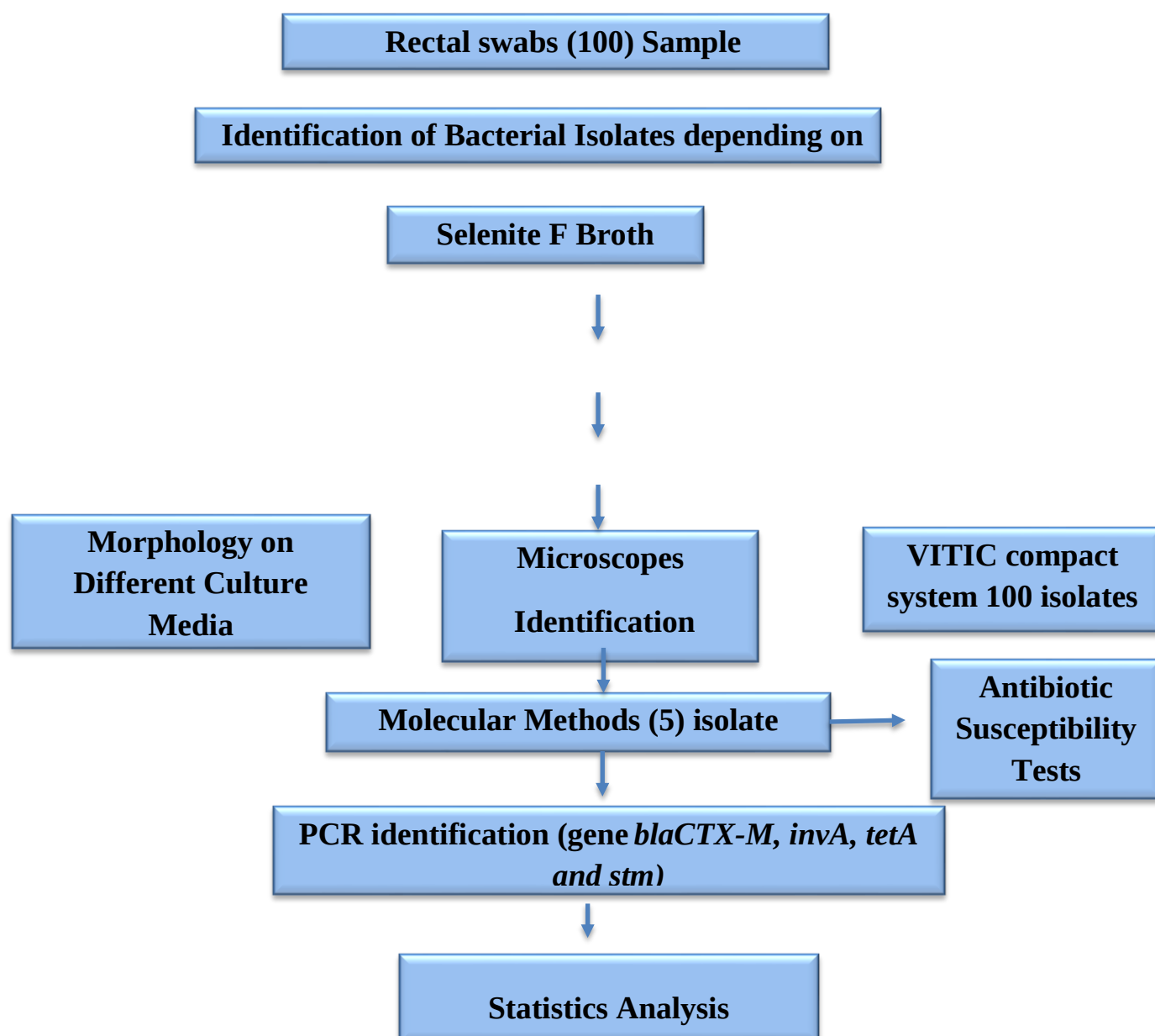


Figure 3.1: Diagrammatic scheme of isolation and identification procedures of *Salmonella*

Table 3.4: Summary of collected pigeon samples by location, age range, distribution, and sample period.

Location	Total Samples	Age Range	Period (Sample Range)
Nasiriyah	60	2 months – 2 years	Sample No. 1 – 60
Al-Gharraf	25	2 months – 1 year	Sample No. 61 – 85
Al-Shatra	15	2 months – 1 year	Sample No. 86 – 100
Total	100		

3.5. Isolation and identification

3.5.1 Preparation of media

The media were obtained from the Oxoid Company and HIMEDIA was prepared to depend on the Manufacturer's direction or protocol.

3.5.1. Selenite F broth

Enrichment medium for primary isolation of *Salmonella* spp. from feces samples and other pathological material (HI MEDIA).

Preparation: The required amount of Selenite F Broth powder was dissolved in the appropriate volume of distilled water according to the manufacturer's instructions. The medium was mixed thoroughly until completely dissolved and then dispensed into sterile tubes or containers under aseptic conditions. The prepared medium was used for enrichment of the collected samples.

3.5.2 *Salmonella*- *Shigella* agar (SS)

Various selective media for the isolation of *Salmonella* spp. And certain *Shigella* spp. From stool samples. (HI MEDIA). Incubation of certain selective enrichment media at increased temperatures (41°C – 43°C) has been suggested in order to limit the proliferation of competing bacteria, particularly from intestinal or fecal specimens (R. Gast and Porter 2020).

Preparation: The required amount of SS Agar dehydrated medium was suspended in the appropriate volume of distilled water according to the manufacturer's instructions. The suspension was mixed thoroughly and heated with continuous stirring until the medium was completely dissolved. The prepared medium was then allowed to cool to an appropriate temperature before being aseptically poured into sterile Petri dishes and allowed to solidify.

3.5.3 xylose-lysine deoxycholate agar (XLD)

This medium is used for the isolation of *Salmonella* from food and clinical specimens. Dissolve the components in water, heat while stirring steadily until the medium starts to boil. Try not to over-heat it though. Also avoid making too large a batch of medium, because that means prolonged heating which is kinda not great. Right away transfer the solution to a water bath around 50C⁰ and keep stirring until the medium has cooled and reached about 50C⁰. The poured agar plates can be kept for up to 14 days, if you store them in plastic bags in the dark at roughly 4C⁰ (World Health Organization Global Foodborne Infections Network, 2010).

Preparation: The required amount of XLD Agar powder was suspended in the appropriate volume of distilled water according to the manufacturer's instructions. The medium was heated with continuous stirring until it was completely dissolved and brought close to boiling. Excessive heating was avoided to prevent deterioration of the medium. The prepared medium was then cooled to approximately 50°C in a water bath with continuous stirring before being aseptically poured into sterile Petri dishes. The plates were allowed to solidify and were stored in sealed plastic bags at approximately 4°C in the dark until use.

3.6. Bacterial identification using the VITEK 2® compact system

All study isolates were confirmed using the VITEK 2® Compact System (bioMérieux, France), which is a fully automated platform that can identify a broad set of clinically relevant bacterial species, including *Salmonella* spp. In practice, the system gives fast answers via biochemical profiling and matching against an extensive reference database, finishing what

used to take more time with traditional approaches, like manual biochemical testing (Melhem *et al.*,2013).

The VITEK 2® Compact System offers accurate and rapid identification, and this is important for correct clinical as well as epidemiological handling of *Salmonella* isolates. Getting to the species level quickly matters most, since distinct *Salmonella* serovars may show different virulence potential, antimicrobial susceptibility patterns, and overall public health consequences. The system was run in a private laboratory in Al-Najaf (Al AMAL Specialist Laboratory), in line with the manufacturer's instructions. The reported identification accuracy for all bacterial isolates , including *Salmonella*, reached 100%, and this was consistent with earlier assessments (Kaur *et al.*,2016; Melhem *et al.*,2013).

3.7. Storage of isolates

3.7.1 Short term storage

The bacterial isolates were maintained for short-term storage at approximately 4°C for periods ranging from one day to one week. Following isolation and identification, the confirmed isolates were maintained under refrigerated conditions to preserve their viability until further processing. Brain Heart Infusion (BHI) broth was used as a suitable enrichment and maintenance medium. BHI broth was prepared according to the manufacturer's instructions by dissolving the required amount of dehydrated medium in distilled water, followed by thorough mixing and sterilization by autoclaving at 121°C for 15 minutes. The prepared medium was allowed to cool before inoculation with the confirmed bacterial isolates. (Melhem *et al.*,2013).

3.7.2 Long term storage

For long-term preservation, the confirmed bacterial isolates were stored at –18 to –20°C for periods ranging from hseveral months to years. Each isolate

was inoculated into sterile Brain Heart Infusion (BHI) broth supplemented with 20% glycerol as a cryoprotective agent. The glycerol-containing preservation medium was prepared by adding sterile glycerol to the cooled BHI broth to obtain a final concentration of 20% (v/v), followed by thorough mixing under aseptic conditions. Each bacterial isolate was transferred separately into a sterile cryovial containing the prepared BHI–glycerol preservation medium, properly labeled, and stored at -20°C until further use (Kaur *et al.*,2016).

3.8 Antimicrobial susceptibility testing

The antimicrobial susceptibility of representative isolates was determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2025) guidelines. Two Mueller–Hinton agar plates were prepared for each isolate to evaluate susceptibility against the tested antimicrobial agents. The antibiotic disks included Ampicillin (10 μg), Amoxicillin–clavulanic acid (20/10 μg), Cefotaxime (30 μg), Ceftazidime (30 μg), Imipenem (10 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Levofloxacin (5 μg), and Ofloxacin (5 μg).

The antimicrobial agents tested included Trimethoprim–Sulfamethoxazole (25 μg) and Doxycycline (30 μg) (Jorgensen *and* Ferraro, 2009).

Representative colonies from each isolate were suspended in 2 mL of sterile nutrient broth and mixed thoroughly to obtain a homogeneous bacterial suspension. The suspension was evenly spread over the surface of Mueller–Hinton agar by gently rotating the plate in multiple directions to ensure uniform inoculation. Excess inoculum was removed, and the plates were allowed to dry at room temperature for approximately 30 minutes before the application of antimicrobial disks (Quinn *et al.*,2016).

Antibiotic disks were aseptically placed on the agar surface using sterile forceps, maintaining an approximate distance of 2 cm between disks and from the edge of the plate to prevent overlapping inhibition zones. The inoculated plates were incubated at 37 °C for 18–24 hours. Following incubation, the diameters of the inhibition zones were measured in millimeters and interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI, 2025) guidelines (Markey *et al.*, 2019).

