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Epidemiology and antimicrobial resistance of dominant *Salmonella* serotypes in Iraqi broiler hatcheries: a comprehensive One Health analysis

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Hatcheries are critical points for *Salmonella* dissemination, and the distribution of its serotypes significantly impacts public health. Despite this global threat, surveillance deficits remain regarding these foodborne pathogens within regional production environments. To address these gaps, this investigation evaluated the occurrence rates, serotype distribution, and antimicrobial resistance profiles of bacterial isolates recovered from commercial broiler production facilities. A multi-facility cross-sectional study was conducted from march to october 2023, encompassing 450 specimens stratified equally into 150 chick cecal contents, 150 eggshells, and 150 worker hand swabs. Microbiological isolation followed international horizontal protocols. Biochemical profiling and slide agglutination methods were employed for serovar identification, while phenotypic susceptibility profiles were determined utilizing the disk diffusion method under clinical laboratory criteria. The analysis detected an overall pathogen prevalence of 8.4 %, corresponding to 38 positive samples out of the 450 tested. Specific isolation rates were distributed across chick ceca at 12.0 % (18 positive samples), eggshells at 7.3 % (11 positive samples), and worker hands at 6.0 % (9 positive samples). Serological typing revealed a predominance of *Salmonella* Enteritidis at 34.2 % (13 isolates) and *Salmonella* Kentucky at 26.3 % (10 isolates), each exhibiting alarming multidrug resistance at 38.5 % (5 isolates) and 60.0 % (6 isolates), respectively. Notably, the recovered *Salmonella* Kentucky strains demonstrated concerning ciprofloxacin resistance at 40.0 % (4 isolates) and cefotaxime resistance at 20.0 % (2 isolates). Both dominant serotypes were consistently recovered from all three sample matrices, demonstrating active circulation within the facilities. In conclusion, commercial broiler facilities serve as reservoirs for highly resistant multidrug-resistant strains. The concurrent presence of a classic egg-associated pandemic variant and an environmentally durable, fluoroquinolone-resistant clone represents a severe threat to food safety. This scenario calls for the immediate implementation of an integrative national health framework incorporating environmental biosecurity measures, strict antimicrobial stewardship, and ongoing systematic monitoring across the production continuum.

Keywords: *Salmonella* Enteritidis, *Salmonella* Kentucky, Iraq, hatchery, multidrug resistance, One Health, antimicrobial resistance.

Епідеміологія та антимікробна резистентність домінуючих серотипів *Salmonella* в бройлерних інкубаторах Іраку: комплексний аналіз «Єдиного здоров'я»

