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Isolation and Diagnosis of *Salmonella Typhimurium* from Pigeon (*Columba livia domestica*) with Diarrheal Disease in Thi-Qar Province, Iraq

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Abstract

Salmonella enterica serovar typhimurium is a major avian enteric pathogen and zoonotic agent, which has considerable veterinary and public health impacts. The synanthropic nature of pigeons may contribute to the spread of multidrug-resistant Salmonella, but their epidemiology in salmonellosis is poorly understood.

Aim: This study aims to detect and isolate S. typhimurium from diarrheic pigeons, to study the antibiotic resistance patterns of the isolated S. typhimurium, and to evaluate the public health issues obtained by the results of the study.

Methods: 100 samples of diarrheic pigeon's fecal matter were aseptically collected. These were cultured on nutrient agar, and then subcultured on Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella agar (SSA). Potential Salmonella colonies were identified by appearance and confirmed using the VITEK 2 identification system. Drug susceptibility patterns were determined and Fisher's exact test was used to measure statistical significance.

Results: Results showed that the most common isolate was Escherichia coli (70%), followed by Klebsiella (15%), Enterobacter spp. (10%), and Salmonella Typhimurium (5%). S. typhimurium appeared as red colonies with a black centre (hydrogen sulfide production) on XLD agar media. A high level of multidrug resistance was observed. Full resistance (100%) was found against Fosfomycin 200 µg, Ampicillin 10 µg, Cefotaxime 30 µg, Gentamicin 10 µg, Trimethoprim-Sulfamethoxazole 1.25/23.75 µg, Ciprofloxacin 5 µg, Amoxicillin-Clavulanic acid 20/10 µg and Imipenem 10 µg ($p < 0.05$). Resistance to Levofloxacin 5 µg (80%), Streptomycin 10 µg (80%), Ceftazidime 30 µg (80%) and Doxycycline 30 µg (60%) was not significant ($p > 0.05$). Conclusion: Diarrheal disease can be caused by multidrug-resistant S. typhimurium in pigeons, suggesting the animals may serve as a reservoir and spreaders of resistant strains. Combining selective media with the VITEK 2 system was a successful strategy. These results highlight the importance of ongoing surveillance of avian salmonellosis, biosecurity, and appropriate use of antibiotics to reduce the zoonotic risk associated with resistant Salmonella.

Keywords: Salmonella Typhimurium; pigeons; diarrheal disease; antimicrobial resistance.

الملخص

إن هذا النوع من هذه البكتيريا **Salmonella enterica** هو من النمط المصلي **Typhimurium** والذي يُعد عامل مُمرض معوي في الطيور ، وتعتبر أيضاً مُسبب مرضي مشترك بين الانسان و الحيوان ، و له تأثير كبير على الصحة البيطرية العامة . و تعايش الحمام مع الانسان يساهم في انتشار السالمونيلا المتعددة المقاومة للمضادات الحيوية ، و لا يزال الدور الوبائي لداء السالمونيلا غير مفهوم بشكل كافٍ .

إن الهدف من هذه الدراسة هو الكشف عن و عزل **Salmonella Typhimurium** من الحمام المصاب بالإسهال ، لغرض تسجيل الأنماط المقاومة للمضادات الحيوية في هذه العزلات ، و تقييم التاثير المحتمل لهذه النتائج على الصحة العامة.

تم جمع 100 عينة من الحمام المصاب بالإسهال بطرق معقمة . تمت زراعة العينات اولياً على وسط الآكار المغذي ، ثم أُعيدت زراعتها على الأوساط الانتقائية و التفرقية و هي كما في التالي

XLD (Xylose Lysine Deoxycholate) SSA (Salmonella-Shigella agar),

و بناءً على مظهر المستعمرات البكتيرية تم التعرف على السالمونيلا المحتملة ، و تم تاكيدها باستخدام نضام **VITEK 2** لغرض التشخيص . و تم تحديد أنماط الحساسية للمضادات الحوية ، و بإستخدام اختبار فيشر الدقيق **(Fisher's exact test)** تم قياس الدلالة الإحصائية .