Table (3.5): Antibiotic discs

Antimicrobial Class	Antibiotic (Disk Content)	Code	Susceptible (S) ≥ mm	Intermediate (I) mm	Resistant (R) ≤ mm
Penicillins	Ampicillin (10 µg)	AMP	17	14–16	13
β-lactam/β-lactamase inhibitor	Amoxicillin–Clavulanic acid (20/10 µg)	AMC	18	14–17	13
Cephalosporins (3rd generation)	Cefotaxime (30 µg)	CTX	26	23–25	22
Cephalosporins (3rd generation)	Ceftazidime (30 µg)	CAZ	≥21	18–20	17
Carbapenems	Imipenem (10 µg)	IPM	23	20–22	19
Aminoglycosides	Gentamicin (10 µg)	GM	15	13–14	12
Fluoroquinolones	Ciprofloxacin (5 µg)	CIP	21	16–20	15
Fluoroquinolones	Levofloxacin (5 µg)	LEV	17	14–16	13
Fluoroquinolones	Ofloxacin (5 µg)	FO	16	13–15	12
Folate pathway antagonists	Trimethoprim–Sulfamethoxazole (25 µg)	COT	16	11–15	10
Tetracyclines	Doxycycline (30 µg)	DO	16	13–15	12

3.9. Polymerase chain Reaction (PCR) assay

3.9.1 Preparation *Salmonella* DNA

Table (3.6) Component for preparation of PCR products.

componant	volume
1. Master mix(2x)	12.5 µl
2. Upstream primer(10)	1.25 µl
3. Downstream primer(10)	1.25 µl
4. DNA template	5 µl
5. Nuclease- Free Water	5 µl

3.9.2 Steps of PCR

3.9.2.1 PCR primer design

PCR primers were designed to amplify genes associated with bacterial identification and genes in *Salmonella Typhimurium*. Primer design criteria included 15–30 bp length, 40–60% GC content, minimal internal secondary structures, and matched melting temperatures (T_m) for efficient annealing. All primers were custom synthesized by Macrogen Company (Macrogen, n.d.).

Table (3.7): Primers used for amplification of the target genes

Target gene	Primer	Primer sequence (5'–3')	Amplicon size (bp)
<i>invA</i>	Forward (F)	GTG AAA ATT ATC GCC ACG TTC GGG CAA	284
<i>invA</i>	Reverse (R)	TCA ATC GTC ACC CGA AAT ACC ATA CCA	284
<i>stm</i>	Forward	TGT TGT TGG AGC CTT CCC TTT	523

	(F)		
<i>stm</i>	Reverse (R)	AGA CGG CTG GTA AGA TCG CAG	523
<i>tetA</i>	Forward (F)	GGC GGT CTT AGT CGG GAA AA	576
<i>tetA</i>	Reverse (R)	ACT CCC ACT GTT CCA CTT CT	576
<i>blaCTX-M</i>	Forward (F)	ATG TGC AGY ACC AGT AAR GTK ATG GC	593
<i>blaCTX-M</i>	Reverse (R)	TGG GTR AAR TAR GTS ACC AGA AYC A	593

3.9.2.2 Detection of *invA* gene

The *invA* gene is a conserved invasion gene used as the gold standard molecular marker for *Salmonella enterica* detection due to its essential role in host cell invasion (Rahn *et al.*,1992). PCR amplification of the *invA* gene was performed using monoplex PCR with a final reaction volume of 50 µl, consisting of 1 µl primer, 5 µl DNA template, 25 µl master mix, and 19 µl distilled water. Amplification was carried out using an Agilent SureCycler 8800 thermal cycler. Thermocycling conditions specific to *invA* are summarized in Table 3-8.

Table (3-8). PCR conditions for *invA* gene

Gene	Initial Denaturation	No. of Cycles	Denaturation	Annealing	Extension	Final Extension
<i>invA</i>	94 °C – 5 min	35	94 °C – 1 min	58 °C – 1min	72 °C – 1 min	72 °C – 10 min

3.9.2.3 Detection of *blaCTX-M* gene

The *blaCTX-M* gene encodes extended-spectrum β -lactamases, enzymes that confer resistance to broad-spectrum cephalosporins (Boyd *et al.*,2004). Detection of this gene was carried out using monoplex PCR. The

final reaction volume was 20 µl, including 1 µl primer, 5 µl DNA template, and 14 µl distilled water. A professional thermocycler system was used for amplification. Thermocycling conditions are provided in Table 3-9.

Table (3.9). PCR conditions for *blaCTX-M* gene

Gene	Initial Denaturation	No. of Cycles	Denaturation Cycles	Annealing	Extension	Final Extension
blaCTX-M	94 °C – 5 min	35	94 °C – 30 sec	54 °C – 40 sec	72 °C – 45 sec	72 °C – 10 min

3.9.2.4 Detection of *tetA* gene

The *tetA* gene confers tetracycline resistance by encoding a membrane-associated efflux pump that exports tetracycline from the bacterial cell (Randall *et al.*,2004). PCR amplification was performed using monoplex PCR in a total reaction volume of 50 µl, consisting of 1 µl primer, 5 µl DNA template, 25 µl master mix, and 19 µl distilled water. The thermocycler conditions specific to *tetA* are shown in Table 3-10.

Table (3.10). PCR conditions for *tetA* gene

Gene	Initial Denaturation	No. of Cycles	Denaturation	Annealing	Extension	Final Extension
tetA	94 °C – 5 min	35	94 °C – 30 sec	50 °C – 40 sec	72 °C – 45 sec	72 °C – 10min

3.9.2.5 Detection of *stm* Gene (*Salmonella Typhimurium* specific)

The *stm* gene serves as a specific molecular marker for *Salmonella enterica* serovar *Typhimurium*, allowing accurate serovar-level identification (Shanmugasundaram *et al.*,2009). PCR amplification was carried out using monoplex PCR in a final volume of 50 µl, consisting of 1 µl primer, 5 µl DNA

Template, 25 µl master mix, and 19 µl distilled water. Amplification was performed using a professional thermocycler. The conditions are listed in Table 3-11.

Table (3.11). PCR conditions for *stm* gene

Gene	Initial Denaturation	No. of Cycles	Denaturation	Annealing	Extension	Final Extension
Stm	94 °C – 5 min	35	94 °C – 1min	62 °C – 2 min]	72 °C – 2 min	72 °C – 10 min

3.10 DNA Extraction and purification:

3.10.1 Kit Contents:

Table (3.12) DNA Extraction materials

Label	Description		Contain
1- G-Buffer	For Gram positive and negative bacteria		20 ml
2- Binding Buffer			15 ml
3-Washing Buffer A	Add 21 EtOH	ml of absolute	9 ml
4- Washing Buffer B	Add 40 EtOH	ml of absolute	10ml
5- Elution Buffer			20 ml
6- Columns	Contain silica member		50 columns
7- Collection tubes	Polypropylene tube for 2 ml		50 tubes
8- RNase A (lyophilized powder)	Dissolve in 300 of Distell water		3 mg
9- Proteinase K (Lyophilized powder)	Dissolve in 88 of Distell water		1.76 mg
10- Lysozyme (Lyophilized powder)	Dissolve in 200 of Distell water		20 mg
11- DNA Marker Solution	(100-1500 bp)		

3.10.2 Preparation of agarose Gel for electrophoresis

A 1% agarose gel was prepared by dissolving 1.5 g of agarose in 100 ml of 0.5× TBE buffer. The mixture was heated until the agarose was completely dissolved, after which 4 µl of ethidium bromide was added for nucleic acid staining. The molten agarose was poured into a gel casting tray and allowed to solidify. Subsequently, 5 µl of each PCR product was loaded into individual wells, while 5 µl of a 1000 bp DNA ladder was loaded into the first well as a molecular size marker. Electrophoresis was carried out at 75 V for 40 minutes, after which the amplified DNA fragments were visualized under ultraviolet (UV) illumination.

3.11 Ethical approval

In presented study, no genetically modified organisms or biological materials were employed, which was conducted under the supervision and recommendations of the Faculty of Veterinary Medicine at the University of AL SHATRA and in accordance with the controls allowed by it. Without the use of any extra materials or modifications, all of the samples utilized in this study were obtained according to the research methods for each kind.

3.12 Statistical analysis

The data obtained from antimicrobial susceptibility testing were analyzed using appropriate statistical procedures. The inhibition zone diameters in millimeters for each antibiotic were recorded and expressed as mean $\pm$ standard deviation (SD). The numbers and percentages of isolates categorized as susceptible, intermediate, or resistant were computed based on the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI, 2025). The Chi-square (χ^2) test was employed to assess differences between categorical variables and to evaluate whether significant changes existed in the distribution of antimicrobial resistance patterns among the isolates. The Pearson correlation coefficient (r) was used to evaluate the strength and direction of the linear relationship between age groups and infection rates. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp 2019; Field, 2018).

Chapter Four

Results

4. Results

4.1 Isolation and Identification of *Salmonella*

A total of 100 isolates from various locations and also age categories of pigeon were gathered. These isolates were identified via cultural, biochemical and molecular methods, kind of a combined technique. In summary, the results indicate that enteric bacteria pathogens are relatively widespread in this study sample set, but there is variation in prevalence across locations.

Amongst the isolated bacteria, *E.coli* is the most commonly found followed by *Klebsiella spp* and *Enterobacter spp*; while *Salmonella Typhimurium* is the least frequently isolated bacteria, quite rare really.

E.coli was present in 70 (70%) out of the 100 samples, suggesting *E.coli* as the main enteric bacteria pathogens in this studied population. *Klebsiella spp* and *Enterobacter spp* were found at moderate prevalence while 5 (5%) *Salmonella Typhimurium* isolates were found (Table 4.1)

Table (4.1): Distribution of enteric bacterial isolates

Bacterial species	No. of isolates	Percentage (%)
<i>Escherichia coli</i>	70	70%
<i>Klebsiella spp.</i>	15	15%
<i>Enterobacter spp.</i>	10	10%
<i>Salmonella Typhimurium</i>	5	5%
Total	100	100%

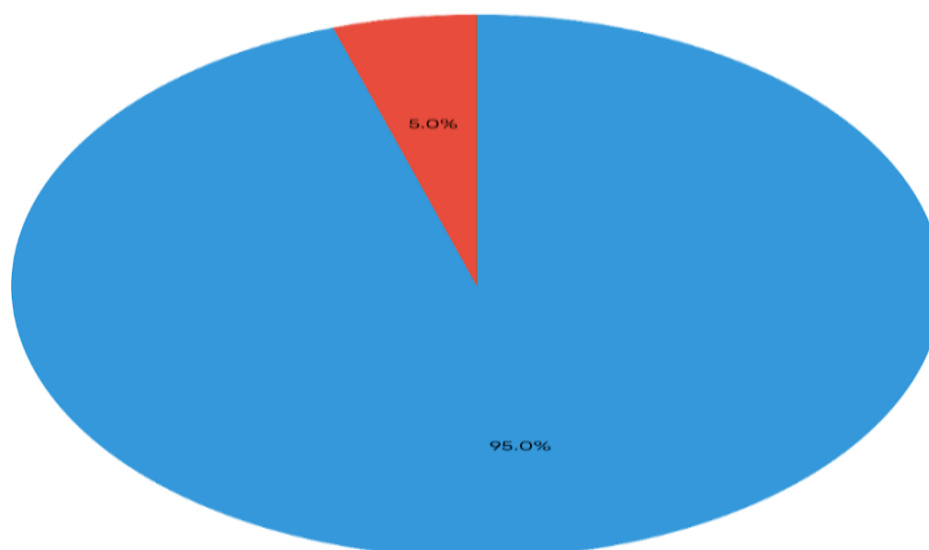


Figure (4.1): Study-wide infection rate. Out of a total of 100 samples studied, 5% were found to be infected with *Salmonella Typhimurium*, while 95% had no infection.