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Птахівничі інкубатори є критичними ланками поширення *Salmonella*, що безпосередньо впливають на епідемічне благополуччя населення. Попри глобальну загрозу сальмонельозу, на регіональному рівні досі бракує комплексного моніторингу біологічних властивостей цих харчових патогенів у промисловому птахівництві. Це дослідження мало на меті заповнити наявні прогалини в знаннях щодо контамінації *Salmonella* в бройлерних інкубаторах Іраку. Дослідження тривало з березня по жовтень 2023 року й охоплювало 450 зразків, які рівномірно розподілили за трьома категоріями: вміст сліпої кишки добових курчат (150 зразків), яєчна шкаралупа з вивідних лотків (150 зразків) та мазки з рук персоналу (150 зразків). Мікробіологічний скринінг здійснювали згідно з чинними міжнародними протоколами щодо горизонтальних методів виявлення патогенів. Для ідентифікації сероварів застосовували класичне біохімічне профілювання та реакцію слайд-аглютинації, тоді як чутливість до протимікробних препаратів визначали диско-дифузійним методом за критеріями Інституту клінічних та лабораторних стандартів. За результатами аналізу загальна частота виділення патогену становила 8,4 % (38 позитивних знахідок із 450 протестованих). Зокрема, рівень контамінації вмісту сліпої кишки курчат сягнув 12,0 % (18 зразків), яєчної шкаралупи – 7,3 % (11 зразків), а змивів з рук працівників – 6,0 % (9 зразків). За результатами серотипування встановлено абсолютне домінування двох варіантів: *Salmonella* Enteritidis – 34,2 % (13 ізолятів) та *Salmonella* Kentucky – 26,3 % (10 ізолятів). Обидва штами продемонстрували критично високі показники множинної стійкості до ліків (мультирезистентності), які склали 38,5 % (5 ізолятів) та 60,0 % (6 ізолятів) відповідно. Особливе занепокоєння викликає виявлена у штамів *Salmonella* Kentucky резистентність до ципрофлоксацину на рівні 40,0 % (4 ізоляти) та до цефотаксиму на рівні 20,0 % (2 ізоляти). Постійне виділення обох домінуючих серотипів з усіх типів біологічного матеріалу свідчить про їхню активну циркуляцію в інкубаторах. Таким чином, сучасні бройлерні підприємства є стійкими резервуарами небезпечних мультирезистентних штамів сальмонел. Одночасна присутність класичного пандемічного серовару, пов'язаного з яйцепродуктами, та екологічно витривалого, стійкого до фторхінолонів клону створює серйозну подвійну загрозу для безпеки харчових продуктів. Ця ситуація вимагає негайного впровадження інтегрованої національної стратегії в рамках концепції «Єдине здоров'я», яка передбачає посилення заходів біобезпеки виробничого середовища, суворий контроль за використанням антибіотиків та безперервний систематичний моніторинг на всіх етапах птахівничого континууму.

Ключові слова: *Salmonella* Enteritidis, *Salmonella* Kentucky, Ірак, інкубатор, мультирезистентність, Єдине здоров'я, антимікробна резистентність.

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Introduction

Non-typhoid *Salmonella* (NTS) is a leading foodborne pathogen globally, causing approximately 95 million cases and 50,000 deaths annually [1]. The primary source of human NTS infection is poultry and poultry-derived products, which significantly contributes to the global disease burden [2]. The diverse serotypes and epidemiology of salmonellosis are closely interlinked. Each serotype, characterized by its unique virulence, host predilection, and antimicrobial resistance (AMR), directly affects salmonellosis transmission dynamics and overall public health [3].

A commercial broiler hatchery represents a key control point, and potentially a point of amplification, within the hatchery segment of the poultry production continuum [13]. Subclinically infected breeder flocks can introduce *Salmonella* into this system through vertically transmitted, contaminated eggs [6]. The hatching phase, with its warm and humid conditions, provides an optimal environment for bacterial proliferation.

The *Salmonella*-laden dust and fluff aerosolized during the pipping phase creates a highly infectious environment, leading to massive colonization of immunologically naive chicks. These chicks, when transferred to grow-out facilities, become *Salmonella* reservoirs and shedders, effectively undermining infection control at the very beginning of the production chain [14].

In Iraq, the poultry industry plays an important role in the agricultural economy as well as national food security. Unfortunately, it remains severely affected by outbreaks of infectious diseases that impact both the economy and public health [4]. Iraq's specific socio-economic contexts, climatic and husbandry stressors, high flock density, and the extensive preventive use of antibiotics create unique ecological niches that select for certain highly resilient *Salmonella* serotypes [5, 6].

In the greater Middle East and North Africa region, certain serotypes, such as *Salmonella* Enteritidis and *Salmonella* Kentucky, have emerged and become dominant; however, major surveillance gaps at the hatchery level in Iraq still need to be addressed [7, 8].

Salmonella Enteritidis, the leading cause of foodborne illness, is a global pandemic clone that colonizes the avian reproductive tract and contaminates the internal contents of eggs [9]. In contrast, the environmentally resilient and multidrug-resistant *Salmonella* Kentucky clone poses a serious threat to infection treatment, especially due to its robust association with fluoroquinolone resistance [10] and the challenges it presents for risk assessment.

