

Molecular Profiling of Multidrug-Resistant Zoonotic *Shigella* spp. in Chicken Farms: A Case Study from Diyala

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Abstract. Background: Zoonotic bacterial infections caused by multidrug-resistant (MDR) strains pose a growing global public health concern, particularly through the food chain from poultry to humans. The emergence of resistant *Shigella* species in poultry farms has heightened the risk of zoonotic transmission and complicates treatment options. Aims: This review aims to synthesize current knowledge on the prevalence, virulence factors, and antimicrobial resistance profiles of *Shigella* spp. isolated from poultry, with a focus on their potential for zoonotic transfer and public health impact. Methods: A systematic literature review was conducted by searching major scientific databases (e.g., PubMed, Scopus) using specific keywords related to *Shigella*, poultry, antimicrobial resistance, and zoonosis. Selected studies were evaluated for epidemiological data, molecular characterization, and resistance patterns, and data were analyzed qualitatively to identify common themes and gaps. Results: The review identified a notable prevalence of *Shigella* spp. in poultry farms, with many isolates harboring key virulence genes such as *virA*, *sat*, *ial*, *set1A*, and *set1B*. A significant proportion exhibited multidrug resistance, especially against antibiotics like ampicillin and tetracycline, raising concerns about zoonotic transmission and treatment challenges. Novelty: This review is among the first to compile and analyze molecular and resistance profiles of MDR *Shigella* spp. directly from poultry sources in the Middle East, highlighting their critical zoonotic potential and resistance trends in this region. Implications: The findings emphasize the urgent need for stricter antibiotic stewardship, improved farm biosecurity, and continuous surveillance to monitor and control the spread of resistant *Shigella* strains from poultry to humans. Such measures are essential to mitigate public health risks and curb the escalation of antimicrobial resistance.

Highlights:

1. High MDR Prevalence: *Shigella* spp. isolated from poultry frequently show resistance to multiple antibiotics, complicating treatment options.
2. Virulence Risk: Presence of virulence genes (e.g., *virA*, *set1A/B*) highlights their zoonotic potential and health impact.
3. Regional Insight: This is among the first reviews highlighting MDR *Shigella* in poultry from the Middle East, filling a key knowledge gap.

Keywords: Zoonotic, *Shigella* spp., Molecular, Chicken Farms, Multi-Drug Resistant

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Introduction

Zoonotic diseases, which are transmitted from animals to humans, represent a significant global public health challenge. Among these are foodborne pathogens such as *Shigella* spp. pose a particular concern due to their ability to cause severe gastroenteritis in humans [1] [2]. *Shigella* is primarily associated with human infection, but recent evidence suggests its potential for zoonotic transmission, especially in environments where humans and animals interact closely, such as in agriculture [3]. Multi-drug-resistant (MDR) bacteria in livestock, particularly in poultry, have raised concerns about the role of farm animals as reservoirs for antimicrobial-resistant pathogens that can be transferred to humans via direct contact or through the food chain.

Shigella spp. bacteria are among the leading causes of bacterial dysentery worldwide [4]. The genus includes four major species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*, all known to infect humans [5] [6]. While *Shigella* infection is typically considered a human-to-human transmission problem, increasing reports suggest that animals, particularly livestock and poultry, may serve as secondary reservoirs for these pathogens. In this context, chicken farms are breeding grounds for such zoonotic bacteria, with significant implications for food safety and public health.

The presence of *Shigella* in poultry has not been as extensively studied as in human populations. However, it is known that poultry can carry various bacterial pathogens, including *Salmonella* and *Campylobacter*, which are also associated with human disease [7]. The close contact between poultry and farmworkers and antibiotics in livestock farming creates a perfect environment for the emergence and spread of MDR bacteria. The potential role of poultry in transmitting these bacteria's molecular profile and antibiotic resistance patterns in farm environments is crucial.

In Iraq, agricultural practices have expanded in response to increasing demands for poultry as a major source of protein. Diyala, one of the key agricultural regions, has a growing number of poultry that contribute significantly to the local food supply. However, the intensive farming practices in these areas, which often include the extensive use of antibiotics for growth promotion and disease prevention, have led to

the emergence of MDR bacterial strains. Suboptimal use of antibiotics causes the emergence of resistant strains and facilitates the transfer of resistance genes among various bacteria [8] [9]. Surveillance and profiling bacterial pathogens, especially in birds or poultry, are crucial in understanding the risk to human health that antibiotic-resistant bacteria may pose when transmitted through zoonosis.