4.1.1 Isolation rate by region

The study samples were collected as follows:

Table 4.2 Regional distribution samples

Region	Sample Size	P Value	Significance
Nasiriyah	60	0.05	S
Al-Gharraf	25	0.05	S
Al-Shatra	15	0.05	S

4.1.2 Isolation Rate According to Age (*Salmonella Typhimurium*)

The samples were collected according to age groups to determine which age group had the highest infection rate, as illustrated in Table 4.3 below.

Table 4.3. Age infection rate

Age Group	No. of <i>S. Typhimurium</i>	Percentage (%)
1month–1 year	3	40%
1–2 years	1	40%
2–4 years	1	20%
Total	5	100%

Table (4.4): The distribution of age with positive cases of *Salmonella Typhimurium*

Age Group	Age	No. of Infections	Percentage (%)	Pearson r
1month–1 year	1	3 (Nasiriyah)	40%	-0.866
1–2 years	2	1(Al-Gharraf)	40%	
2–4 years	3	1 Al-Shatra)	20%	
Overall		5	100%	r = -0.87

4.2 identification of *Salmonella*

The study revealed that five isolated from 100 samples included in the study which appeared as positive characteristics of *Salmonella* in culture.

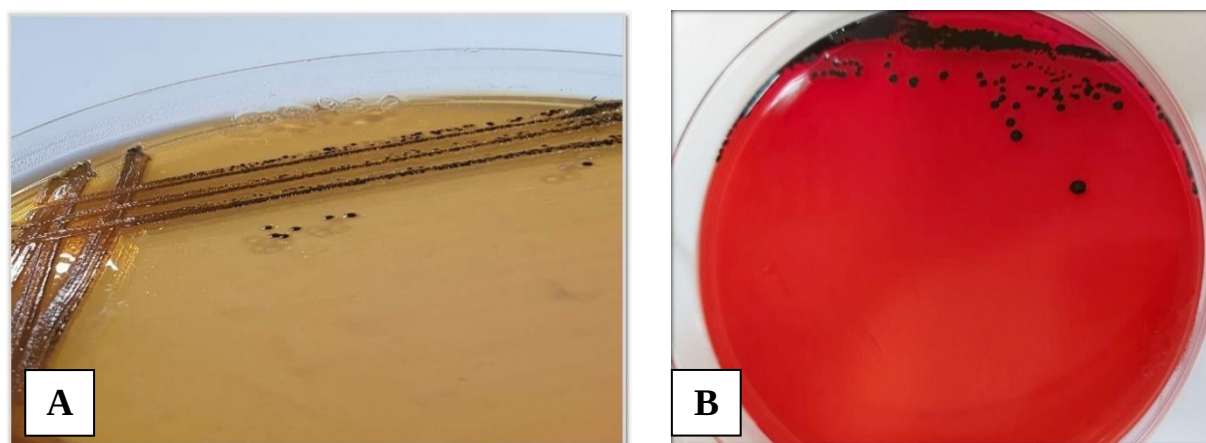


Figure (4-2) Isolated *Salmonella* on SS agar and XLD agar. (A) Shows the positive result of a *Salmonella* isolate on an SS agar. (B) Shows the positive result of a *Salmonella* isolate on an XLD agar.

They were dark pink in selective broth. Isolates were cultured on *Salmonella*-Shigella agar and the colonies were circular, smooth, convex, and pale in color with a black center. Then sub-cultured by using selective media Xylose-Lysine-Deoxycholate (XLD) agar for confirmation of the xylose fermentation, lysine decarboxylation, and production of hydrogen sulfide, recovered bacteria appeared on XLD agar as a small red colony with a black center as shown in Figure (4-1).

4.3 Microscope examination

Gram staining revealed that all isolates were **Gram-negative bacilli**, appearing as **pink rods arranged singly or in short chains**. The cells were **non-spore-forming** and exhibited the typical morphological characteristics of *Salmonella* spp.

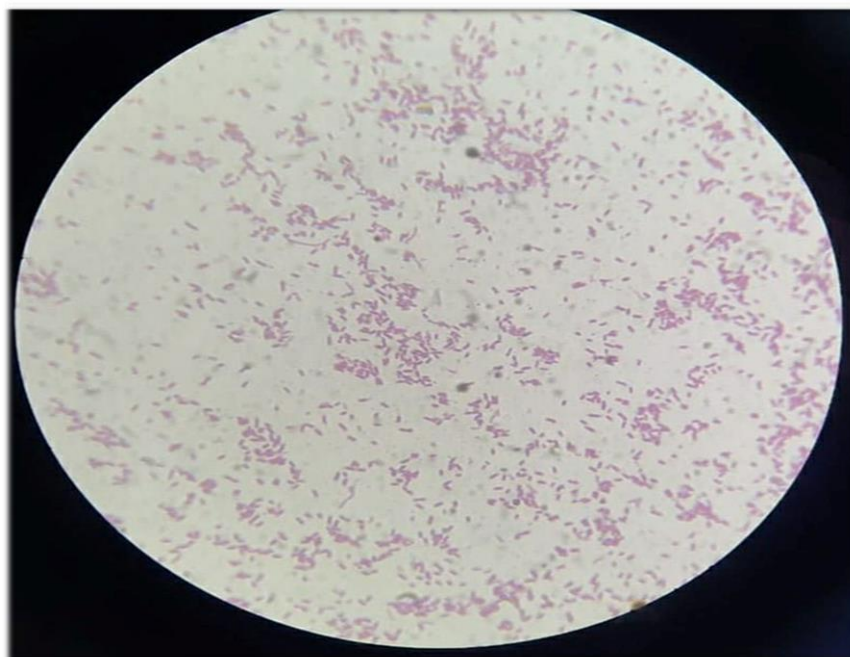


Figure (4.3). Microscopic Gram-stained image showing *Salmonella* as Gram-negative rods.

4.4 VITEK 2 compact (automated identification)

The VITEK 2 biochemical profile showed high-confidence identification for *Salmonella* spp. 98%. The system used micro-well cards containing multiple biochemical substrates, confirming the identity through carbohydrates utilization, amino acid reactions, and enzyme activity all results cards show in (Appendix 1)

4.5 Molecular identification of *Salmonella*

4.5.1 Polymerase chain reaction analysis of *Salmonella* isolates

Following PCR amplification of 5 isolates of *Salmonella*, all strains yielded PCR products indicative of the presence of the *invA* gene. The band size obtained for confirmation was 284 bp, corresponding to the expected size of the *invA* gene (Figure 4.4).



Figure (4.4): Ethidium bromide-stained agarose gel (75 V for 1 hr) of the *invA* gene of *Salmonella* spp. isolates. Lane (M): DNA molecular size marker (100 bp ladder). Lanes (1, 2, 3, 4, and 5) show positive amplification of the *invA* gene (284 bp).

4.5.2 Serogroups of non-typhoidal *Salmonella*

All five isolates tested positive for the *Salmonella*-specific *Stm* gene (523bp). Subsequent molecular analysis using the *stm* gene successfully identified the strains as *Salmonella* Typhimurium, as evidenced by the characteristic band size shown in Figure (4.5).

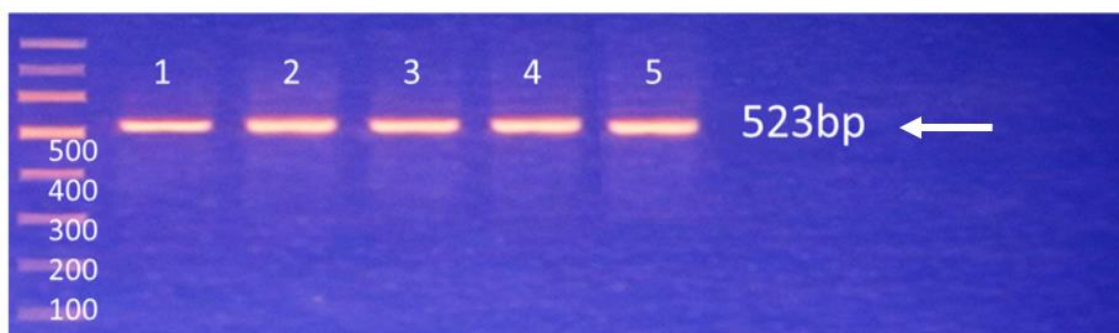


Figure (4-5): Ethidium bromide-stained agarose gel (75 volt at 1 hr) of *stm* gene of *Salmonella* spp. isolates. Lane (M), DNA molecular size marker (100 bp ladder), Lanes (1, 2, 3, 4 and 5) show positive results with *stm* gene (523 bp).

4.5.3 Detection of tetracycline resistance genes

All 5 isolates resistant to tetracycline by disk diffusion method, five (100%), were amplified *tetA* gene (molecular size 576 bp) as shown in figure (4-6).

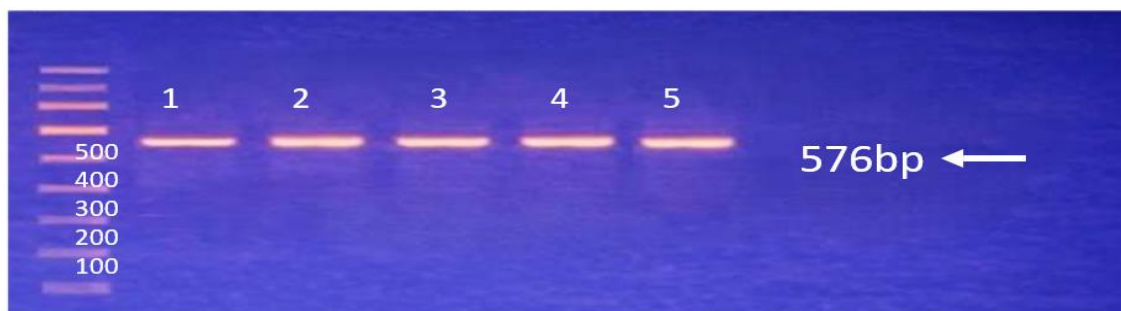


Figure (4-6): Ethidium bromide-stained agarose gel (75 volt at 1 hr) of *tetA* gene of *Salmonella*. isolates. Lane (M), DNA molecular size marker (100 bp ladder), Lanes (1, 2, 3, 4 and 5) show positive results with *tetA* gene (576 bp).