The aim of the study

This study aimed to bridge the existing knowledge gaps regarding *Salmonella* contamination within Iraq's broiler hatcheries.

To achieve this, the investigation set out to document: i) the prevalence and distribution of *Salmonella* serotypes across chicks, eggshells, and hatchery personnel, and ii) the specific patterns of antimicrobial resistance and multidrug resistance (MDR) among the recovered isolates, highlighting their critical implications for both public health and veterinary sectors.

Materials and methods

Study Design and Sample Collection

A cross-sectional study was conducted over an eight-month period from March to October 2023. The study encompassed five of the largest commercial broiler hatcheries in Iraq, located within the central geographical regions of the country, specifically in the provinces of Baghdad, Babil, and Wasit. The selection of these specific broiler hatcheries was based on their market significance and their extensive distribution of day-old chicks across a wide geographical range.

In total, four hundred and fifty (450) samples were collected aseptically, with each hatchery contributing ninety (90) samples. The collected samples were stratified as follows:

- chicks (n=150): Thirty (30) newly hatched chicks (less than 24 hours old) per hatchery were randomly selected. These chicks were humanely euthanized in strict accordance with the American Veterinary Medical Association (AVMA) guidelines, and their cecal contents were collected directly into sterile, pre-labeled 15 ml conical tubes;

- eggshells (n=150): From each of the hatcher trays, thirty (30) pieces of eggshells (approximately 4 cm^2 each) with attached membranes were collected from various positions immediately after the chicks were removed

- workers (n=150): Hand swabs were collected from thirty (30) consenting workers (six workers from each of the five hatcheries) at the end of their work shifts. Informed consent was obtained from each participant prior to swab collection. For this procedure, a standardized protocol was followed: a sterile gauze swab, pre-moistened with 5 ml of Buffered Peptone Water (BPW), was used to thoroughly swab the entire surface of the hands, including the fingers and beneath the fingernails.

Following collection, all samples were immediately placed into a cooler equipped with ice packs and were transported to the laboratory to be processed within 4 hours of collection.

Microbiological isolation and biochemical identification

Salmonella isolation and identification followed the International Organization for Standardization protocol ISO 6579-1:2017 [11] with minor adjustments. In brief, every sample was homogenized and subsequently non-selectively pre-enriched by incubation in BPW at 37 °C for 18±2 hours. Thereafter, 0.1 ml of the pre-enrichment culture was transferred to 10 ml of Rappaport-Vassiliadis (RV) broth and incubated for 24 hours at 41.5 °C. Afterward, a loopful of the RV broth was streaked onto both xylose lysine deoxycholate (XLD) agar and Hektoen enteric (HE) agar and incubated at 37 °C for 24±3 hours. Of the presumptive *Salmonella* colonies (black-centered on XLD, green with black centers on HE), the rest were purified on tryptic soy agar (TSA) for additional studies.

Systematic biochemical confirmation was performed. Characterization of purified isolates was initially conducted using conventional tubed media. Each isolate was subjected to triple sugar iron (TSI) agar in order to assess its reactions in glucose and lactose/sucrose fermentation and to evaluate hydrogen sulfide production. The lysine iron agar (LIA) was used to assess lysine decarboxylation, while urea broth was utilized to rule out urease-positive organisms, and assessment of Simmons citrate agar was performed to evaluate the ability to use citrate as the sole carbon source.

Presumptive *Salmonella* isolates would usually display TSI reactions with an alkaline (red) slant and acid (yellow) butt, with H2S blackening, while LIA shows H2S and a purple butt, and urea color change is absent, with variable reactions regarding citrate utilization. All remaining biochemically typical isolates were then confirmed using the API 20E system (bioMérieux, France) as per the guidelines, and the serotype VITEK 2 Compact System (bioMérieux, France) with the GN ID card.