أظهرت الفحوصات المايكروبايلوجية أن اكثر العزلات شيوعاً كانت **Escherichia coli** بنسبة 70% ، ثم **Klebsiella spp.** بنسبة 15% ، ثم **Enterobacter spp.**بنسبة 10%، سُجلت **Salmonella enterica serovar Typhimurium** بنسبة 5% . و قد أظهرت عزلات **Salmonella Typhimurium** نمواً مميزاً على وسط **XLD** بشكل مستعمرات حمراء ذات مراكز سوداء ، وهذا يشير الى قدرتها على انتاج كبريتيد الهايدروجين. كما بينت نتائج اختبار الحساسية للمضادات الحيويه وجود مستوى عالي من المقاومة المتعددة، إذ سُجلت مقاومه كاملة بنسبة 100% تجاه الفوسفوميسين، الامبسيلين، السيفوتاكسيم، الجنتاميسين، الترايميثوبريم-سلفاميثوكسازول، السيبروفلوكساسين، الاموكسيسيلين-كلافولانيك، الإيميبينيم، وكانت هذه المقاومة ذات دلالة إحصائية عند مستوى **$p < 0.05$** . في المقابل ، أظهرت العزلات مقاومة بنسبة 80% لكل من الليفوفلوكساسين، الستربتوميسين، و السيفتازيديم، وبنسبة 60% تجاه الدوكسيسيكلين، إلا ان هذه النتائج لم تكن ذات دلالة إحصائية **$p > 0.05$** .

اشارت النتائج هي هذه الدراسة الى ان **Salmonella Typhimurium** المقاومة لعدة مضادات حيوية قد تكون من العوامل البكتيرية المرتبطة بحالات الإسهال في الحمام ، مما يعزز احتمالية دور هذه الطيور كمستودع و ناقل محتمل للسلالات المقاومه . كما اظهر الجمع بين الأوساط الزرعية الانتقائية و نظام **VITEK2** كفاءة واضحة في عزل و تشخيص العزلات البكتيرية . و تؤكدهذه النتائج أهمية اعتماد برامج مراقبة مستمرة لداء الساموتيلا في الطيور ، مع تعزيز إجراءات الأمن الحيوي و ترشيد استخدام المضادات الحيوية ، بهدف الحد من انتشار السلالات المقاومة و تقليل المخاطر الصحية المشتركة بين الانسان و الحيوان .

الكلمات المفتاحة **Salmonella enterica serovar Typhimurium** : ؛ الحمام ؛ الإسهال ؛ مقاومة المضادات الحيوية ؛ المقاومة المتعددة ؛ الامراض المشتركة بين و الحيوان ؛ **VITEK2** ؛ الصحة العامة

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1 .Introduction

Salmonella enterica serovar Typhimurium is a highly important human and animal pathogen of worldwide epidemiological importance. It is a major cause of non-typhoidal salmonellosis, causing a spectrum of clinical disease ranging from self-limiting gastroenteritis to severe systemic disease and death in at-risk individuals [1-4]. *Salmonella* belongs to the family *Enterobacteriaceae* and consists of Gram-negative, motile, facultative anaerobic bacilli. While over 2,500 serovars have been described, only a select few - namely *S. Typhimurium* and *S. Enteritidis* - are responsible for the bulk of human and animal infections and food-borne outbreaks globally [1-3, 5-8].

Non-typhoidal Salmonella is commonly found in poultry and other birds, and contaminated meat, eggs and egg products are major sources of human infection [1, 5, 6, 9-13]. Infection in birds, known as avian salmonellosis, is typically enteric and results in diarrhoea, dehydration, weight loss and may cause mortality. But subclinical infections with extended shedding of the bacteria are also common, making infections difficult to detect and control [6, 14, 15]. In experimental infections of chicks with *S. Typhimurium*, the pathogen has been shown to cause acute enteritis, severe histopathological damage in the small and large intestine and profound changes in the gut microbiome, all in line with the clinical signs of severe diarrheal disease[15, 16].