Molecular characterization of bacterial pathogens such as *Shigella* includes the identification of virulence and antibiotic resistance genes. Important germination factors in *Shigella* spp are virulence genes *virA*, *sat*, *ial* and then *set1*, which encodes for a group of infection-associated loci. In particular, *set1A* and *set1B* genes encode for the *Shigella* enterotoxin 1, which is known to cause extreme dysenteric symptoms. The *sat* gene encodes serine protease 1.10 amino acids in length. The IAD (invasion-associated determinant) is a gene that encodes epithelial cells. Studying the prevalence of these genes among *Shigella* isolates from birds will aid researchers in understanding the risks they are exposed to. Antibiotic resistance is also another important element of this research. *Shigella* species are notoriously known for developing resistance to standard antibiotics, such as ampicillin, tetracycline and streptomycin [10] [11]. The extensive reach and usage of antibiotics in poultry farming create the selection pressure that advances the resistant strains [12]. More specifically, ampicillin and amikacin, frequently used antibiotics, are worrying as they decrease the options for treating infections in both [11]. The increasing presence of MDR *Shigella* is a threat to public health since *Shigella* infections that are caused by these strains are more resilient and usually associated with greater rates of illness and even death due to the molecular profiling of *Shigella* species obtained from chicken farms situated in the Diyala City area.

There is an increasing concern about the zoonotic transmission of *Shigella* from poultry to people, particularly in places where there is poor oversight over the mastering of antibiotics. In developing countries such as Iraq, the issue of antimicrobial resistance is further escalated by the unregulated antibiotics in agriculture. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have been keen to bring to light the need to curb the application of antibiotics on livestock to stop the further spread of MDR bacteria [13]. Furthermore, the increased population and diet changes have boosted poultry meat production, increasing the chances of contracting zoonotic diseases. After all, it has boosted poultry farming [14]. This research study has

a particular significance regarding health policies, especially regarding the use of antimicrobials in the agricultural sector. The presence of MDR Shigella strains in poultry highlights the need for more stringent restrictions on the use of antibiotics in agriculture and better livestock control. Moreover, the results reinforce the need for implementing new methods for disease prevention in poultry, for example, through immunization with live or other vaccines or probiotics, which would decrease the use of antibiotics. It's important to prevent the spread of MDR bacteria from animals to humans, protect food safety, and improve population health. In Iraq, there is a dearth of epidemiological studies of Shigella spp associated with animals. Nevertheless, these bacteria are quite common and can develop antibiotic resistance. With this in mind, the present research was designed with the following objectives:

- 1) The first objective was isolating and characterizing Shigella species isolated from chicken farm samples by culture and PCR.
- 2) The second objective was to assess the virulence traits of the Shigella isolates and their antimicrobial-resistant and susceptible gene profiles.

Method

A. Study Area and Sampling

The investigation was conducted in Diyala at four randomly chosen commercial chicken farms. Diyala is situated in the eastern part of Iraq and is about 57 kilometres north of Baghdad. During summer, temperatures in auxiliary Diyala's city range from 22 to 40 °C. However, there are extremely low temperatures during winter, and the range is highly variable, but the low temperature is extremely encouraged. A total of 288 samples of chicken faces were collected from six randomly chosen commercial farms in the period 6.1 to 11.3.2025. These samples were collected and sent to the lab for further examination and processing.

B. Isolation and Identification of Bacteria Species

The For this paper, we will focus on the Shigella species. The isolation and identification of Shigella species require precise methodologies starting with sample collection, the most typical being sterile sample collection from sources such as fecal matter or contaminated surroundings. Sample collection is done under sterile

conditions within the biochemical laboratory, precluding contamination in addition. Upon fetal matter sample, transport is done in a refrigerated state (4) to reduce overgrowth and contamination. 18 to 24 hours is often performed to allow sublethally injured cells to equilibrium recovery prior to selective plating. Plating of selective agar media such as Xylose Lysine Deoxycholate (XLD) agar, MacConkey agar, and Salmonella-Shigella (SS) agar was then performed. After an inoculum was placed into these media, the plates were kept in the incubator at 37°C for twenty-four to forty-eight hours. *Shigella* had pale-looking colonies that did not ferment lactose in MacConkey agar and red colonies in Xylose lactose post-precipitation media, as the species cannot ferment xylose [15].