Serological typing

All confirmed *Salmonella* isolates were serotyped at the National Reference Laboratory for Salmonella in Baghdad. Slide agglutination tests were subsequently carried out according to the White-Kauffmann-Le Minor scheme using specific somatic (O) and flagellar (H) antisera (Statens Serum Institut, Denmark) [11]. All serotyping procedures were performed by trained technicians to ensure quality assurance.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing for all confirmed *Salmonella* isolates was carried out using the disc diffusion method on Mueller-Hinton agar per CLSI recommendations. A panel of twelve antimicrobial agents representing nine classes was selected due to their relevance in human and veterinary medicine: ampicillin (AMP, 10 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), cefotaxime (CTX, 5 µg), ceftazidime (CAZ, 30 µg), aztreonam (ATM, 30 µg), imipenem (IPM, 10 µg), gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), azithromycin (AZM, 15 µg), and chloramphenicol (C, 30 µg).

Inoculum preparation followed the 0.5 McFarland standard. Plates were incubated for 16–18 hours at 35±2 °C. Following the measurement of the zones of inhibition, the results were categorized as susceptible (S), intermediate (I), or resistant (R), in accordance with CLSI criteria. Quality control consisted of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Multidrug resistance (MDR) was defined as non-susceptibility to at least three antimicrobial classes.

Data analysis

Data were analyzed using SPSS statistical software (version 28.0, IBM Corp., USA). Prevalence was expressed alongside a 95 % confidence interval (CI). To assess differences in prevalence across sample types and hatcheries, the chi-square test or Fisher's exact test was employed. A p-value of less than 0.05 was considered statistically significant.

Results and discussion

Prevalence of Salmonella and distribution by sample type

The comprehensive microbiological analysis confirmed the presence of *Salmonella* in 38 of the 450 collected samples, representing an overall prevalence of 8.4 % (95% CI: 6.1–11.4%), as detailed in ***Table 1***. Varying prevalence rates were observed across the different sample types, with the highest isolation rate recorded in chick cecal contents at 12.0 % (18/150). This finding indicates that a significant proportion of the progeny were colonized at an early developmental stage.

Table 1

Prevalence of *Salmonella* by sample type in Iraqi broiler hatcheries

Sample type	Number tested	Number positive	Prevalence, %	95% confidence interval (CI)
Chick cecal contents	150	18	12.0	7.3–18.3
Eggshells	150	11	7.3	3.7–12.7
Worker hand swabs	150	9	6.0	2.8–11.1
Total	450	38	8.4	6.1–11.4

Furthermore, *Salmonella* was detected on 7.3% (11/150) of the evaluated eggshells, pointing toward potential vertical transmission or environmental contamination within the hatcher trays. Regarding public health implications and potential occupational exposure, the pathogen was also isolated from 6.0 % (9/150) of worker hand swabs, highlighting a critical interface for zoonotic transmission.

Definition and distribution of dominant serotypes

The 38 isolates that underwent serological characterization showed that most belonged to two major serotypes, as demonstrated in **Table 2**. *Salmonella* Enteritidis and *Salmonella* Kentucky together comprised more than 60 % of all isolates. Other clinically and poultry-relevant serovars, including *S.* Infantis, *S.* Typhimurium, *S.* Mbandaka, and *S.* Virchow, were also recognized, albeit in much less predominant numbers.

Table 2

Serotype distribution of *Salmonella* isolates from Iraqi hatcheries

Salmonella serovar	Number of isolates	Percentage, %
S. Enteritidis	13	34.2
S. Kentucky	10	26.3
S. Infantis	5	13.2
S. Typhimurium	4	10.5
S. Mbandaka	3	7.9
S. Virchow	3	7.9
Total	38	100

It is important to highlight that the two dominant serotypes were not restricted to a single source but were

recovered from all three sample matrices, demonstrating their active circulation and persistence in the hatchery environment. Specifically, *S.* Enteritidis was retrieved from 6 chick ceca, 4 eggshells, and 3 worker hand swabs, whereas *S.* Kentucky was recovered from 4 chick ceca, 3 eggshells, and 3 worker hand swabs.

