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Research Article

Detection of Salmonella and Escherichia coli in Chickens, Eggshells, and the Hands of Egg Handlers in Poultry Farms and Marketplaces in Khartoum, Sudan

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Abstract

Background: *Salmonella* and *Escherichia coli* are major foodborne pathogens that pose serious public health risks through the consumption of contaminated eggs and poultry. This study aimed to assess the prevalence of *E. coli* and *Salmonella spp*. in poultry and humans across four locations in Khartoum State, Sudan, focusing on different production systems and contamination sources.

Methods: A total of 100 swabs were aseptically collected from caged and cage-free farms, local markets, and the hands of egg handlers. The sample sources included chicken cloaca (n = 20), freshly laid eggs (n = 20), hands of poultry farm employees (n = 20), eggs in storage (n = 20), and commercial eggs from markets (n = 20). Samples were tested for *Salmonella spp*. and *E. coli* using Gram staining, culture-based isolation, and biochemical identification tests.

Results: Among the 100 samples examined, 59% tested positive for *E. coli*, while 42% were positive for Salmonella spp.. A co-infection of both bacteria was detected in 1% of samples. *E. coli* prevalence was highest in cloacal swabs (70%), followed by employee hands (65%), eggs after laying and eggs in the market (55%), and eggs in storage (50%). Salmonella spp. was most frequently detected in stored eggs (50%), followed by eggs after laying and eggs from the market (45%). The lowest prevalence was found in employee hands (40%) and cloacal swabs (30%). Comparison of battery and floor production systems revealed no significant difference, with *E. coli* prevalence at 60% in battery farms and 58% in floor farms, while *Salmonella* spp. was detected at 42% in both systems.

Conclusion: The findings highlight widespread bacterial contamination in eggs, poultry environments, and handlers, with high contamination rates from birds' cloaca and farm workers' hands. This necessitates a need for enhanced biosecurity measures, strict hygiene practices, and microbial monitoring throughout the poultry production and supply chain. Implementing a One Health approach is essential to mitigate the risk of foodborne illnesses and safeguard public health.

Keywords: *Salmonella*; *Escherichia coli*, Eggs; Foodborne Diseases; Poultry Farms; Biosecurity; Sudan

Introduction

Food safety is a critical global concern, with the World Health Organization (WHO) linking over 200 diseases to the consumption of contaminated food each year [1]. These contaminants-including bacteria, viruses, parasites, and chemical substances-can enter the food chain at virtually any point, from production and processing to retail and final handling. The public health impact is immense, especially in developing countries where monitoring systems and regulatory frameworks may be inadequate [2].

Among the most notable contributors to foodborne diseases are zoonotic pathogens originating from poultry, which is widely consumed and economically significant across the globe. Poultry serves as a major reservoir for foodborne bacteria such as *Salmonella spp.* and *Escherichia coli*, both of which are frequently associated with outbreaks of gastroenteritis and other systemic infections in humans [2,3]. These pathogens may contaminate poultry products during slaughter, processing, or even during packaging and sale.

Eggs, while highly valued for their nutritional profile and affordability, also present a critical food safety challenge. Contamination can occur internally (transovarian transmission) or externally through contact with fecal matter, contaminated nesting environments, or poor handling practices [4,5]. Contamination of eggs and egg products by microorganisms significantly impacts egg quality, leading to spoilage and the transmission of pathogens [6]. Bacteria such as *Escherichia coli* (*E. coli*) and *Salmonella* spp. are among the primary organisms that colonize eggs easily, contributing to foodborne illnesses that cause premature deaths, severe health complications like typhoid fever and gastroenteritis, and significant productivity losses [7]. Most *Salmonella* infections in humans stem from the ingestion of contaminated poultry, particularly from consuming raw eggs or foods containing raw eggs [8]. Globally, *Salmonella* contamination of eggs and eggshells presents a serious public health challenge. As consumer preferences shift toward cage-free egg production and raw, unprocessed foods, the risk of salmonellosis increases [9]. The contamination of eggs during production is influenced by factors such as flock size, age, stress, feeding practices, vaccination, and cleaning routines [9].