4.5.4 Molecular detection of extended spectrum β -Lactamase gene

All 5 analyzing isolates resistant to amoxicillin/clavulanic acid by the Kirby-Bauer method, *blaCTX-M* gene was detected. Specifically, 5 (100%) isolates showed positive PCR amplification for *blaCTX-M* with a molecular weight of 593 bp, as shown in Figure 4-7.

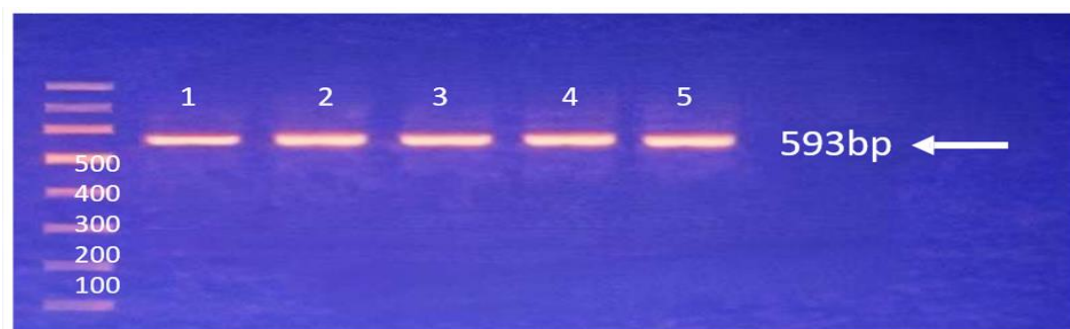


Figure (4-7): Ethidium bromide-stained agarose gel (75 volt at 1 hr) of *blaCTX-M* gene of *Salmonella* spp isolates. Lane (M), DNA molecular size marker (100 bp ladder), Lanes (1, 2, 3, 4 and 5) show positive results with *blaCTX-M* gene (593 bp).

4.6 Antibiotic sensitivity test

4.6.1 Evaluation of antibiotic susceptibility test

The study was also conducted to highlight the current antibiotic-resistant profile of 5 *Salmonella* isolates to screen the prevalence of Resistance to antibiotics in *Salmonella* in pigeon. All 5 isolates were tested for their susceptibility to 12 antimicrobial drugs classified as susceptible, intermediate, and resistant CLSI (2025). Figure (4-1).

4.6.2 According to the antibiotic susceptibility test, *Salmonella* isolates:

The study examined 5 positive isolates which demonstrated resistance patterns that included complete resistance to ampicillin 5 (100%) and 80% resistance to amoxicillin 4 (80%) and complete resistance to ceftriaxone 0 (0%) and 80% resistance to cephalexin 4 (80%) and 60% resistance to gentamicin 3 (60%) and 20% resistance to amikacin 1 (20%) and 20% resistance to trimethoprim 1 (20%) and 60% resistance to sulfamethoxazole 3 (60%) and complete resistance to tetracycline 5 (100%) and complete resistance to erythromycin 5 (100%) and 40% resistance to nalidixic acid 2 (40%) and 60% resistance to ciprofloxacin 5 (100%) and 60% resistance to chloramphenicol 3 (60%). Table 4-5 displays the rates at which tested isolates show resistance against antibiotics.

Table (4-5). Antibiotic resistance profile.

AB	S n (%)	R n (%)	P-value
LEV	1 (20%)	4 (80%)	0.37
FO	0 (0%)	5 (100%)	0.03*
AMP	0 (0%)	5 (100%)	0.03*
CTX	0 (0%)	5 (100%)	0.03*
S	1 (20%)	4 (80%)	0.37
GM	0 (0%)	5 (100%)	0.03*
COT	0 (0%)	5 (100%)	0.03*
CIP	0 (0%)	5 (100%)	0.03*
DO	2 (40%)	3 (60%)	1.00
AMC	0 (0%)	5 (100%)	0.03*
IPM	0 (0%)	5 (100%)	0.03*
CAZ	1 (20%)	4 (80%)	0.37

Table (4-5): The antimicrobial susceptibility pattern of *Salmonella Typhimurium* isolates (n = 5) exhibited high resistance rates to most antibiotics, with significant resistance ($p < 0.05$) observed for FO, AMP, CTX, GM, COT, CIP, AMC, and IPM. Other antibiotics showed non-significant resistance patterns. These findings indicate a multidrug-resistant profile among the isolates.

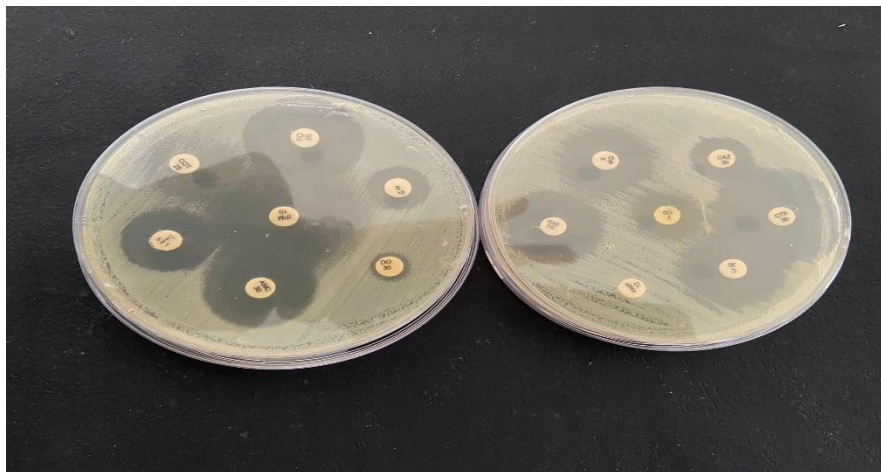


Figure (4-8) Disc diffusion method. The figure demonstrates the antimicrobial susceptibility patterns of *Salmonella Typhimurium* isolates. The isolates exhibited high sensitivity to Amoxicillin–Clavulanic acid, Fosfomycin, Ceftazidime, and Nitrofurantoin, while lower sensitivity was observed against Ampicillin, Streptomycin, Doxycycline, and Oxacillin. The variation in inhibition zone diameters indicates the presence of differential resistance profiles among the tested isolates.

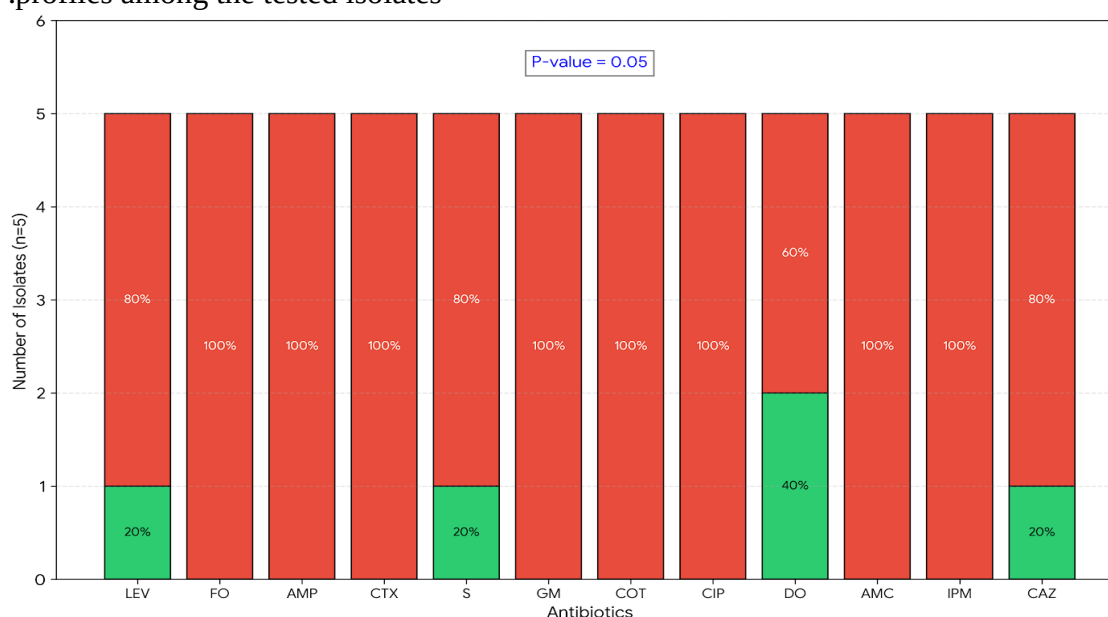


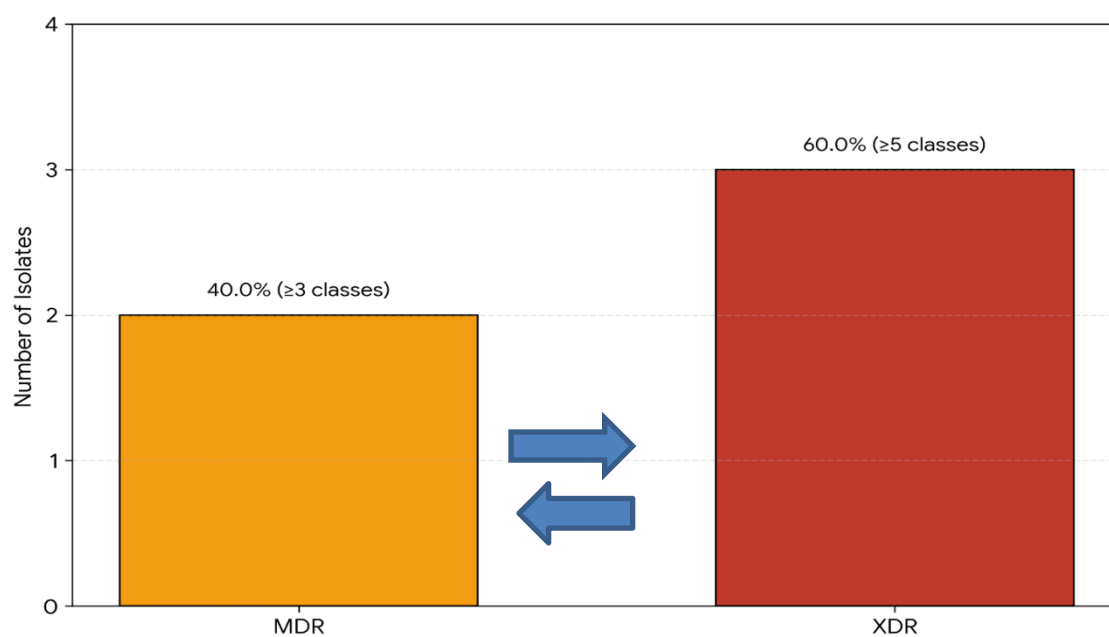
Figure (4.9): Antibiotics distribution. The figure illustrates the sensitivity (S) and resistance (R) patterns for 12 different antibiotics. There is a high level of multi-drug resistance across the isolates. Most antibiotics including FO (Fosfomycin), AMP (Ampicillin), CTX (Cefotaxime), GM (Gentamicin), COT (Trimethoprim-sulfamethoxazole), CIP (Ciprofloxacin), AMC (Amoxicillin/Clavulanate), and IPM (Imipenem) show 100% resistance among the tested isolates. DO (Doxycycline) shows the highest sensitivity at 40%, followed by LEV (Levofloxacin), S (Streptomycin), and CAZ (Ceftazidime) at 20%. The statistical significance is noted with a P-value of 0.05.