Antimicrobial resistance patterns and multidrug resistance

The AST results exhibited concerning antimicrobial resistance rates among the isolates, with the dominant pattern of each serotype outlining a specific profile. The descriptive resistance data are summarized in **Table 3**, indicating that the most common resistance profiles were directed against tetracycline and ampicillin.

Table 3

Antimicrobial resistance profile of all *Salmonella* isolates (n=38)

Antimicrobial agent (class)	Number of resistant isolates	Resistance, %
Tetracycline (tetracyclines)	28	73.7
Ampicillin (penicillins)	25	65.8
Trimethoprim-sulfamethoxazole (diaminopyrimidines/sulfonamides)	14	36.8
Chloramphenicol (amphenicols)	11	28.9
Amoxicillin-clavulanic acid (beta-lactam/beta-lactamase inhibitor)	8	21.1
Ciprofloxacin (fluoroquinolones)	6	15.8
Gentamicin (aminoglycosides)	5	13.2
Cefotaxime (3rd gen. cephalosporins)	4	10.5
Ceftazidime (3rd gen. cephalosporins)	3	7.9
Azithromycin (macrolides)	3	7.9
Aztreonam (monobactams)	2	5.3

Thus, the AST results demonstrate a high frequency of resistance to older, commonly used antimicrobials, particularly tetracycline and ampicillin, among the isolates. Conversely, lower resistance rates were observed against critically important antimicrobials, including third-generation cephalosporins, macrolides, and monobactams. Notably, no resistance was detected against imipenem, which indicates that carbapenems remain fully effective against all recovered *Salmonella* strains. These findings underline a concerning trend toward multi-drug resistance (MDR) within the hatchery environment, highlighting the urgent need for targeted antimicrobial stewardship in the poultry sector.

A detailed comparison of the phenotypic resistance characteristics revealed that antimicrobial resistance profiles in *S.* Kentucky were consistently higher than those observed in *S.* Enteritidis, with particularly pronounced disparities regarding tetracycline and ciprofloxacin susceptibility rates. While both serotypes demonstrated extensive resistance toward older first-line antimicrobials, the exact quantitative distribution of these resistance traits varied significantly. These comparative resistance patterns and their respective stratification among the 23 dominant isolates are detailed in **Table 4**.

Table 4

Comparative antimicrobial resistance profiles of the dominant *Salmonella* serotypes

Antimicrobial agent	*S.* Enteritidis (n=13) resistant, %	*S.* Kentucky (n=10) resistant, %
Ampicillin (AMP)	69.2 (9)	80.0 (8)
Tetracycline (TE)	76.9 (10)	90.0 (9)
Ciprofloxacin (CIP)	7.7 (1)	40.0 (4)
Cefotaxime (CTX)	7.7 (1)	20.0 (2)
Trimethoprim-sulfa (SXT)	30.8 (4)	50.0 (5)
MDR rate	38.5 (5)	60.0 (6)

The overall MDR rate calculated for all 38 recovered isolates from the hatcheries was 39.5 % (15/38). As demonstrated in **Table 4**, *S.* Kentucky carries the highest burden of MDR within the monitored facilities, with a remarkably high proportion of isolates resistant to multiple critical drug classes.

Thus, the comparative data indicate that while *S.* Enteritidis remains a highly prevalent threat with high resistance to ampicillin (69.2 %) and tetracycline (76.9 %), it still retains relatively high susceptibility to critical fluoroquinolones and third-generation cephalosporins. Conversely, *S.* Kentucky demonstrates an alarming expansion of resistance phenotypes, exhibiting a 40.0 % resistance rate to ciprofloxacin and a 20.0 % resistance rate to cefotaxime, alongside a staggering 60.0 % multidrug resistance rate. These findings emphasize that *S.* Kentucky represents a highly resilient, dangerous vehicle for critical resistance genes within the Iraqi poultry production continuum, demanding immediate targeted surveillance.