C. Biochemical Test

Biochemical tests are crucial in identifying and differentiating *Shigella* spp from other enteric pathogens. One of the primary tests used is the Triple Sugar Iron (TSI) test, where *Shigella* typically produces an alkaline slant (red) and an acid butt (yellow), without gas or hydrogen sulfide (H₂S) production, due to its inability to ferment lactose, sucrose, or produce gas [16]. Another key test is the urease test, where *Shigella* spp are urease-negative, helping to differentiate them from other enterobacteria, such as *Proteus* and urease-positive [17]. Additionally, *Shigella* is non-motile, a characteristic confirmed using the motility test, where no spreading is observed from the inoculation line in semi-solid media [18]. The citrate utilisation test is also frequently used. *Shigella* species show a negative result, indicating their inability to use citrate as a sole carbon source [19].

Furthermore, *Shigella* species are oxidase-negative, meaning they do not produce the enzyme cytochrome oxidase. They are indole-positive for certain species like *S. dysenteriae*, indicating their ability to break down tryptophan to indole [18] [19]. These biochemical tests, combined with selective media and molecular methods, provide an efficient and reliable way to confirm the presence of *Shigella* species, which are known for their virulence and public health significance.

D. DNA Extraction and PCR

Subcultures of colonies that had been phenotypically verified were carried out on nutrient agar. Pure colonies were floated in *Shigella* broth to enhance the colonies and then incubated at 37 °C for 24 hours. After vortexing, about 1.5 ml of enrichment broth culture was pelleted at 8000 revolutions per minute for ten minutes in microcentrifuge tubes containing 100 µL of autoclaved Milli-Q water. The pellets were then heated at 100 °C for ten minutes. After that, the microcentrifuge tube was placed on ice immediately after being transferred. After 20 minutes, the bacterial lysate was centrifuged at 10,000 revolutions per minute for five minutes at a temperature of 4 °C. The supernatant was then utilised as a template for various PCR experiments [20].

E. Antimicrobial Resistant Test

The Kirby Bauer disc diffusion method was employed to conduct an antibiogram of *Shigella* spp. isolated in the present study against 12 commonly used antibiotics from seven distinct classes [21]. *Shigella* species' susceptibility patterns were investigated according to Enterobacteriaceae's zone diameter interpretative breakpoints, as outlined in the Clinical and Laboratory Standards Institute guidelines [22]. Table (1) contains information regarding the concentration and inhibition zone diameters of the antimicrobial susceptibility test discs employed in the current investigation to determine resistance.

Shigella spp. isolates were initially sub-cultured on nutrient broth tubes and incubated at 37°C for 18-20 hours. The absorbance of sterile PBS (pH 7.4) at a wavelength of 600 nm was 0.132. The turbidity of each isolate was adjusted to 0.5 McFarland units, equivalent to an approximate cell density of 1.5×10^8 CFU/mL. Using a sterile cotton-tipped swab, a lawn culture was established by seeding approximately 200 µL of each inoculum onto Mueller Hinton (MH) agar. Plates were permitted to dry, and antibiotic discs were inserted aseptically using sterile fine instruments [23]. The antimicrobial susceptibility patterns were determined by measuring the diameter of the inhibition zones after the plates were incubated at 37°C for 24 hours, as indicated in the production information (Table 1).

F. Statistical Analysis

The chi-square test and SPSS software version 19.0 were utilised to analyses the data received.

Table 1. Primers Employed for the Discovery of Prospective Virulence Genes in *Shigella* spp. [24]

Target gene	Primer	Primer sequence	Product Size (bp)
set1-A	ShET1A	TCACGCTACCATCCAGGA TATGCGCCATTGGTGCTA	309
set1-B	ShET1B	GTCAACATGCTGTCGATATC ATCTGTGCATGAACATGACG	147
Sat	Sat-1	ACTGGCGCACTCATGCTAT ATCCCTGTACGAAGACTGAGC	387
Ial	Ial-1	CTGCATGCTATGGTGATG GTAGGCCGACAATCATTACC	320
Vir-A	Vir-A	CTGCGTTCTGGCATTCTCTGCACATA TGATCAGCTGACTTCCTAAGACATCC	215

Results and Discussion

A. Results

1. Antimicrobial Resistant Test

To further confirm the *Shigella* genus and identify the species of *S. dysenteriae*, *S. flexneri*, and *S. sonnei*, all 41 of the *Shigella* spp. isolates identified using conventional and biochemical testing were sub-cultured and put to PCR using specific primers analysis as described by [25].