S. Typhimurium is of particular concern as it has been responsible for outbreaks with high morbidity and mortality in captive and wild passerine birds. Some of these outbreaks have been linked to human salmonellosis, highlighting the zoonotic potential of avian-adapted strains [10, 17-19]. Avian-related strains often display host-adaptive characteristics, specific virulence factors and modulate biofilm formation to promote persistence among avian hosts [5, 14, 17, 20-22]. Moreover, these strains are increasingly exhibiting multidrug resistant phenotypes, which not only pose significant challenges for treatment strategies but also present a serious public health burden [4, 14, 20, 21, 23-25]

Pigeons (*Columba livia domestica*), because they are synanthropic, have high population sizes in urban environments and are in close proximity to human dwellings, food storage areas, and water sources, are potentially an important but overlooked source of Salmonella. Although ubiquitous, there has been little focus on the occurrence and resistance profiles of *S. Typhimurium* in pigeon populations, especially those showing clinical signs of gastrointestinal disease. Thus, studying *S. Typhimurium* from pigeons is crucial to better understand its prevalence, virulence and antibiotic resistance, as well as the potential of these birds to serve as a source of infection for humans and animals.

This information is critical for design and implementation of One Health-driven control measures, such as enhanced biosecurity and surveillance, prudent use of antimicrobials and vaccination strategies, to limit the impact of *avian salmonella* and its associated public health issues [1, 2, 6, 9, 10, 20]. This study aims to recover and identify *Salmonella enterica* serovar *typhimurium* from diarrheic pigeons by conventional and computer-based (VITEK 2) identification systems. To establish the resistance patterns of the isolated *S. Typhimurium* and to determine the statistical significance of patterns of resistance, and to assess the role of pigeons as potential reservoirs of multidrug-resistant *Salmonella* and the veterinary and human health risks.

2 . Materials and Methods

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2.1. Sample Collection

One hundred (100) fecal samples were collected from pigeons with diarrheal disease at the period from October 6, 2025 to February 2, 2026. The samples were collected with sterile cotton swabs and placed into sterile, leak-proof containers. To prevent bacterial proliferation and preserve sample quality, samples were stored and transported to the laboratory in cool storage (4°C) and processed within 4 hours of collection. These methods align with best practices for the detection of Salmonella in animal fecal samples [11, 13, 26]. The permission and ethical approval were received from University of Shatrah (number SU-EC-2025-10, on October 6, 2025).

2.2Sample Preparation

Ten-fold serial dilutions were prepared from each fecal sample to obtain isolated colonies for subsequent analysis. Briefly, 1 g of fecal material was homogenized in 9 mL of sterile physiological saline (0.85% NaCl) to prepare a 10^{-1} suspension. Serial dilutions were subsequently performed up to 10^{-6} as required to achieve countable, well-isolated colonies [24, 27].

2.3. Primary Culture

Aliquots (0.1 mL) from appropriate dilutions were spread onto Nutrient Agar (NA) plates to activate and recover viable bacteria. Plates were incubated aerobically at 37°C for 18–24 hours[13, 24].

2.4. Selective Isolation of *Salmonella Typhimurium*

After initial incubation, colonies with different morphologies were transferred onto selective and differential culture media: Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella agar (SSA). Inoculated plates were incubated at 37°C for 24 hours[13, 27, 28]

Presumptive *Salmonella Typhimurium* colonies were selected based on their typical appearance: red colonies with black centers on XLD agar (xylose fermentation and hydrogen sulfide [H_2S] production) and colorless colonies with black centers on SSA (non-lactose fermentation and H_2S production)[13, 27, 28].

2.5. Confirmatory Identification Using VITEK 2 System

Suspected Salmonella colonies were confirmed by the VITEK 2 automated bacterial identification system (bioMérieux, Marcy-l'Étoile, France). The VITEK 2 system uses miniaturized biochemical assays with enhanced colorimetric readings to assess the biochemical profile of bacterial isolates. Bacterial suspensions were dispensed into VITEK 2 Gram-Negative identification cards, which contain a series of substrates. The VITEK 2 system automatically tracks and analyzes biochemical reactions, and matches the metabolic patterns to a detailed database for species-level identification[29-32]

This approach, which combines traditional morphological identification on selective agar with automated biochemical identification, is in line with international standards such as ISO 6579 for the detection of Salmonella in foods and animal faeces, and offers a robust and standardized testing approach [11, 13, 33].