This detailed study sheds significant light on the *Salmonella* dynamics within the Iraqi broiler hatchery system, demonstrating that the environment is dominated by two high-risk serotypes characterized by concerning levels of antimicrobial resistance.

Though the overall prevalence of 8.4 % is moderate, it carries substantial epidemiological significance, as it confirms that the hatchery environment acts as a critical *Salmonella* reservoir and a primary dissemination node at the very beginning of the production chain. The 12.0 % prevalence observed in chick ceca serves as a clear indicator of successful vertical transmission from breeder flocks and immediate post-hatch colonization, which represents a primary determinant for persistent bacterial shedding and downstream contamination [13, 14].

Furthermore, the detection of *Salmonella* in 6.0 % of worker hand swabs constitutes a major public health finding. This result quantifies the direct occupational hazard and aligns with global studies describing poultry workers as a high-risk population for exposure to enteric pathogens [15]. These findings emphasize an urgent need for targeted biosecurity education, particularly within Iraqi facilities where stringent hygiene practices are most critical, necessitating

the enforcement of strict handwashing protocols and the mandatory use of personal protective equipment, such as gloves.

The most notable outcome of this investigation is the clear predominance of two specific serotypes: *Salmonella* Enteritidis (34.2 %) and *Salmonella* Kentucky (26.3 %). The prevalence of *S.* Enteritidis is particularly concerning, as this serotype remains the leading cause of egg-associated salmonellosis globally [9, 16]. It poses a unique threat due to its specific capacity to colonize the avian reproductive tract, specifically the oviduct, thereby contaminating the internal contents of intact eggs via vertical transmission.

Its persistent presence during hatchery operations indicates active circulation within the breeder flocks supplying the hatching eggs. Moreover, the concurrent detection of *S.* Enteritidis on eggshells and worker hands illustrates the ease with which the pathogen disseminates within the hatchery facility, highlighting multiple interconnected routes for potential occupational and environmental exposure.

The widespread incidence of *S.* Kentucky suggests another serious issue, highlighting a substantial epidemiological challenge. This serotype is highly adaptive, successful, and environmentally resilient. Furthermore, *S.* Kentucky is frequently characterized by multidrug resistance [10, 17]. In our study, the resistance profile of *S.* Kentucky is highly concerning, particularly the 40.0 % resistance rate to ciprofloxacin. Ciprofloxacin is a fluoroquinolone that is critically important to human medicine. In humans, infections caused by resistant *Salmonella* strains are more likely to be severely invasive and exhibit a poor response to standard treatments [18]. Consequently, the high multidrug resistance rate (60.0%) found among the *S.* Kentucky isolates severely limits therapeutic options in both human and veterinary medicine.

The high background level of antimicrobial resistance, especially to tetracycline and ampicillin, is indicative of the prolonged and often unregulated use of these antibiotics for growth promotion and disease prevention in the Iraqi poultry industry, as documented in previous studies [5, 19]. This widespread usage creates a powerful selective pressure that favors the emergence and persistence of the multidrug-resistant strains of *S.* Kentucky identified in this research. Within the hatcheries, the high chick densities and potential biosecurity lapses further facilitate the horizontal dissemination of these resilient clones [20].

In conclusion, the hatchery environment in Iraq does not represent a random, unorganized collection of *Salmonella*. Instead, it is dominated by two highly specialized, high-risk strains: *S.* Enteritidis, acting as the classic egg-borne threat, and multidrug-resistant *S.* Kentucky, representing a modern antimicrobial-resistant threat. The co-existence of these two strains severely jeopardizes food safety and public health. Consequently, *S.* Enteritidis will require compartmentalized monitoring and control within breeder flocks. Conversely, *S.* Kentucky will necessitate enhanced environmental sanitation, robust biosecurity measures to rupture the contamination cycle, and significant restrictions on the use of critical antimicrobials, such as fluoroquinolones.