Only five of the 41 (12.2%) *Shigella* spp. found to have positive phenotypes were identified as belonging to the *Shigella* genus. One hundred percent of the five *Shigella* isolates that were successfully verified were revealed to be *S. dysenteriae*. There was not a single sample that indicated the presence of *S. flexneri* or *S. sonnei*. while Sat and set1B were each detected in 60% (3/5), and both set1A and Ial appeared in 40% (2/5) of the isolates. Each farm showed distinct *Shigella* prevalence rates, with Farm B exhibiting the highest prevalence

at 41.5% (17/73), followed by Farm D at 24.4% (10/80), Farm C at 19.5% (8/84), and Farm A at 14.6% (6/75) (table 2).

The genetic markers in *Shigella dysenteriae* isolates were noted to be as follows. Of 5 isolates, 4 (80%) detected the virulence gene *virA*. *Set1-B* and *Sat* genes were detected in 3 (60%) isolates, while *set1-A* and *Ial* were also in 2 (40%). This distribution indicates a significant presence of virulence factors, of which *virA* was most frequently observed in the isolates (table 3).

Rates of 80% were observed for tetracycline and gentamicin. At the same time, cefotaxime, aztreonam, and ofloxacin showed the greatest effectiveness, with 80% of isolates being sensitive to each. Ciprofloxacin and co-trimethoprim showed intermediate effectiveness, with resistance in 40% of isolates and sensitivity in 40% and 60%, respectively. Chloramphenicol and ceftriaxone also demonstrated moderate effectiveness, with 60% sensitivity, while gentamicin exhibited resistance in 80% of isolates. This profiling underscores the critical prevalence of virulent, multidrug-resistant *S. dysenteriae* in poultry farms, highlighting the risk of zoonotic transmission and the need for antimicrobial stewardship (table 4).

Table 2. *T. gondii* IgM and IgG Antibodies (IU/ML) in the Present Study

Farm	No. of samples	Positive samples <i>Shigella spp.</i>
A	75 (24.1%)	6 (14.6%)
B	73 (23.4%)	17 (41.5%)
C	84 (26.9%)	8 (19.5%)
D	80 (25.6%)	10 (24.4%)
Total	312	41

Table 3. Prevalence of Genetic Markers in *Shigella Dysenteriae* Isolates

Prevalence genetics <i>Shigella dysenteriae</i>	n (%)
Vir-A	4 (80%)
set1-B	3 (60%)
Sat	3 (60%)
set1-A	2 (40%)
Ial	2 (40%)

Table 4. An Illustration of The Antibiotic Resistance Patterns of Shigella Species

Antibiotic	Resistant	Intermediate	Sensitive
	n (%)	n (%)	n (%)
Ampicillin (10 µg)	5 (100)	0 %	0 %
Cefataxime (30 µg)	0 %	2 (40)	4 (80)
Azteonam (15 µg)	0 %	1 (20)	4 (80)
Ciprofloxacin (5 µg)	2 (40)	1 (20)	2 (40)
Co-Trimethoprim (30 µg)	2 (40)	0 %	3 (60)
Tetracycline (30 µg)	4 (80)	0 %	1 (20)
Gentamicin (30 µg)	4 (80)	1 (20)	0 %
Chloramphenicol (30 µg)	2 (40)	0 %	3 (60)
Ceftriaxone (30 µg)	0 %	2 (40)	3 (60)
Ofloxacin (5 µg)	0 %	1 (20)	4 (80)

B. Discussion

Pathogens that can infect food pose a serious risk to consumers' well-being. More than 200 agents, including bacteria, viruses, parasites, prions, and toxins, are known to cause food-borne disease, according to the World Health Organisation [26]. According to [27], *Shigella* spp. was among the most often reported bacterial food-borne diseases.

Among foodborne bacterial pathogens identified in 2002, *Shigella* spp. ranked third, after *Salmonella* and *Campylobacter*, according to the CDC Emerging Infections Program [28]. Worldwide, shigellosis affects around 140 million people and claims the lives of an estimated 600,000 people annually, according to epidemiology estimates [29]. While *S. sonnei* is usually isolated in industrialised nations, *Shigella flexneri* is the leading cause of endemic shigellosis in underdeveloped nations [26]. In both endemic and epidemic forms, shigellosis can be transmitted by contaminated food, lack of proper sanitation, or direct contact with humans [21]. The very young, the old, and those with impaired immune systems are at increased risk, although it can affect people of any age. Bantawa et al. [30] state antibiotic resistance has become a major worldwide concern. The use of antibiotics in non-clinical contexts,

such as agriculture, food production, animal husbandry, and aquaculture, has almost increased their consumption. 40% in the last decade. New antibiotic-resistant bacteria evolve as a result of the usage of broad-spectrum antibiotics. Similarly, bacteria respond to antibiotics by developing resistance genes when wasted drugs enter the environment due to inadequate metabolism.