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on all confirmed S. Typhimurium isolates using the Kirby-Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antimicrobial agents were tested: levofloxacin (LEV, 5 µg), fosfomycin (FO, 200 µg), ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), streptomycin (S, 10 µg), gentamicin (GM, 10 µg), trimethoprim-sulfamethoxazole (COT, 1.25/23.75 µg),

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ciprofloxacin (CIP, 5 µg), doxycycline (DO, 30 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), imipenem (IPM, 10 µg), and ceftazidime (CAZ, 30 µg). The antibiotic disks used in this study were commercially obtained from HiMedia Laboratories.

2.7. Statistical Analysis

Data were organized using Microsoft Excel and analyzed with IBM SPSS Statistics. Descriptive statistics were used to calculate the number and percentage of bacterial isolates from diarrheic pigeons, while antimicrobial susceptibility results for *S. Typhimurium* isolates were expressed as frequencies and percentages of resistant and susceptible isolates. Due to the small number of confirmed isolates (n = 5), Fisher's exact test was applied, with $p \leq 0.05$ considered statistically significant; however, the findings should be interpreted cautiously because of the limited sample size.

3 . Results

3.1. Isolation and Identification of Bacterial Pathogens

Bacteriological examination of the 100 fecal samples collected from diarrheic pigeons revealed the presence of multiple enteric bacterial species. Table 1 summarizes the prevalence and distribution of bacterial isolates recovered from the examined samples

.

Table 1. Prevalence of Bacterial Isolates Recovered from Diarrheic Pigeons (n = 100)

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

The most commonly isolated bacterium was *Escherichia coli*, which accounted for 70% of the isolates. *Klebsiella spp*. and *Enterobacter spp*. were isolated from 15% and 10% of the samples, respectively. *Salmonella enterica* serovar Typhimurium was isolated from 5% (5/100) of the samples.

3.2Colony Morphology on Selective Media

After 24 hours at 37°C on XLD agar, the five S. Typhimurium isolates formed typical red colonies with black centers (Figure 1a). The colonies' appearance is indicative of xylose fermentation and hydrogen sulfide (H_2S) production, as H_2S reacts with ferric ammonium citrate in the agar to produce a black precipitate.

On SSA, the isolates showed non-lactose fermenting colonies with black centers, indicative of H_2S production. On nutrient agar, *S. typhimurium* colonies appeared as smooth, circular, moist, and slightly raised colonies with entire margins. The colonies were translucent to opaque and creamy grayish in color after incubation at 37°C for 24 hours (Figure 1b).

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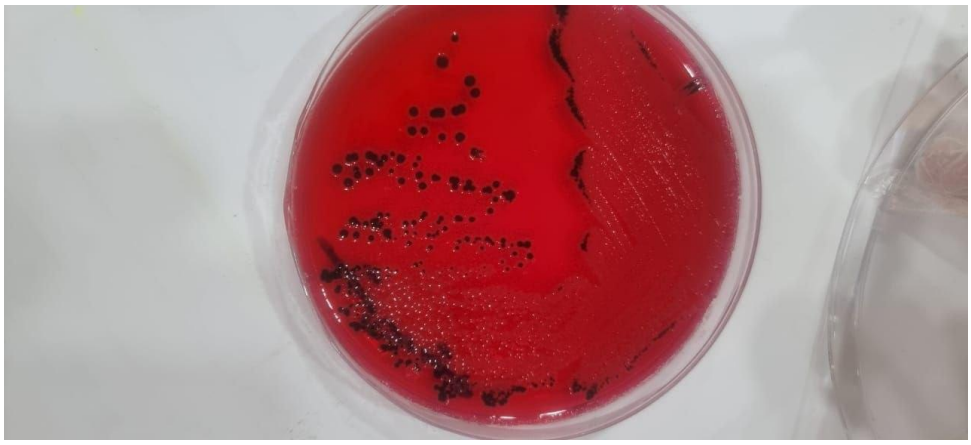

Figure 1a: *S. typhimurium* colonies on XLD agar showing red colonies with black centers.