Globally, the burden of foodborne illnesses is staggering. WHO estimates that approximately 600 million people-nearly 1 in 10 individuals worldwide-suffer from foodborne diseases each year, with *Salmonella*, *E. coli*, and *Campylobacter* among the leading organisms [10]. Given these vulnerabilities, strict hygiene protocols are essential throughout the production chain to mitigate microbial risks by establishing sanitation checkpoints and monitoring pathogen presence during processing and transportation [11,12].

In Africa, the issue is particularly pronounced. Studies have reported poultry contamination rates ranging between 5% and 30%, with the presence of *Salmonella enterica* serovars and pathogenic E. coli strains frequently detected in raw meat, eggs, and processing surfaces [13-15]. These pathogens pose a dual threat to both consumer health and food security. Moreover, increasing reports of antimicrobial-resistant (AMR) strains add another layer of complexity to the management and treatment of foodborne infections [16].

In Sudan, published data on poultry-borne pathogens are relatively sparse. However, existing research has demonstrated noteworthy contamination levels. For example, *Salmonella* was detected in 5% of eggs and 4.4% of handlers, while *E. coli* was present in 24.4% of eggs sampled from various supply chains in Khartoum [17,18]. Yet, the epidemiological scope of contamination across poultry farms, wet markets, and handlers' hands-an important transmission vector-remains largely underexplored.

This study seeks to address this critical knowledge gap by systematically investigating the presence of *Salmonella* and *E. coli* in chicken meat, eggshells, and the hands of egg handlers within poultry farms and marketplaces across Khartoum, Sudan.

Materials and Methods

Study area and sample collection

A cross-sectional study was conducted from June to August 2021 in four locations of Khartoum State, Sudan-namely Omdurman, Tibna, Halfaya, and Kuku-covering key areas for poultry farming and distribution.

A total of 100 swab samples were aseptically collected from various sources including: Chicken cloaca, freshly laid eggs, Hands of poultry farm workers, Eggs in storage, and Commercial eggs from marketplaces. Twenty samples from each location. Samples were taken using sterile cotton swabs from chickens, eggshell surfaces, and handlers' hands. All samples were transported in ice containers and processed within six hours at the Bacteriology Laboratory, Department of Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology.

Bacteriological analysis

Bacterial isolation was performed using the swab method as described by [17], with modifications in media selection. After initial dilution in normal saline, samples underwent serial dilutions and were plated on nutrient agar, followed by incubation at 35 ± 2°C for 24-48 hours. Presumptive colonies were distinguished based on morphology and Gram staining, then sub cultured on Eosin Methylene Blue (EMB) agar and Salmonella-Shigella (S.S) agar (Oxoid). Media were prepared and sterilized per manufacturer instructions.

Bacterial identification to genus level was achieved using standard biochemical tests, including: catalase, oxidase, coagulase, methyl red, Voges-Proskauer, urease, indole, citrate, motility, lysine decarboxylase, lysine deaminase, hydrogen sulfide production, glucose and lactose fermentation. Identification was carried out in accordance with the protocols outlined by [19].

Data management and statistical analysis

All data were analysed using SPSS version 22. The Chi-square test was applied to assess statistically significant differences in contamination rates among the various sample types. A p-value < 0.05 was considered statistically significant.

Results

Bacteriological identification

Two bacterial species were successfully isolated and identified across all collected samples: *Salmonella spp.* and *Escherichia coli* (*E. coli*). On Salmonella-Shigella (S.S) agar, *Salmonella* colonies appeared with black centers due to hydrogen sulfide (H_2S) production. In contrast, *E. coli* formed characteristic green metallic sheen colonies on Eosin Methylene Blue (EMB) agar, indicative of strong lactose fermentation and acid production. Biochemical test and sugar fermentation were used to identify the bacteria.

Production systems and farm distribution

A total of 100 samples were collected from farms employing either battery or floor systems. Halfaya and Omdurman farms exclusively utilized battery systems, while Kuku and Tibna used floor-based systems. Each production system was equally represented (n = 50 per system).