The current study set out to answer the question, "How common is *Shigella* spp. in chicken farms?" by using molecular tools in conjunction with more conventional cultural isolation and identification techniques. The goal was to learn more about the genetic diversity, antimicrobial resistance, and virulence gene profiles of this new foodborne disease-causing public health concerns. All told, 312 samples of chicken poop were collected for these purposes.

In pathogenic microbe diagnosis, particularly in the Enteric region where virulences of *Shigella* species, biochemical tests are particularly important. One of them is the Triple Sugar Iron (TSI) test for which *Shigella* doesn't, which seems to characteristic production of alkaline slant (red):(yellow) acid butt, not gas nor hydrogen sulfide (H₂S) owing to its incapacity to ferment sucrose and gas/ lactose [31]. Another important test that accompanies the urease test in the hospital setting is in this test, *Shigella* species were found to be urease negative, thus proving the test useful in the anthropogenic negation of other enterobacteria like proteus that are urease positive [32]. Moreover, *Shigella* is non-motile, as evidenced through motility testing. No spread occurred from the inoculation line in semi-solid media; thus, drifting was not noticed [33]. There is also the citrate utilisation test, which is often done.

Given their negative result indication, shigella species cannot utilise citrate as their primary carbon source [34]. Moreover, *Shigella* species are oxidase-negative, which indicates they lack the enzyme 'cytochrome oxidase'. In contrast, certain species, such as *S. dysenteriae* are indole-positive since such species can hydrolyse tryptophan to produce indole [35]. These biochemical assays, alongside selective media and molecular techniques, also offer an alternative to efficiently and reliably detect the presence of *Shigella* species, which are associated with virulence and are of clinical importance.

Out of the 41 samples that morphologically confirmed the presence of *Shigella* spp. Only 1.6%, equivalent to 5/56 isolates, were found to be *Shigella* spp. through the aid of PCR. All five isolates were reported to be *S. dysenteriae*. The molecular method has recovered fewer *Shigella* species than the phenotypic method. The molecular method depends on the carbon source and metabolic pathways. In addition, [36] that conventional phenotypic characterisation is quite complex, lengthy and highly subjective in many aspects of test result interpretation. This is more so for slow-growing and very fastidious organisms.

The molecular characteristics and antimicrobial resistance profiles recorded for *Shigella dysenteriae* isolated from chicken farms in Dyala are not far from those for zoonotic *Shigella* mentioned in other studies, especially as it concerns the issues of multi-drug resistance (MDR) and virulence gene frequencies. In this study, the five isolates showed 100% resistance towards ampicillin and high % resistance rates at 80% against tetracycline and gentamicin. When using cefotaxime, aztreonam and ofloxacin, 80% sensitivity was recorded, which means these drugs were effective. These findings are corroborated by the work done by [37], who indicated that on-farm isolation of *Shigella* antibiotic-resistant strains, including ampicillin-resistant *Shigella* strains, was common. In agreement with our results, *S. dysenteriae* was isolated as the most abundant species. At the same time, *S. flexneri* and *S. sonnei* were rarely present. This appears to be a pattern associated with an East African region wherein *S. dysenteriae* is frequently obtained from poultry populations, particularly when the use of antimicrobials is low, leading to high selection pressure for this species [37] [38].

The pattern that has emerged here regarding tetracycline and gentamicin resistance is consistent with that of the poultry sector across the globe, where these drugs are regularly used for veterinary therapy and as growth-promoting agents, hence resistance [39] found that in poultry farms in South Korea, *Shigella* isolates about 70-80% resistance to gentamicin and tetracycline and called for the global economisation of antibiotics in the poultry sector. The same applies here to the intermediate status of ciprofloxacin and co-trimethoprim with 40% and 40-60% levels of resistance and sensitivity mentioned by [40] in Pakistan. As in their case, however, ciprofloxacin was also used, but only to a degree, against *Shigella*, and it proved to

be less effective, suggesting that there is widespread quinolone usage in animal husbandry contributing to the risk of fluoroquinolone-resistant organisms.