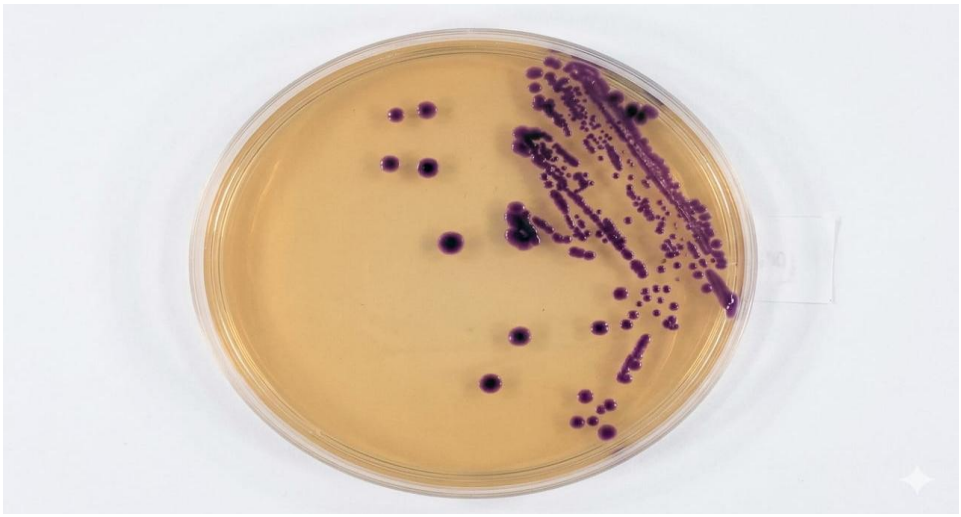

Figure 1b: Colonies of *S. typhimurium* grown on nutrient agar after 24 h incubation at 37°C.

Figure 1c: Colonies of *S. typhimurium* grown on SS agar after 24 h incubation at 37°C.

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3.3. Distribution of *Salmonella Typhimurium* According to Sex

The distribution of *S. typhimurium* isolates according to the sex of sampled pigeons is presented in Table 2.

Table 2. Distribution of *Salmonella Typhimurium* Isolates According to Sex

Sex	No	%
Male	4	4%
Female	1	1%

3.4Antimicrobial Resistance Profiles

The antimicrobial susceptibility of the five confirmed *S. Typhimurium* isolates is shown in Table 3 and Figure 2. The isolates demonstrated an extremely resistant multidrug-resistant (MDR) pattern with 100% resistance to eight of the twelve antibiotics tested

.

Table 3. Antimicrobial Susceptibility Profile of *Salmonella Typhimurium* Isolates (n = 5) Recovered from Diarrheic Pigeons

no.	Antibiotic	No tested	Sensitive	Percent	No tested	Resistant	Percent
1	LEV	5	**1**	**20%**	5	**4**	**80%**
2	FO	5	**0**	**0%**	5	**5**	**100%**
3	AMP	5	**0**	**0%**	5	**5**	**100%**
4	CTX	5	**0**	**0%**	5	**5**	**100%**
5	S	5	**1**	**20%**	5	**4**	**80%**
6	GM	5	**0**	**0%**	5	**5**	**100%**
7	COT	5	**0**	**0%**	5	**5**	**100%**
8	CIP	5	**0**	**0%**	5	**5**	**100%**
9	DO	5	**2**	**40%**	5	**3**	**60%**
10	AMC	5	**0**	**0%**	5	**5**	**100%**
11	IPM	5	**0**	**0%**	5	**5**	**100%**
12	CAZ	5	**1**	**20%**	5	**4**	**80%**

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Statistically significant difference between resistant and susceptible frequencies ($p < 0.05$, Fisher's exact test). All eight strains (100%) were resistant to fosfomycin, ampicillin, cefotaxime, gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin, amoxicillin-clavulanic acid and imipenem. Fisher's exact test analysis showed that the resistance to these eight antibiotics was significantly greater than the susceptibility ($p = 0.03$ for each). Resistance to levofloxacin (80%, $p = 0.37$), streptomycin (80%, $p = 0.37$), ceftazidime (80%, $p = 0.37$) and doxycycline (60%, $p = 1.00$) were not statistically significant (probably due to the small number of samples).

Collectively, these findings indicate that all five *S. typhimurium* isolates exhibited a multidrug-resistant phenotype, defined as resistance to three or more classes of antimicrobial agents.