This is the first study to present commercial broiler hatcheries in Iraq as reservoirs of multidrug-resistant *Salmonella*, predominantly *S.* Enteritidis and *S.* Kentucky. The contamination of workers' hands with these pathogens poses a distinct occupational and zoonotic risk. However, this contamination also presents a strategic opportunity to intervene in the poultry production chain. Addressing these challenges effectively will require an integrated and proactive One Health approach.

To manage the major risks outlined in this study, the adoption of a comprehensive risk mitigation framework is essential. This process must start with the implementation of measures to strengthen biosecurity, maintained through regular auditing of breeding farms and hatcheries to avoid the initial introduction and subsequent spread of *Salmonella*. In the same vein, the introduction of antimicrobial stewardship at the national level must be prioritized, particularly regarding the control of non-therapeutic use of critically important antibiotics, such as fluoroquinolones, to reduce the selective pressure associated with the escalation of multidrug-resistant strains.

Additionally, the risk associated with direct zoonotic transmission calls for the introduction of occupational health and safety programs to ensure and monitor the compliance of hatchery personnel with the use of personal protective equipment and the adoption of effective hand hygiene. Lastly, the implementation of a national system for active surveillance, which entails *Salmonella* serotype and antimicrobial resistance pattern profiling within hatcheries, will guide these actions and assess their long-term impact. The rationale for this framework is the precise management of rapidly changing public health and veterinary challenges, which is a direct function of an active surveillance system. Ultimately, to safeguard the Iraqi poultry sector, and by extension the human population, from the risks of salmonellosis and antimicrobial resistance, it is vital to respond to these challenges directly at the hatchery level.

Conclusions

This study demonstrates that commercial broiler hatcheries in Iraq serve as critical reservoirs for *Salmonella*, with an overall isolation prevalence of 8.4 % (38/450). The contamination burden was heavily driven by two dominant high-risk serotypes, namely *Salmonella* Enteritidis (34.2 %) and multidrug-resistant *Salmonella* Kentucky (26.3 %). Alarmingly, the highest colonization rate was recorded in chick cecal contents at 12.0 %, while environmental and occupational transmission routes were confirmed via contaminated eggshells (7.3 %) and worker hand swabs (6.0 %). The recovered isolates exhibited extensive phenotypic resistance to older first-line antimicrobials, particularly tetracycline (73.7 %) and ampicillin (65.8 %). Furthermore, *Salmonella* Kentucky displayed a concerning 40.0 % resistance rate to ciprofloxacin, which is critically important for human medicine. With an overall multidrug resistance rate of

39.5 % that escalates to 60.0 % specifically in *Salmonella* Kentucky clones, these findings highlight an urgent need for national One Health surveillance and strict antimicrobial stewardship at the hatchery level.

DECLARATIONS

Ethical Statement

The author declares that the conducted studies fully comply with generally accepted norms of humane treatment of animals, the principles of bioethics, and the ethical principles of the Declaration of Helsinki for human-related research.

This study was officially approved by the Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq (Approval No: Vet Medicine (222); November 2025, b, A and A).

To minimize animal stress and discomfort, all handling, maintenance, and experimental sampling procedures involving day-old chicks were performed in strict accordance with standard veterinary guidelines, current international principles of animal welfare, and recommendations outlined by the American Veterinary Medical Association (AVMA).

For the human infrastructure segment involving hatchery personnel, formal institutional ethical clearance for hand swab collection was obtained prior to the commencement of the study. Written or verbal informed consent was explicitly obtained from the participating hatchery workers before any swab collection was performed, and all participants were fully informed about the specific purpose of the study and the strict confidentiality of their data.

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Conflict of Interest

The author states that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declares that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the researcher, who takes sole responsibility for the work.

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