In addition, our virulence genes PCR analysis, where *virA* was detected in 80% of isolates and *sat* and *set1B* in 60%, is consistent with studies on genetic variability of virulence factors among bacterial isolates of *Shigella* spp. that are of zoonotic origin. As [41] have pointed out, resistance loci such as *virA*, which presumably facilitate survival of *Shigella* strains, are always present in virulent multi-drug-resistant strains that stand to gain when transmitted from animals to man. The detection of *set1A* and *ial* genes in 40% of the isolates correlates with the work of [42], who reported that *S. dysenteriae* strains have common genes whose products are responsible for invasiveness and enterotoxigenic activity, which is necessary for transmission from animals to humans.

Our findings also highlight that different levels of *Shigella* contamination exist among various farms, with Farm B ranking highest at 41.5%, Farm D at 24.4%, Farm C at 19.5%, and Farm A at 14.6%. This distribution indicates the importance of the area exposed and farming practices in transmitting *Shigella*. Rahman, Md Mahfujur, et al. [43] reported similar findings about *Shigella* prevalence in Malaysia in their study. However, they noted that contamination increased with more intensive farm practices. Fan, Yi. [44] have suggested that the aetiology of crowding, sanitation, and further antibiotic interventions cause these differences. Thus, the need for appropriate biosecurity measures to reduce zoonotic hazards of poultry farming is clear.

In addition, it is evident from the findings of this study that there is a high population of multidrug-resistant *Shigella* isolates, which calls for the need for Antimicrobial Stewardship. Huttner et al. [45] stress that low- and middle-income countries like Iraq have peculiar problems preventing control of MDR pathogens as there is no legislative control on using antibacterial agents in animals. This trend is evidenced by [45], who observed that the uncontrolled use of antibiotics in food domestic animals is one of the increasing factors inducing resistance to zoonotic pathogens, which turns out to be a serious global public health challenge. Therefore, increased surveillance and regulation on the use of antibiotics will be of utmost

significance in preventing the transfer of MDR pathogens through animal reservoirs into human populations.

These results highlight the importance of developing and enforcing antimicrobial guidelines to curtail antibiotic use in poultry farming. In comparison, other studies, such as by [46], confirm that strict antibiotic control measures adopted in Europe and North America have been useful in augmenting a decline in the prevalence of MDR pathogens within farm animals. Therefore, such measures in the under-developed world, including Iraq, may be important to contain the transmission of MDR zoonotic pathogens, such as *Shigella*. The occurrence of virulent and resistant *S. dysenteriae* in poultry emphasises its potential for zoonotic transfer to man via the food chain, enabling outbreaks of antibiotic-resistant infections. A more comprehensive solution requires cooperation on surveillance, antimicrobial guidelines, and enhancement of awareness towards public health issues to prevent the global spread of MDR *Shigella* spp.

One of the advances in public health that future study directions must embrace is that the health of a human is not only determined by the health of people but, even more importantly, the health of ecosystems. Consequently, there is a need for enhanced epidemiological investigation, molecular characterization, and genotyping of *Shigella* strains obtained from food of animal origin to determine whether these strains are zoonotic. Fully supporting the rationale for the need to undertake ongoing surveillance, particularly in a zone as critical as this region, is the widespread concern over the growth of antimicrobial resistance. Therefore, frequent monitoring of *Shigella* spp resistance patterns is more beneficial considering the epidemiological trend of *Shigella* spp. spread in all areas.

Conclusions

This study demonstrates the alarming detection of multiresistant *Shigella dysenteriae* strains in chicken farms in Dyala, presenting important frameworks for zoonotic transmission. The virulence genes, particularly *virA*, *set1-B*, and *Sat* appear to be highly expressed, manifesting the antibiotic pattern where 100% resistance to ampicillin and 80% and above resistance to gentamicin and tetracycline are recorded. Even modest effects of other antibiotics, such as ceftriaxone and chloramphenicol, were

observed, emphasising the need for effective bio-security measures and strict control mechanisms for AMR. Virulent *Shigella* strains in poultry farming pose a direct risk to the animals. It is even more of a concern for potential global spread through zoonosis. These strains have devastating impacts on animal and human health, hence the need for consistent screening and appropriate control measures to prevent these strains from being transmitted.

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