Prevalence of *E. coli* across locations

Out of 100 samples, *E. coli* was detected in 59% (59/100), while 41% were negative. Halfaya Poultry Farm exhibited the highest *E. coli* prevalence (76%), followed by Tibna (60%), Kuku (56%), and Omdurman (44%). Despite this variation, statistical analysis indicated no significant association between location and *E. coli* infection (p = 0.134). (Table 1)

Location	Negative (%)	Positive (%)	p-value
Halfaya	6 (24.0%)	19 (76.0%)	0.134
Kuku	11 (44.0%)	14 (56.0%)	
Omdurman	14 (56.0%)	11 (44.0%)	
Tibna	10 (40.0%)	15 (60.0%)	
Total	41 (41.0%)	59 (59.0%)	

Table 1: Association between location and *E. coli* prevalence.

Prevalence of *salmonella spp.* across locations

Salmonella was isolated from 42% (42/100) of samples. The highest prevalence was observed in Omdurman (56%), followed by Kuku (44%), Tibna (40%), and Halfaya (28%). Again, no statistically significant association was found between location and *Salmonella* presence (p = 0.25). (Table 2)

Location	Negative (%)	Positive (%)	p-value
Halfaya	18 (72.0%)	7 (28.0%)	0.250
Kuku	14 (56.0%)	11 (44.0%)	
Omdurman	11 (44.0%)	14 (56.0%)	
Tibna	15 (60.0%)	10 (40.0%)	
Total	58 (58.0%)	42 (42.0%)	

Table 2: Association between location and *Salmonella spp.* Prevalence.

Prevalence of *E. coli* across sample sites

Different sample sources showed varied *E. coli* contamination rates. Cloacal swabs recorded the highest prevalence (70%), followed by hands of employees (65%). Eggs in markets and immediately after laying showed 55% positivity, while eggs in storage showed the lowest (50%). There was no statistically significant difference among sample sites (p = 0.693). (Table 3)

Sample Source	Negative (%)	Positive (%)	p-value
Cloaca	6 (30.0%)	14 (70.0%)	0.693
Eggs after laying	9 (45.0%)	11 (55.0%)	
Eggs in storage	10 (50.0%)	10 (50.0%)	
Eggs in market	9 (45.0%)	11 (55.0%)	
Hands of employee	7 (35.0%)	13 (65.0%)	
Total	41 (41.0%)	59 (59.0%)	

Table 3: Prevalence of *E. coli* across sample sites.

Prevalence of *salmonella spp.* across sample sites

Overall, *Salmonella spp.* was isolated in 42% of samples. Eggs in storage had the highest prevalence (50%), followed by eggs after laying and eggs in markets (45% each). The lowest prevalence was observed in cloacal samples (30%). The association between source and prevalence was not statistically significant (p = 0.753). (Table 4)

Sample Source	Negative (%)	Positive (%)	p-value
Cloaca	14 (70.0%)	6 (30.0%)	0.753
Eggs after laying	11 (55.0%)	9 (45.0%)	
Eggs in storage	10 (50.0%)	10 (50.0%)	
Eggs in market	11 (55.0%)	9 (45.0%)	
Hands of employee	12 (60.0%)	8 (40.0%)	
Total	58 (58.0%)	42 (42.0%)	

Table 4: Prevalence of *Salmonella spp.* across sample sites.

Relationship between *E. coli* and production system

There was no significant difference in *E. coli* prevalence between battery (60%) and floor (58%) systems (p = 0.5). The relative risk (RR = 2.043) indicated a slightly higher likelihood of *E. coli* presence in the battery system, although not statistically meaningful. (Table 5)

Production System	Negative (%)	Positive (%)	p-value	RR
Battery	20 (40.0%)	30 (60.0%)	0.500	2.043
Floor	21 (42.0%)	29 (58.0%)		
Total	41 (41.0%)	59 (59.0%)		

Table 5: Association between production system and *E. coli* prevalence.

Relationship between *salmonella spp.* and production system

Both battery and floor systems had identical *Salmonella* prevalence rates of 42%. There was no significant association between production system and *Salmonella* contamination (p = 0.58). The relative risk (RR = 2.213) indicated an equal risk between systems. (Table 6)

Production System	Negative (%)	Positive (%)	p-value	RR
Battery	29 (58.0%)	21 (42.0%)	0.580	2.213
Floor	29 (58.0%)	21 (42.0%)		
Total	58 (58.0%)	42 (42.0%)		

Table 6: Association between production system and *Salmonella spp.* Prevalence.