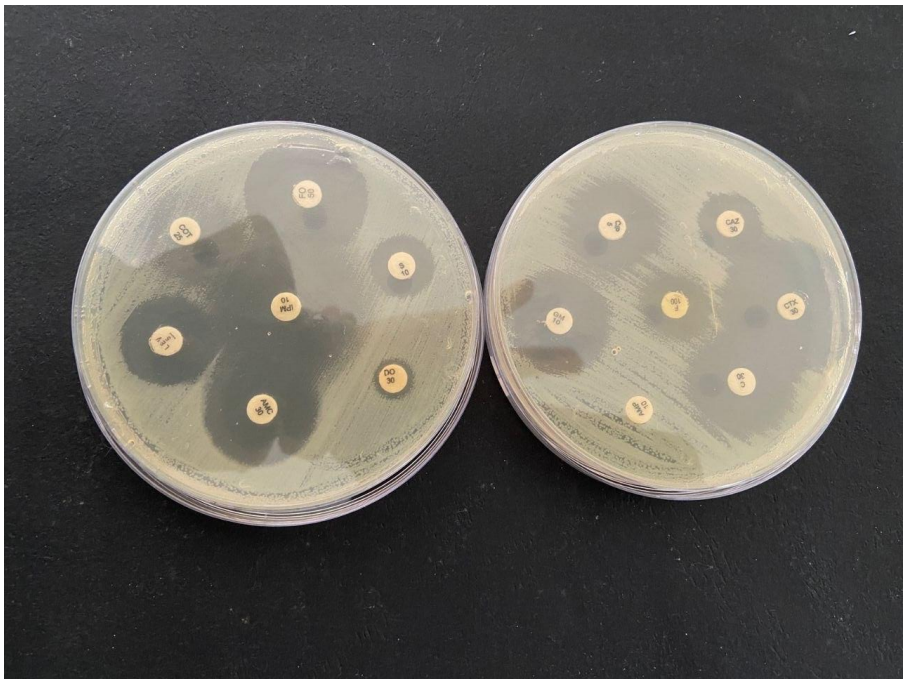

Figure 2: Antimicrobial resistance patterns of *S. typhimurium* isolates

4 .Discussion

In the present study, pigeons with diarrheal disease were found to carry *Salmonella enterica* serovar Typhimurium, although at a lower isolation rate (5%) compared with other enteric bacteria, particularly *Escherichia coli* (70%). This finding reflects the polymicrobial nature of enteric disease in pigeons, were diarrhea may results from bacterial, viral, parasitic, and environmental factors. The predominance of *E. coli*, followed by *Klebsiella spp*. and *Enterobacter spp*. Indicates an imbalance in the intestinal microbiota and suggests that diarrheal disease in pigeon cannot be attributed to *Salmonella* infections alone.

While the isolation frequency of *S. Typhimurium* was low, detection in affected birds has important epidemiological implications. *Salmonella Typhimurium* is one of the most significant avian-associated non-typhoidal Salmonella serovars with a clear zoonotic potential [10, 17, 18]. The relatively low rate could be due to intermittent shedding

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of Salmonella in the faeces, the difference in bacterial numbers of

infected individuals, or the presence of other members of the Enterobacteriaceae species in primary culture, or the lack of pre-enrichment step in the protocol of isolation. It's also possible that some of the infected pigeons were sub-clinical carriers, where Salmonella is shed intermittently at levels that may not be detected by direct plating [6, 14, 15].

Pre-enrichment in Buffered Peptone Water followed by selective enrichment in Rappaport-Vassiliadis broth may have improved the isolation rate of Salmonella from these samples. The lack of enrichment in the current study is a limitation that must be taken into account in future studies. However, the ability to recover *S. Typhimurium* from 5% of samples by direct culture methods indicates a relatively high bacterial load in these samples, which could have implications for health.

The typical colonial appearance on XLD agar, with red colonies and black centers (due to H_2S production), was as expected for *Salmonella*. The use of the VITEK 2 system for confirmation of the presumptive diagnosis improved the accuracy of the diagnosis and eliminated the possibility of errors associated with subjective interpretation of colony morphology and biochemical reactions [29, 30, 32, 33]. The combination of culture on selective media and automated biochemical testing used to diagnose Salmonella in this study is a feasible and efficient approach for routine Salmonella detection in bird samples.