4.6.3 Multidrug resistance (MDR) profiles

The current study demonstrated that all 5 (100%) *Salmonella* isolates were non-susceptible to at least one agent in three or more antimicrobial categories, confirming a multidrug-resistant profile. Among these isolates, 1 (20%) exhibited resistance to 4 antibiotic classes, while 2 (40%) were resistant to five classes. Moreover, 1 (20%) isolate showed resistance to 6 antimicrobial classes, and 1 (20%) sample demonstrated resistance to 7 classes. These findings are shown in (4-6).

Table (4.6) MDR, XDR of salmonella

Multidrug resistance profile	No. (%) of <i>Salmonella</i> isolates (n=5)	No. of antibiotic classes
M.D.R (%)	(100%)5	≥ 3
XDR (%)	(80%)4	≥ 5



(Figure 4.10): Of the five *Salmonella* strains, two (40%) were categorized as multidrug resistant, being resistant to three or more types of antimicrobials, while three (60%) were categorized as extensively drug-resistant, being resistant to five or more types of antimicrobials. The predominance of extensively drug-resistant strains suggests a heavy burden of antimicrobial resistance.

Chapter Five
Discussions

5. Discussion

5.1 Prevalence and distribution bacteria

The present study recovered four enteric bacterial groups from 100 rectal swab samples collected from pigeons in Thi-Qar Province. *Escherichia coli* was the predominant isolate (70%), followed by *Klebsiella* spp. (15%), *Enterobacter* spp. (10%), and *Salmonella Typhimurium* (5%). These findings indicate that the pigeon population examined in this study was exposed to a broad range of enteric bacteria, although the prevalence of *S. Typhimurium* was lower than that of the other bacterial groups. This observation agrees generally with the findings discussed by Roth *et al.* (2019), who emphasized the frequent recovery of enteric bacteria from avian populations, and with Founou *et al.* (2021), who linked food-chain and environmental contamination with the circulation of resistant enteric organisms. The present result differs in magnitude from studies reporting higher *Salmonella* prevalence in birds; this difference may be explained by variation in location, season, sample type, bird population, and the intermittent shedding of *Salmonella*, rather than by a contradiction in the biological role of pigeons as potential carriers (Roth *et al.*, 2019; Founou *et al.*, 2021).

The predominance of *E. coli* should not be interpreted solely as evidence of disease. Because *E. coli* is commonly associated with the intestinal microbiota of birds, its frequent recovery may reflect normal colonization as well as fecal contamination of the environment. Nevertheless, the high proportion observed in the present study may also indicate repeated exposure to contaminated feed, water, soil, or nesting material. In the researcher's view, the result is therefore best understood as an indicator of substantial enteric bacterial circulation rather than as proof that all recovered *E. coli* isolates were pathogenic. Pathotype-specific virulence testing would be required to

distinguish commensal strains from diarrheagenic or extraintestinal pathogenic strains (Roth *et al.*, 2019; Founou *et al.*, 2021; Jajere, 2019).

The detection of *Klebsiella spp.* and *Enterobacter spp.* further supports the possibility of environmental contamination. These organisms may persist in moist organic material and can be introduced into pigeon habitats through wastewater, refuse, contaminated feed, and contact with other animals. Thus, their recovery alongside *E. coli* suggests that the sampled pigeons were participating in a wider environmental microbial cycle. This interpretation is consistent with reports that urban and free-ranging birds can acquire enteric bacteria from contaminated surroundings, but the present study cannot establish whether the birds were the original source of contamination or merely exposed carriers (Roth *et al.*, 2019; Founou *et al.*, 2021; Jajere, 2019).

Although *S. Typhimurium* was detected in only 5% of the samples, the finding remains epidemiologically important. Prevalence alone does not measure public-health significance; the potential risk also depends on serotype, virulence determinants, antimicrobial resistance, shedding, and opportunities for contact with humans and domestic animals. In this study, all *Salmonella* isolates carried the *invA* marker and were identified molecularly as *S. Typhimurium* using the *stm* marker. Therefore, the low isolation rate should not be taken to mean that the organism is unimportant. Rather, it suggests that a relatively small carrier population may still represent a source of environmental contamination, particularly because pigeons move between residential, agricultural, and public areas (Roth *et al.*, 2019; Founou *et al.*, 2021; Jajere, 2019).

The prevalence observed here may differ from results reported in other regions because of differences in sampling season, sample type, bird origin, population density, culture protocols, and the intermittent nature of *Salmonella* shedding. In this respect, the present finding is consistent with

Jajere (2019), who described geographic and ecological variation in *Salmonella* occurrence, but it should not be considered numerically identical to findings from poultry or other avian hosts. Thus, the agreement is mainly epidemiological, whereas the difference concerns the measured prevalence under different study conditions. A rectal swab provides evidence of carriage at the time of sampling but may not detect organisms when shedding is absent or below the analytical detection limit. The researcher therefore considers the 5% prevalence a conservative estimate of detectable carriage in the sampled population, not necessarily the absolute prevalence in all pigeons of Thi-Qar Province. Seasonal sampling, repeated sampling of the same birds, and examination of environmental specimens would provide a stronger estimate of transmission dynamics (Jajere, 2019).

Overall, the results support the need for surveillance of pigeons as potential carriers of zoonotic bacteria. However, the findings should be interpreted within the limits of the sample size and study design. The study demonstrates the presence of *S. Typhimurium* and resistant determinants in pigeons, but it does not prove direct transmission from pigeons to humans. That risk should be investigated through epidemiological linkage, environmental sampling, and genomic comparison of isolates from birds, animals, humans, and shared environments (Roth *et al.*, 2019; Founou *et al.*, 2021; Jajere, 2019).

5.2 Regional distribution of infection

The positive *S. Typhimurium* samples were distributed among Nasiriyah, Al-Gharraf, and Al-Shatra, with three positive samples from Nasiriyah and one sample from each of Al-Gharraf and Al-Shatra. Although this pattern suggests some regional variation in the occurrence of *S. Typhimurium*, the unequal numbers of samples collected from each region should be considered when interpreting the findings. A higher absolute number of positive samples in Nasiriyah may simply reflect the larger number of samples collected from this

region rather than a genuinely higher prevalence. Therefore, the observed regional differences should not be interpreted as evidence of differences in infection risk without further investigation (Prestinaci *et al.*, 2015; Eng *et al.*, 2015).

Regional variation in *S. Typhimurium* occurrence may plausibly be associated with several environmental and anthropogenic factors, including pigeon density, accumulation of waste, access to contaminated water, proximity to livestock or poultry farms, and the availability of food refuse. Urban pigeons frequently exploit human-generated food sources and may come into contact with contaminated surfaces, sewage, or wastewater. In agricultural areas, interactions with livestock facilities, manure, irrigation water, and feed-storage areas may provide additional opportunities for exposure to enteric pathogens. These factors provide plausible explanations for the observed regional distribution; however, they remain hypothetical in the present study because sanitation conditions, bird density, environmental bacterial loads, and antimicrobial residues were not directly measured at the sampling sites (Prestinaci *et al.*, 2015; Eng *et al.*, 2015).

The present findings therefore suggest that geographical location may function primarily as a proxy for several interacting environmental and management-related factors rather than as an independent biological determinant of *S. Typhimurium* occurrence. Accordingly, the observed regional variation should support the implementation of site-specific surveillance rather than lead to the conclusion that any particular location represents a definitively higher-risk source. Future studies should report prevalence as a proportion within each region, provide corresponding confidence intervals, use more comparable sample sizes among locations, and apply appropriate statistical models to account for potential confounding factors such as age, season, and habitat characteristics.

The observed regional pattern may also have implications for antimicrobial resistance. Pigeons living near livestock operations, waste-disposal

sites, or densely populated communities may have greater opportunities to encounter antimicrobial-resistant bacteria or antimicrobial residues. Such exposure could contribute to the persistence and dissemination of antimicrobial resistance determinants among pigeon-associated bacterial populations. However, the present study did not measure antimicrobial residues, environmental bacterial contamination, or the genetic mechanisms and mobile genetic elements responsible for resistance transmission. Therefore, any association between geographical location and antimicrobial resistance should be regarded as biologically plausible but not directly demonstrated by the current data (Prestinaci *et al.*, 2015; Eng *et al.*, 2015).

5.3 Age as a risk factor for *Salmonella* infection

Most positive samples were obtained from younger pigeons, with 80% of the *Salmonella*-positive birds belonging to the younger age categories and the remaining 20% occurring in older birds. The observed negative correlation between age and *Salmonella* occurrence ($r = -0.87$) is consistent with a possible decline in detectable bacterial carriage with increasing age. However, this finding should be interpreted with caution because only five *Salmonella*-positive birds were identified. Therefore, the correlation coefficient may be unstable and should not be considered definitive evidence of a causal relationship between age and *Salmonella* occurrence (Wigley, 2018; Barrow & Freitas Neto, 2018; Jajere, 2019).

Several biological and environmental factors may contribute to the observed age-related pattern. The findings are consistent with the general interpretation of Wigley (2018) and Barrow and Freitas Neto (2018), who associated the increased susceptibility of young birds to enteric infections with the immaturity of their host defense mechanisms. Similarly, Jajere (2019) reported age-related differences in susceptibility to *Salmonella* infection among avian populations. Nevertheless, the present study differs from these broader

studies in terms of sample size and host species; therefore, the cited literature provides biological support for the observed pattern but does not establish that the same mechanisms were responsible for the findings in the pigeons examined in the present study.

Young pigeons may have less-developed immune responses, an incompletely established intestinal microbiota, and greater susceptibility to intestinal colonization following exposure. In addition, they may spend more time in crowded nesting environments and have more frequent contact with fecal material, contaminated feed, and water. These conditions may increase both the likelihood of exposure and the probability of recovering *Salmonella* from cloacal swabs. As pigeons mature, progressive development of immune function, changes in diet and behavior, and reduced dependence on crowded nesting environments may contribute to a reduction in detectable bacterial carriage (Jajere, 2019; Wigley, 2018; Barrow & Freitas Neto, 2018).

The present findings are broadly compatible with observations from other avian hosts in which younger age has been associated with increased susceptibility to enteric infections. However, quantitative comparisons with poultry or other bird species should be made cautiously because differences in maternal immunity, husbandry practices, diet, nesting behavior, and patterns of pathogen exposure may influence age-related susceptibility. Accordingly, age is considered a potential risk indicator in the present pigeon population rather than an independent determinant of *Salmonella* infection (Wigley, 2018; Barrow & Freitas Neto, 2018; Jajere, 2019).