Discussion

This study underscores a major public health concern, the widespread of *Escherichia coli* (59%) and *Salmonella* spp. (42%) in poultry production environments. These pathogens, were detected across multiple points in the poultry supply chain-including birds, eggs, handlers, and storage facilities-suggesting persistent contamination risks from production to consumer level.

Bacterial prevalence varied across farms location: *E. coli* was highest at Halfaya (76%) and lowest in Omdurman (44%), while *Salmonella* spp. was most frequent in Omdurman (56%) and least in Halfaya (28%). However, statistical analysis found no significant association between contamination and farm location ($p > 0.05$), suggesting that despite observable differences, high contamination risk exists across all farms. This contrasts with findings in Nigeria [20], where farm location strongly influenced bacterial prevalence due to differential biosecurity enforcement.

The similarity in contamination between battery (60% *E. coli*, 42% *Salmonella*) and floor systems (58% and 42%, respectively) challenges assumptions about the hygienic superiority of battery cages. These results indicate that management practices, not housing systems alone, are the main determinants of contamination levels-particularly worker hygiene. This is supported by high contamination rates on handlers' hands and is consistent with [21] and [22]. However, studies like [23] report higher contamination in deep-litter systems due to increased fecal contact, indicating that housing, when combined with poor hygiene, can exacerbate risks, supporting findings from [24], who also reported no significant impact of housing systems on Salmonella prevalence.

High contamination levels were recorded across several sampling points: E. coli: Highest in cloacal swabs (70%), followed by employee hands (65%) and market/laid/stored eggs (~50-55%). In this study, the highest prevalence of *E. coli* (70%) was observed in samples collected from the cloaca, which aligns with higher prevalence rates reported by [25] in Malaysia (83%), and [26] in Indonesia (90%). However, our findings are higher than those reported by [21] in Nigeria (1.94%), [27] Ethiopia (13.4%), [28] in Malaysia (17.8%), [29] in Nigeria (23.3%), and [30] in Bangladesh (60%), indicating regional variations in prevalence due to differences in hygiene practices, environmental conditions, and biosecurity measures.

For *E. coli* isolated from eggs after laying, the present study reveals a prevalence of 55%. This is slightly lower than the rates reported by [14] in Nigeria (61.5%) and [31] in Assiut, Egypt (62.0%), but notably higher than the prevalence observed by [17] in Khartoum, Sudan (21.9%). Regarding *E. coli* prevalence in market samples, our study reported 55%, which is higher than the rates observed by [32] in North India (11.8%), [33] in Shahrekord, Iran (19.0%), and [34] in Lusaka, Zambia (34.26%). The lowest prevalence in our study was found in stored eggs with a prevalence of 50%. This is higher compared to findings by [23] in Giza, Egypt (36.0%), and [14] in Enugu, Nigeria (32.7%).

Salmonella was most prevalent in stored eggs (50%), laid eggs (45%), and market eggs (45%). These results point to multiple transmission pathways-including fecal shedding, poor handling practices, and inadequate storage hygiene. Alarmingly, contamination on workers' hands was substantial (65% for *E. coli*, 40% for *Salmonella*), implicating human vectors in cross-contamination. Studies in Egypt [35] reported significantly lower contamination on handlers' hands (20%), demonstrating the protective effect of better hygiene training and sanitation infrastructure.

In this study, the overall prevalence of *Salmonella* spp. was 42%, which is higher than reported by [36] in India (21.7%), [37] in Karbala, Iraq (21.1%), [38] in Marodi Jeh Region, Somalia (8.9%), and [17] in Khartoum, Sudan (5.0%). These variations may be due to differences in regional biosecurity measures, sampling techniques, or environmental conditions that influence the persistence and spread of Salmonella. The highest prevalence of Salmonella (50%) was observed in samples collected from the store, surpassing findings by [17] in Sudan (5.0%), [23] in Egypt (20.0%), and [39] (6.7%). Salmonella prevalence from eggs after laying was 45%, higher than the prevalence reported by [40] in London, UK (3.2%), [8] in North India (3.84%), and [41] in Ethiopia (4.5%).