The high level of multidrug resistance observed in the five S. *typhimurium* isolates was a significant and alarming finding of this study. Resistance was observed in 100% of the isolates to eight different antimicrobial agents from several classes, including β-lactams (ampicillin, cefotaxime, amoxicillin-clavulanic acid), carbapenems (imipenem), aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), folate pathway inhibitors (trimethoprim-sulfamethoxazole) and phosphonic acid derivatives (fosfomycin). This high degree of resistance is concerning for many reasons.

First, it suggests there is considerable selective pressure, presumably resulting from the misuse and overuse of antimicrobial agents in veterinary and agricultural practices. Second, it implies that widely used therapeutic agents may be ineffective or ineffective against S. Typhimurium strains isolated from pigeons, thus complicating the treatment of clinical infections in avian and potentially, human hosts. Third, the resistance profile raises concern about the potential spread and horizontal transmission of mobile genetic elements harbouring resistance genes among enteric bacteria in pigeon populations and to environmental interfaces [14, 20, 21].

The notable resistance to ciprofloxacin (a fluoroquinolone) and cefotaxime (a third-generation cephalosporin) is an important finding. These antibiotics (or their structural relatives) are listed by the World Health Organization as critically important medicines for human use, and are often used as first-line or last-chance treatment for severe infections caused by Gram-negative bacteria. The combination of fluoroquinolone and extended-spectrum cephalosporin resistance, along with resistance to a carbapenem (imipenem), is a pattern that is consistent with a combination of resistance mechanisms, including extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and/or carbapenemases, although this requires molecular confirmation and was not performed in the current study.

The resistance to amoxicillin-clavulanic acid (a β-lactam/β-lactamase inhibitor) also suggests the presence of ESBL or β-lactamases resistant to β-lactamase inhibitors. While the number of confirmed S. Typhimurium isolates was limited

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(n = 5), the consistent resistance observed among a number of the antibiotics evaluated is highly suggestive of a multidrug-resistant (MDR) strain rather than isolate-specific resistance. This observation has clinical and epidemiological ramifications that should be considered.

Epidemiologically, pigeons should not be considered simply as "spill-over" hosts for *Salmonella*. Their synanthropy, mobility, urban distribution, and potential access to human food storage areas, markets and water bodies make them potential reservoirs and mechanical vectors of pathogenic and antibiotic-resistant enteric bacteria [17, 19, 22]. Pigeons infected with resistant strains can potentially spread pathogens into the environment through their feces, which can then lead to the spread of resistant pathogens to animals and humans. This is especially pertinent in the urban and peri-urban environment, where people and pigeons interact. The results of this study are largely in agreement with published reports. Several studies have reported the presence of multidrug-resistant Salmonella in different avian species, including chickens, wild birds and pigeons [5, 14, 20-22]. The 5% isolation rate of the present study is similar to some studies but lower than others, which could be due to variations in sampling, study sites, populations, and diagnostic techniques, including the inclusion or exclusion of enrichment steps.

The finding of E. coli as the most commonly isolated enteric pathogen is consistent with many reports of enteric pathogens in pigeons and other birds. This highlights the need for broad diagnostic strategies that take into account multiple possible causes of diarrheal disease in birds.

5 .Conclusions

This study found that Salmonella enterica serovar Typhimurium is present in pigeons with diarrheal disease, but at a lower rate of isolation (5%) than other enteric bacteria, especially Escherichia coli (70%), Klebsiella spp. (15%), and Enterobacter spp. (10%). Although the occurrence of S. Typhimurium was low, the isolated strains displayed an alarmingly high level of multidrug-resistant phenotype; all of the isolated S. Typhimurium strains were resistant to eight veterinary and human important antibiotics (ampicillin, cefotaxime, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, fosfomycin, and imipenem).

The results of the present study have significant veterinary and public health consequences. Pigeons may play a role in the environmental spread of multidrug-resistant Salmonella strains, and may be an overlooked reservoir in the epidemiology of salmonellosis. Combining traditional bacteriological methods with automated biochemical analysis by the VITEK 2 system was a successful approach to the laboratory diagnosis of S. Typhimurium strains in bird faeces.

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