Environmental exposure may also have contributed to the apparent association between age and *Salmonella* occurrence. Overcrowded nesting sites, accumulated fecal material, contaminated water, and inadequate nutritional conditions may disproportionately affect younger birds. Improving environmental sanitation, reducing overcrowding, and providing clean feed and

water are therefore reasonable preventive measures. However, these recommendations should be evaluated in future longitudinal studies that follow pigeons across different age groups and distinguish newly acquired infection from persistent bacterial carriage (Wigley, 2018; Barrow & Freitas Neto, 2018; Jajere, 2019).

5.4 Conventional and automated identification of *Salmonella*

The combined use of enrichment, selective culture, microscopy, and automated identification provided a sequential diagnostic approach for the recovery and identification of *Salmonella*. Selenite F enrichment was used to enhance the recovery of *Salmonella* from specimens containing competing intestinal bacteria, while SS and XLD agars facilitated the preliminary differentiation of suspected *Salmonella* colonies. The recovery of pale colonies with black centers on SS agar and red colonies with black centers on XLD agar was compatible with hydrogen sulfide (H₂S) production, a characteristic commonly observed in *Salmonella*. However, these colonial characteristics are not species-specific and therefore should be regarded as presumptive rather than confirmatory when used alone (Funke *et al.*, 2019; Elkenany *et al.*, 2019; ISO 6579-1, 2017).

Microscopic examination revealed Gram-negative bacilli, which is consistent with the general characteristics of *Salmonella* but may also be observed in several other members of the Enterobacterales. Therefore, microscopy supported the culture findings but could not independently establish the identity of the isolates. The diagnostic reliability of the present study was enhanced by combining phenotypic observations with automated biochemical identification and molecular methods rather than relying on a single diagnostic technique (Funke *et al.*, 2019; Elkenany *et al.*, 2019; ISO 6579-1, 2017).

The VITEK 2 Compact system identified the isolates as *Salmonella* spp. with a reported identification confidence of 98%. This finding is consistent with Funke *et al.* (2019) and Elkenany *et al.* (2019), who reported that automated biochemical identification systems can improve the speed and reproducibility of bacterial identification. Unlike approaches based exclusively on conventional biochemical testing, the present study used VITEK 2 as an automated biochemical identification step, followed by molecular verification. Thus, the agreement with previous studies mainly concerns the usefulness of automated identification, whereas the methodological distinction lies in the sequential combination of culture, VITEK 2, and PCR used in the present study.

Automated biochemical identification offers advantages in terms of reproducibility and reduced processing time compared with extensive panels of manual biochemical tests. Nevertheless, a high identification confidence does not completely exclude the possibility of misidentification, particularly among closely related members of the *Enterobacterales* or atypical isolates. In the present study, molecular detection of the *invA* and *stm* genes provided an additional level of verification and increased confidence in the identification of the recovered isolates (Funke *et al.*, 2019; Elkenany *et al.*, 2019).

The culture–VITEK–PCR sequence can therefore be considered a strength of the diagnostic approach used in this study. Culture demonstrates the presence of viable organisms, VITEK 2 provides rapid biochemical classification based on phenotypic characteristics, and PCR detects specific genetic targets. However, these methods address different diagnostic aspects and should not be considered interchangeable. Culture may fail to recover organisms present at low levels or shed intermittently, VITEK 2 relies on biochemical profiles for identification, whereas PCR detects target DNA

regardless of the viability of the organism. Therefore, the most appropriate interpretation is that the concordance of the culture, automated identification, and molecular findings increased confidence in the identification of the recovered *Salmonella* isolates (Funke *et al.*, 2019; Elkenany *et al.*, 2019; ISO 6579-1, 2017).

5.5 Molecular identification by using *InvA* and *Stm* Gene

All five isolates produced the expected 284-bp PCR product corresponding to the *invA* gene. The detection of *invA* in all recovered isolates supports their identification as *Salmonella*, as this gene is widely used as a conserved molecular target for the detection of the genus and is associated with the invasion system encoded within *Salmonella* pathogenicity island 1 (SPI-1). This finding is consistent with previous reports by Eng *et al.* (2015), Elkenany *et al.* (2019), and Lopez *et al.* (2022), which described *invA* as a useful molecular target for the detection of *Salmonella*. The present findings therefore extend the application of this molecular marker to pigeon-derived isolates from Thi-Qar Province. However, detection of *invA* should be interpreted as evidence of the presence of a virulence-associated determinant rather than as a direct measurement of virulence expression or clinical severity. The agreement between the *invA* PCR results and the culture and VITEK 2 findings further supports the reliability of the identification process in the isolates examined (Eng *et al.*, 2015; Elkenany *et al.*, 2019; Lopez *et al.*, 2022).

The *invA* findings should therefore be interpreted as molecular evidence supporting the identification of *Salmonella* and the presence of a virulence-associated genetic determinant, rather than as a complete assessment of pathogenicity. The presence of a virulence-associated gene does not necessarily indicate its expression, the severity of infection, or the

capacity of an isolate to cause disease in humans or other hosts. A more comprehensive evaluation of pathogenic potential would require additional characterization of virulence-associated genes, gene expression, serological characteristics, and genomic relatedness (Eng *et al.*, 2015; Elkenany *et al.*, 2019; Lopez *et al.*, 2022).

All five isolates also yielded the expected PCR product for the *stm* marker, providing additional molecular evidence supporting the identification of the isolates as *S. Typhimurium* when interpreted together with the culture, biochemical, and *invA* findings. This result is epidemiologically relevant because *S. Typhimurium* has a broad host range and is frequently associated with foodborne and zoonotic infections. Therefore, the molecular findings extend the interpretation beyond the detection of *Salmonella* spp. to a serotype of considerable veterinary and public-health importance (Eng *et al.*, 2015; Elkenany *et al.*, 2019; Lopez *et al.*, 2022).

The concordant detection of both molecular targets strengthens the internal consistency of the identification process. Nevertheless, PCR detection alone cannot establish the direction or source of transmission between pigeons, humans, livestock, or other animals. The detection of *S. Typhimurium* in pigeons demonstrates carriage and indicates the potential for environmental shedding, but it does not by itself establish an outbreak or a direct zoonotic transmission event. Demonstrating such transmission would require comparative epidemiological investigations, source attribution, and preferably whole-genome sequencing to determine genetic relatedness among isolates from different hosts and environments (Eng *et al.*, 2015; Elkenany *et al.*, 2019; Lopez *et al.*, 2022).

5.6 Detection of antibiotic resistance genes (*tetA* and *blaCTX-M*)

The *tetA* gene was detected in all five isolates, and all isolates exhibited phenotypic resistance to tetracycline based on the disk diffusion test. This concordance is consistent with previous reports by Chopra and Roberts (2001), Roberts and Schwarz (2016), and Elkenany *et al.* (2019), which associated *tetA* with tetracycline resistance through an efflux mechanism and reported its occurrence among enteric bacteria of animal origin. The complete detection of *tetA* in the present isolates provides a plausible molecular explanation for the observed tetracycline-resistant phenotype. However, this finding does not establish that *tetA* was the sole determinant responsible for resistance, as other resistance genes, regulatory mechanisms, permeability changes, and efflux systems may also contribute to the phenotype (Elkenany *et al.*, 2019; Chopra & Roberts, 2001; Roberts & Schwarz, 2016).

The *tetA* gene encodes an efflux system that decreases the intracellular concentration of tetracycline, thereby reducing its antibacterial activity. Its detection in all isolates is consistent with the widespread distribution of tetracycline-resistance determinants among bacteria associated with animal and agricultural environments. Nevertheless, PCR detection demonstrates the presence of the gene but does not provide information about its level of expression or functional activity. Furthermore, the present study did not determine whether *tetA* was located on a transferable plasmid or another mobile genetic element. Plasmid profiling, sequencing, and conjugation experiments would therefore be required to evaluate its potential for horizontal transfer (Chopra & Roberts, 2001; Bevan *et al.*, 2017; Founou *et al.*, 2021).

The *blaCTX-M* gene was also detected in all five isolates. This finding is consistent with Bevan *et al.* (2017), who described CTX-M enzymes as globally important extended-spectrum β -lactamase (ESBL) determinants, and with Founou *et al.* (2021), who highlighted the contribution of animal and environmental reservoirs to the maintenance and dissemination of antimicrobial resistance. Although the 100% detection rate observed in the present study may appear higher than rates reported elsewhere, this comparison should be interpreted cautiously because only five isolates were examined. Thus, the finding represents the proportion of *blaCTX-M*-positive isolates within the recovered *S. Typhimurium* isolates and should not be interpreted as the prevalence of this gene in the overall pigeon population.

CTX-M enzymes are ESBLs capable of hydrolyzing a range of β -lactam antibiotics, particularly extended-spectrum cephalosporins. Therefore, the detection of *blaCTX-M* provides a plausible molecular explanation for resistance to relevant β -lactam agents observed in the antimicrobial susceptibility testing. However, the presence of the gene alone does not establish its expression level or exclude the contribution of other β -lactam resistance mechanisms. Accordingly, the result demonstrates the presence of an important antimicrobial-resistance determinant but does not, by itself, fully explain the observed resistance phenotype (Founou *et al.*, 2021; Bevan *et al.*, 2017).

The coexistence of *tetA* and *blaCTX-M* in all five isolates is particularly noteworthy because it indicates the simultaneous presence of resistance determinants associated with different antimicrobial classes. This pattern may reflect exposure to antimicrobial selection pressure or acquisition of multiple resistance determinants. However, the present findings do not demonstrate that the two genes are physically linked, located on the same plasmid or mobile genetic element, or transferred together. The study

therefore demonstrates co-occurrence within the same bacterial isolates, whereas the genetic linkage and mechanisms underlying their dissemination remain unresolved (Chopra & Roberts, 2001; Bevan *et al.*, 2017; Founou *et al.*, 2021).

From an epidemiological perspective, pigeons may serve as potential sentinels of antimicrobial-resistance circulation at the animal–environment interface. This interpretation is more appropriate than suggesting that pigeons are the original source of the resistance determinants, because the present study did not investigate their origin or transmission pathways. Determining the direction and extent of dissemination would require sampling from environmental and animal sources, including wastewater, feed, soil, livestock, poultry, and human-associated isolates, followed by comparative genomic analysis. Such investigations could help clarify whether the resistance determinants detected in pigeon-derived *S. Typhimurium* are shared with bacterial populations circulating in other hosts and environmental compartments (Chopra & Roberts, 2001; Bevan *et al.*, 2017; Founou *et al.*, 2021).