The prevalence of Salmonella in market samples in this study was 45%, significantly higher than [34] in Lusaka, Zambia (2.31%), and [40] in the UK (3.2%). Market-specific factors, such as differing sanitation standards, storage conditions, and levels of regulation, likely contribute to these differences. Notably, [42] in Bangladesh reported a similar prevalence (45.8%), suggesting that high Salmonella prevalence can be consistent in markets with comparable hygiene challenges. The prevalence of Salmonella on the hands of employees was 40%, aligning with [43] in Ethiopia (33.3%) but higher than the 14.8% reported by [41] in Ethiopia and 2% by [35] in Egypt. Variations in personal hygiene practices, training, and access to sanitation facilities among employees could influence these rates, with studies like [44] in Ethiopia finding no Salmonella on handlers' hands, further highlighting the impact of hygiene practices. Salmonella isolated from cloaca samples showed a prevalence of 30%, which is lower than the 71.1% reported by [45] in Vietnam and 46.3% by [46] in Malaysia. This difference could stem from environmental factors, bird species, or the sampling method. The current prevalence was higher than rates reported by [37] in Iraq (21.7%), suggesting that localized environmental conditions and farm practices significantly influence pathogen prevalence.

The *E. coli* prevalence in this study (59%) was comparable to Egypt [47] (57.1%), higher than in Ethiopia [48] (31.8%), [29] in Gusau, Nigeria (22.1%), and [35] in Dakahlia, Egypt (37.4%), which could reflect differences in local poultry management or the effectiveness of disease control measures. *Salmonella* prevalence (42%) exceeded findings from India [36] (21.7%), Iraq [37] (21.1%), and Sudan [17] (5%). Such variation reflects differences in farm management, hygiene protocols, and national biosecurity policies. Countries with stricter standards generally report lower prevalence rates.

Contamination on farm workers hands was particularly concerning, it highlights poor adherence to hand hygiene protocols, reinforcing the need for targeted hygiene training and stricter enforcement of sanitation practices on farms.

The presence of *E. coli* and *Salmonella* in eggs-especially post-laying and in market samples-poses a direct threat to consumers. Undercooked or mishandled eggs could lead to serious gastrointestinal illness, particularly in vulnerable populations. Additionally, frequent antibiotic use to manage infections may promote AMR, compounding public health risks. The detection of pathogens on handlers' hands and in multiple environmental points underscores the interconnectedness of human, animal, and environmental health. A One Health approach-integrating veterinary, public health, and environmental interventions-is essential. This includes: Strengthening biosecurity on farms. Conducting hygiene education for poultry workers, promoting intersectoral collaboration in disease surveillance and response

Study Limitations

Despite its significant findings, the study had several limitations

- Sample size may not reflect broader trends
- Seasonal variations were not assessed
- Culture-based methods lack molecular diagnostic precision
- AMR patterns were not analysed

Conclusion

In conclusion, the study revealed a significant prevalence of *Salmonella* spp. and *E. coli* in various samples from cloaca, eggs, employee hands, and market environments, highlighting the persistent risk of contamination across the poultry production and supply chain. The findings underscore the need for improved monitoring and management strategies to mitigate the public health risks associated with these pathogens in poultry. From a One Health perspective, addressing this issue requires an integrated approach that considers the interconnectedness of human, animal, and environmental health. Enhancing biosecurity measures across farms and markets, including better waste management and disinfection practices, is essential to reduce contamination risks. Additionally, training farmers and employees on hygiene and handling, coupled with improved surveillance systems, will help monitor and control pathogen prevalence, ultimately safeguarding public health and improving poultry production sustainability.

Recommendations

Future research should incorporate molecular tools (e.g., PCR), seasonal data, and antimicrobial resistance profiling for deeper epidemiological insight.

Declaration of Competing Interest

The authors have no competing financial interests to declare.

Data Availability Statement

The data supporting the conclusions of this study can be obtained from the corresponding authors upon a reasonable request.

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