5.7 Antibiotic susceptibility patterns of *Salmonella Typhimurium*

The antimicrobial susceptibility results demonstrated resistance to several tested antimicrobial agents, indicating a substantial antimicrobial-resistance burden among the five *S. Typhimurium* isolates. This finding is broadly consistent with reports by Elkenany *et al.* (2019), Founou *et al.* (2021), and Prestinaci *et al.* (2015), which described increasing antimicrobial resistance among *Salmonella* and other enteric bacteria originating from animal and environmental sources. However, the specific resistance percentages observed in the present study should not be directly compared with those reported in other studies because differences in antimicrobial

panels, interpretive breakpoints, sampling populations, geographical settings, and antimicrobial-use practices may substantially influence the results. Therefore, the agreement with previous studies mainly concerns the presence of multidrug resistance, whereas the magnitude and distribution of resistance are specific to the present study population.

An important finding was the concordance between the phenotypic antimicrobial susceptibility patterns and the detection of specific resistance genes. The presence of *bla*CTX-M provides a plausible molecular basis for resistance to relevant β -lactam antimicrobial agents, particularly extended-spectrum cephalosporins, whereas the detection of *tetA* is consistent with the observed tetracycline-resistant phenotype. Nevertheless, phenotypic resistance may result from multiple mechanisms, and the detection of a resistance gene does not necessarily establish that it is solely responsible for the observed phenotype (Founou *et al.*, 2021; Prestinaci *et al.*, 2015; Elkenany *et al.*, 2019).

The observed resistance profile may reflect antimicrobial selection pressure in environments affected by antimicrobial use, animal waste, contaminated water, or pharmaceutical residues. These factors represent plausible ecological contributors to the maintenance and dissemination of antimicrobial-resistant bacteria; however, they cannot be confirmed from the present cross-sectional study because antimicrobial exposure and environmental contamination were not directly measured. Other possible explanations include acquisition of resistant strains from other animal hosts, persistence of mobile genetic elements, and dissemination of genetically related resistant strains (Elkenany *et al.*, 2019; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

Resistance to clinically important antimicrobial groups, particularly fluoroquinolones and aminoglycosides, is of concern because it may reduce

the number of effective therapeutic options. The resistance pattern observed in the present study indicates that the recovered pigeon-associated *S. Typhimurium* isolates carried resistance to multiple antimicrobial classes rather than resistance being restricted to a single therapeutic group. These findings highlight the importance of continued antimicrobial-resistance surveillance and the use of standardized antimicrobial susceptibility testing methods and quality-control procedures in future studies (Elkenany *et al.*, 2019; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

The partial susceptibility observed for doxycycline should also be interpreted cautiously. Because only five isolates were examined, the susceptibility observed in a small proportion of isolates cannot be used to make general treatment recommendations. Moreover, the presence of *tetA* in all isolates indicates that tetracycline resistance determinants were present despite the observed phenotypic variation. Antimicrobial susceptibility results may provide useful laboratory information; however, treatment decisions should be based on validated veterinary interpretive standards, the clinical condition of the animal, the infection site, and appropriate professional judgment.

The small number of *Salmonella* isolates is an important limitation when interpreting the reported percentages. In a sample of five isolates, each individual isolate represents 20% of the total; therefore, the addition or removal of a single isolate can substantially change the reported resistance percentage. Accordingly, individual isolate resistance profiles should be presented alongside overall percentages, and the findings should not be generalized to the prevalence of antimicrobial resistance among all pigeons in Thi-Qar Province. The principal conclusion is that multidrug-resistant *S. Typhimurium* was detected among the examined pigeon isolates, highlighting the need for continued surveillance and further investigation of the sources

and mechanisms of antimicrobial resistance (Elkenany *et al.*, 2019; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

5.8 Multidrug-resistant and extensively drug-resistant

All five *S. Typhimurium* isolates were classified as multidrug-resistant (MDR) according to the operational definition applied in the present study, with resistance to antimicrobial agents belonging to at least three antimicrobial categories. This finding is consistent with the clinical and epidemiological concern regarding resistance across multiple antimicrobial categories described by Magiorakos *et al.* (2012), and is broadly compatible with the resistance patterns reported by Founou *et al.* (2021) and Prestinaci *et al.* (2015). However, the observed MDR proportion of 100% should be interpreted cautiously because it was calculated from only five isolates and therefore should not be generalized to the wider pigeon population of Thi-Qar Province. The finding indicates that resistance occurred across multiple antimicrobial categories rather than being restricted to a single antimicrobial agent or class. Such resistance may limit therapeutic options and facilitate the persistence of resistant organisms under antimicrobial selection pressure (Founou *et al.*, 2021; Prestinaci *et al.*, 2015; Magiorakos *et al.*, 2012).

Three of the five isolates were classified as extensively drug-resistant (XDR), representing 60% of the recovered isolates. This relatively high proportion indicates a substantial resistance burden among the *S. Typhimurium* isolates examined. However, XDR classification depends on the number of antimicrobial categories tested and the specific criteria applied to determine non-susceptibility. Therefore, the interpretation should be based on the complete antimicrobial susceptibility matrix and the operational definition specified in the methodology. In particular, the antimicrobial categories included in the analysis and the handling of intermediate susceptibility results should be clearly stated, as differences in antimicrobial panels and

classification criteria may affect MDR and XDR categorization (Magiorakos *et al.*, 2012; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

The coexistence of MDR and XDR phenotypes with the *tetA* and *blaCTX-M* genes supports the presence of multiple antimicrobial-resistance determinants within the examined isolates. Nevertheless, these two genes cannot account for the complete resistance phenotype. Resistance to fluoroquinolones, aminoglycosides, sulfonamides, and other antimicrobial agents may involve additional acquired resistance genes, chromosomal mutations, altered permeability, or other resistance mechanisms that were not investigated in the present study. Whole-genome sequencing would provide a more comprehensive characterization of the resistance determinants and could also help determine the genetic relatedness of the isolates (Magiorakos *et al.*, 2012; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

The detection of MDR and XDR *S. Typhimurium* among pigeons represents a potential One Health concern because pigeons can move between human settlements, farms, markets, and waste-disposal areas. Shedding of resistant bacteria through feces may contribute to environmental contamination and create opportunities for indirect exposure of other animals or humans. However, this represents a potential transmission pathway rather than direct evidence of zoonotic transmission. Effective control therefore requires a combination of prudent antimicrobial use, environmental sanitation, appropriate biosecurity, targeted antimicrobial-resistance surveillance, and collaboration among veterinary, public-health, and environmental sectors (Magiorakos *et al.*, 2012; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

Overall, the present study demonstrates the occurrence of MDR and XDR *S. Typhimurium* carrying important antimicrobial-resistance determinants among pigeons in Thi-Qar Province. The principal strength of the study is the integration of prevalence data, conventional and automated

identification, molecular confirmation, virulence-gene detection, and antimicrobial-resistance analysis. However, the findings should be interpreted in light of the small number of confirmed isolates, the cross-sectional study design, and the absence of comparative environmental, animal, or human isolates. Addressing these limitations in future investigations would help distinguish local bacterial carriage from environmental persistence and provide stronger evidence regarding potential zoonotic transmission and the dissemination of antimicrobial-resistance determinants (Magiorakos *et al.*, 2012; Founou *et al.*, 2021; Prestinaci *et al.*, 2015).

Conclusions and Recommendations

Conclusions

1. *Salmonella Typhimurium* was detected among pigeons at 5% from 100 samples collected from different regions of Thi-Qar province.
2. The identification of *Salmonella Typhimurium* was successfully done using conventional and bacteriological methods , VITEK2 and molecular detection of the *invA* and *stm* genes confirming the accuracy and reliability of the identification process.
3. Despite 40% phenotypic susceptibility to doxycycline, the presence of the *tetA* gene indicates a high genetic resistance risk, making tetracyclines unsuitable as first-line therapy and requiring cautious interpretation of susceptibility results.
4. Each isolate had *tetA* and *blaCTX-M* resistance genes, which indicates the genetic cause of tetracycline and β -lactam resistance, and is consistent with the multidrug-resistant pattern predicted by antimicrobial susceptibility tests.

Recommendations

Recommendations

1. Routine molecular surveillance of *Salmonella* spp. and antimicrobial resistance determinants, including *invA*, *stm*, *tetA*, and *blaCTX-M*, should be systematically implemented in pigeon and other avian populations to facilitate the early detection of emerging antimicrobial resistance patterns and monitor their epidemiological trends.
2. Enhanced biosecurity, hygienic practices, and environmental sanitation should be implemented in pigeon-rearing facilities to minimize fecal contamination, limit bacterial transmission, and reduce the potential dissemination of zoonotic pathogens.
3. An integrated diagnostic approach combining conventional culture, biochemical identification systems, and PCR-based molecular techniques should be standardized and adopted in diagnostic laboratories to improve the sensitivity, specificity, and timeliness of zoonotic pathogen detection.
4. A coordinated One Health surveillance and control strategy integrating veterinary, medical, and environmental sectors should be established to strengthen the monitoring and control of multidrug-resistant *Salmonella*, thereby reducing its transmission and minimizing associated public health risks.
5. The use of appropriate and standardized diagnostic methods, including culture, biochemical identification, automated systems, and PCR, is recommended to ensure accurate and reliable bacterial identification.

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Appendix

Comments:	

Identification Information	Card:	GN	Lot Number:	2413167503	Expires:	Jul 29, 2026 13:00 CDT
	Status:	Final	Analysis Time:	3.88 hours	Completed:	Jan 12, 2025 23:10 CST
Organism Origin	VITEK 2					
Selected Organism	99% Probability		Salmonella group			
	Bionumber: 0015610460006210		Confidence: Excellent identification			
Analysis Organisms and Tests to Separate:						
Salmonella group						
Salmonella spp						
Salmonella ser.Paratyphi B						
Salmonella ser.Typhimurium						
Salmonella enterica ssp enterica						
Salmonella ser.Enteritidis						
Salmonella ser.Paratyphi C						
Analysis Messages:						
Confirm by serological tests						
Contraindicating Typical Biopattern(s)						

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

Laboratory Report

Printed by: System
Patient ID: 43Nasiriya

System #:

Patient Name: dr, safaa

Isolate: 43Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011549 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0015610460006210

Organism Quantity:

Selected Organism: Salmonella group

Action	Name (User ID)	Date/Time	Comment
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Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

Page 2 of 2

Comments:	

Identification Information	Card: GN	Lot Number: 2413167503	Expires: Jul 29, 2026 13:00 CDT
	Status: Final	Analysis Time: 3.88 hours	Completed: Jan 12, 2025 23:10 CST
Organism Origin	VITEK 2		
Selected Organism	99% Probability	Salmonella group	
	Bionumber: 0015610460006210	Confidence: Excellent identification	
Analysis Organisms and Tests to Separate:			
Salmonella group			
Salmonella spp			
Salmonella ser.Paratyphi B			
Salmonella ser.Typhimurium			
Salmonella enterica ssp enterica			
Salmonella ser.Enteritidis			
Salmonella ser.Paratyphi C			
Analysis Messages:			
Confirm by serological tests			
Contraindicating Typical Biopattern(s)			

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

System #:

Patient Name: dr. safaa

Isolate: 44Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011550 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: System

Patient ID: 44Nasiriya

Bionumber: 0015610460006210

Organism Quantity:

Selected Organism: Salmonella group

Action	Name (User ID)	Date/Time	Comment
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Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

bioMérieux Customer:
System #:
Patient Name: dr. safaa
Isolate: 45Nasiriya-1 (Approved)
Card Type: GN Bar Code: 2413167503011551 Testing Instrument: 000014EEE4E4 (9513)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: System
Patient ID: 45Nasiriya

Bionumber: 0015610460006210
Organism Quantity:

Selected Organism: Salmonella group

Comments:	

Identification Information	Card: GN	Lot Number: 2413167503	Expires: Jul 29, 2026 13:00 CDT
	Status: Final	Analysis Time: 3.87 hours	Completed: Jan 12, 2025 23:09 CST
Organism Origin	VITEK 2		
Selected Organism	99% Probability	Salmonella group	
	Bionumber: 0015610460006210	Confidence: Excellent identification	

Analysis Organisms and Tests to Separate:

Salmonella group
Salmonella spp
Salmonella ser. Paratyphi B
Salmonella ser. Typhimurium
Salmonella enterica ssp enterica
Salmonella ser. Enteritidis
Salmonella ser. Paratyphi C

Analysis Messages:

Confirm by serological tests

Contraindicating Typical Biopattern(s)

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Installed VITEK 2 Systems Version: 9.02
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMérieux Customer:

System #:

Patient Name: dr. safaa

Isolate: 45Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011551 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator (Labadmin)

Laboratory Report

Printed by: System

Patient ID: 45Nasiriya

Bionumber: 0015610460006210

Organism Quantity:

Selected Organism: Salmonella group

Action	Name (User ID)	Date/Time	Comment
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Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

Page 2 of 2

bioMérieux Customer:

Laboratory Report

System #:

Printed by: System

Patient Name: dr. sasa

Patient ID: 46Nasiriya

Isolate: 46Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011559 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0015610461006210

Organism Quantity:

Selected Organism: Salmonella group

Comments:	

Identification Information	Card:	GN	Lot Number:	2413167503	Expires:	Jul 29, 2026 13:00 CDT
	Status:	Final	Analysis Time:	3.97 hours	Completed:	Jan 12, 2025 23:08 CST

Organism Origin	VITEK 2
-----------------	---------

Selected Organism	99% Probability	Salmonella group
	Bionumber: 0015610461006210	Confidence: Excellent identification

Analysis Organisms and Tests to Separate:

Salmonella group
Salmonella spp
Salmonella ser.Paratyphi B
Salmonella ser.Typhimurium
Salmonella ser.Paratyphi C
Salmonella ser.Enteritidis
Salmonella enterica ssp enterica

Analysis Messages:

Confirm by serological tests

Contraindicating Typical Biopattern(s)

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

bioMérieux Customer:

Laboratory Report

System #:

Printed by: System

Patient Name: dr, sasa

Patient ID: 46Nasiriya

Isolate: 46Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011559 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0015610461006210

Selected Organism: Salmonella group

Organism Quantity:

Action	Name (User ID)	Date/Time	Comment
--------	----------------	-----------	---------

Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

bioMérieux Customer:

Laboratory Report

System #:

Patient Name: dr. safaa

Isolate: 47Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011560 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Printed by: System

Patient ID: 47Nasiriya

Bionumber: 0015610460006210

Organism Quantity:

Selected Organism: Salmonella group

Comments:	

Identification Information	Card:	GN	Lot Number:	2413167503	Expires:	Jul 29, 2026 13:00 CDT
	Status:	Final	Analysis Time:	3.95 hours	Completed:	Jan 12, 2025 23:07 CST
Organism Origin	VITEK 2					
Selected Organism	99% Probability		Salmonella group		Confidence: Excellent identification	
	Bionumber: 0015610460006210					
Analysis Organisms and Tests to Separate:						
Salmonella group						
Salmonella spp						
Salmonella ser.Paratyphi B						
Salmonella ser.Typhimurium						
Salmonella ser.Paratyphi C						
Salmonella ser.Enteritidis						
Salmonella enterica ssp enterica						
Analysis Messages:						
Confirm by serological tests						
Contraindicating Typical Biopattern(s)						

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

bioMérieux Customer:

System #:

Patient Name: dr. safaa

Isolate: 47Nasiriya-1 (Approved)

Card Type: GN Bar Code: 2413167503011560 Testing Instrument: 000014EEE4E4 (9513)

Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: System

Patient ID: 47Nasiriya

Bionumber: 0015610460006210

Organism Quantity:

Selected Organism: Salmonella group

Action	Name (User ID)	Date/Time	Comment
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Installed VITEK 2 Systems Version: 9.02

MIC Interpretation Guideline:

AES Parameter Set Name:

Therapeutic Interpretation Guideline:

AES Parameter Last Modified:

Page 2 of 2

الملخص

تُعد بكتيريا *Salmonella* spp، ولا سيما *Salmonella Typhimurium*، من أبرز أنواع البكتيريا الممرضة ذات الأهمية الحيوانية المنشأ. وتُعد الحمام مستودعات وحوامل لهذه الممرضات، إذ يمكنها طرح البكتيريا مع البراز دون ظهور أعراض سريرية واضحة، مما قد يؤدي إلى زيادة معدلات التلوث البيئي ويسهم في انتشار العدوى بين أنواع الطيور الأخرى وحيوانات المزرعة.

ونظرًا لانتشار الحمام في كلٍّ من المناطق الحضرية والريفية، فإنه يمثل عاملاً مهمًا في وبائيات داء السلمونييلات. كما أن ظهور سلالات *Salmonella* المقاومة للمضادات الميكروبية أصبح من أبرز مشكلات الصحة العامة، لما يسببه من تضيق نطاق الخيارات العلاجية وتعزيز إمكانية الانتقال الأفقي لجينات المقاومة. لذلك، تبرز أهمية التشخيص الدقيق لـ *Salmonella Typhimurium*، وتحديد أنماط مقاومتها للمضادات الحيوية، ودراسة توزيعها الوبائي بين الحمام.

في هذه الدراسة، أُخضعت العينات أولاً للإغناء في مرق Selenite F، ثم زُرعت العزلات على الأوساط الزرع الانتقائية، بما في ذلك *Salmonella-Shigella* (SS) agar و Xylose Lysine Deoxycholate (XLD) agar. وتميزت مستعمرات *Salmonella* على وسط XLD بمظهر باهت مع مراكز سوداء نتيجة إنتاج كبريتيد الهيدروجين (H_2S)، في حين أظهر الفحص المجهرى عصيات سالبة لصبغة غرام.

تم التعرف على العزلات باستخدام نظام VITEK 2 Compact، والذي أظهر نسبة تأكيد بلغت 98% لعزلات *Salmonella* spp. وقد جُمعت وفُحصت 100 عينة مسحة للكشف عن الممرضات البكتيرية. كما استُخدمت تقنية تفاعل البوليميراز المتسلسل (PCR) للكشف عن جين *invA* بحجم 284 bp، والذي ظهر في 100% من العزلات. وأظهر الكشف عن جين *stm* بحجم 523 bp أن جميع العزلات كانت تعود إلى *Salmonella Typhimurium*. وبلغت نسبة الانتشار الكلية للممرض (5/100) 5% في المقابل، كانت *Escherichia coli* أكثر العزلات شيوعًا بنسبة 70%، تلتها *Klebsiella* spp بنسبة 15% و *Enterobacter* spp بنسبة 10%.

وبحسب العمر، سُجلت نسبة 80% من العزلات الإيجابية في الحمام اليافع الذي تراوحت أعمارها بين شهر واحد وستين، مما يشير إلى زيادة قابلية الحمام الأصغر عمرًا للإصابة. أما فيما يتعلق بمواقع أخذ العينات، فقد توزعت العينات الإيجابية بواقع 3 حالات في الناصرية، وحالة واحدة في الغراف، وحالة واحدة في الشطرة، مع تسجيل دلالة إحصائية عند $p = 0.05$ وفقًا للتحليل المستخدم.

وأظهرت نتائج اختبار الحساسية للمضادات الميكروبية ارتفاعاً ملحوظاً في مستويات مقاومة عزلات *Salmonella Typhimurium*. إذ سجلت العزلات مقاومة بنسبة 100% لكل من trimethoprim-، gentamicin، cefotaxime، ampicillin، fosfomycin، amoxicillin/clavulanic acid، ciprofloxacin، sulfamethoxazole و imipenem. كما أظهرت مستويات مرتفعة من المقاومة تجاه levofloxacin و streptomycin و ceftazidime، حيث بلغت نسبة المقاومة حوالي 80% في حين كان doxycycline الأقل من حيث مستوى المقاومة، إذ بلغت نسبة العزلات المقاومة له 60%.

وأظهر التحليل الجزيئي أن جميع العزلات (100%) كانت حاملة لجين المقاومة *tetA* بحجم 576 bp، والمرتبطة بمقاومة التتراسيكلينات، بالإضافة إلى جين *blaCTX-M* بحجم 593 bp، والذي يرتبط بصورة رئيسية بمقاومة المضادات الحيوية من مجموعة البيتا-لاكتام. كما تبين أن جميع العزلات كانت متعددة المقاومة للمضادات الحيوية (MDR) بنسبة 100%، في حين صُنفت 60% منها على أنها مقاومة على نطاق واسع للمضادات الحيوية (XDR).

وبصورة عامة، تشير هذه النتائج إلى ارتفاع مستويات مقاومة *Salmonella Typhimurium* المعزولة من الحمام، مما يؤكد إمكانية إسهام الحمام في حمل ونقل الممرضات البكتيرية المقاومة للمضادات ذات الأهمية في مجال الصحة العامة.

وفي الختام، أظهرت الدراسة الحالية وجود سلالات عالية المقاومة من *Salmonella Typhimurium* في الحمام، مما قد يجعل هذه الطيور مصدراً أو حاملاً محتملاً للممرضات المقاومة للمضادات الميكروبية ذات المنشأ الحيواني. وعليه، يُوصى بإجراء مراقبة وبائية مستمرة، إلى جانب تطبيق ممارسات صارمة لترشيد استخدام المضادات الميكروبية، للحد من انتشار السلالات المقاومة وتقليل المخاطر المحتملة على الصحة العامة.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة الشطرة كلية الطب البيطري

فرع الأحياء المجهرية البيطرية

دراسة جزيئية لبعض جينات ضراوة السالمونيلا غير التيفية المعزولة من الحمام من محافظة ذي قار

رسالة ماجستير مقدمة إلى مجلس كلية الطب البيطري، جامعة الشطرة، استكمالاً
لمتطلبات الحصول على درجة الماجستير في علم الأحياء الدقيقة.

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