



# Microbial Screening of Housing and Equipment Before the Arrival of Day-Old Chickens

Chi Mai Duong\*, Nour Al-Din K. N. Houbari, Nguyen Tieu Quynh Du, Huu Ngoc Le, and Thi Kim Hoa Ho

Department of Veterinary Biosciences, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Thu Duc, Ho Chi Minh City, 70000, Vietnam

\*Corresponding author's E-mail: [mai.duongchi@hcmuaf.edu.vn](mailto:mai.duongchi@hcmuaf.edu.vn)

Received: December 23, 2025, Revised: January 21, 2026, Accepted: February 24, 2026, Published: March 25, 2026



## ABSTRACT

Cleaning and disinfection are essential parts of farm management biosecurity. The present study aimed to evaluate the presence of opportunistic bacteria on environmental surfaces and equipment in broiler houses on Day 0, just prior to the arrival of day-old chickens. Samples were collected from nine broiler houses, located on different farms in two provinces in Southern Vietnam. Five swab samples were randomly collected from each house, focusing on ceiling and wall surfaces, fan blades, feeders, and drinkers' heaters. Each environmental sample was analyzed for coliform and *Staphylococcus aureus* (*S. aureus*) counts and for the presence of *Clostridium perfringens* (*C. perfringens*). Before placing chickens in the houses, samples were collected from 10-day-old chickens on each farm. Feather swabs were collected from the body surface for *Escherichia coli* (*E. coli*), *Salmonella* species, and *S. aureus*, while yolk samples were screened for *E. coli* and *Salmonella* species. High bacterial loads were detected in all environmental samples. Specifically, coliform and *S. aureus* counts reached  $6 \log_{10}$  CFU/cm<sup>2</sup> from ceilings and walls. Additionally, equipment surfaces demonstrated substantial bacterial contamination, with counts of 6-11  $\log_{10}$  CFU per feeder or drinker, 9-11  $\log_{10}$  CFU per fan blade, and 6-10  $\log_{10}$  CFU per heater. *Clostridium perfringens* was found on environmental surfaces and equipment in most broiler chicken houses, except for houses 1, 3, and 5, and in a feather sample from a day-old chick. *Escherichia coli* was identified in all chicken samples. *Salmonella* spp. were found in the yolk samples at six out of nine farms (66.7%), whereas *S. aureus* was isolated from 17 of 18 feather samples (99.4%). The presence of these enteric bacteria and *S. aureus* on the environment and equipment surfaces indicated that microorganisms from the previous flock persisted despite thorough cleaning and disinfection. This residual contamination indicated that bacteria persisted mainly during the broiler cycle due to insufficient environmental sanitation and the presence of already infected chickens. The current results demonstrated that existing disinfection methods are insufficient to protect newly stocked flocks. These findings underscore the need for improved hygiene standards to reduce the prevalence of zoonotic pathogens in poultry production.

**Keywords:** Biosecurity, Broiler house, Cleaning, Disinfection, *Escherichia coli*, *Salmonella* spp.

## INTRODUCTION

Effective cleaning and disinfection are essential procedures in farm biosecurity. Facilities and equipment are thoroughly sanitized between rotations to eliminate the risk of pathogen transmission to new flocks. The purpose of cleaning and disinfection is not solely to protect young chickens from infection risks but also to mitigate antibiotic use in disease control (Dhaka et al., 2023; Pinto Jimenez et al., 2023). Chickens are susceptible to infections from hens through vertical and horizontal transmission pathways (Oikarainen et al., 2019; Shaji et al., 2023).

Infections and contamination can occur at hatcheries and during transportation (Khan et al., 2022). Furthermore, the internal farm environment serves as a significant reservoir of bacterial pathogens that can infect chickens immediately after placement (Zhai et al., 2020). The effectiveness of routine cleaning and disinfection in removing residual bacteria from broiler houses before chick placement and its impact on the microbial status of day-old chickens (DOCs) remain poorly characterized. Therefore, ongoing dependence on early antibiotic use, especially in developing countries, not only harms the

young chickens' health but also worsens the global problem of antimicrobial resistance (Liang et al., 2023; Ibeagha-Awemu et al., 2025). Thus, a risk-based approach was used to select representative bacterial indicators and pathogens associated with poultry to evaluate residual contamination and potential early-detection exposure. The current study aimed to evaluate bacterial contamination in farm environments and equipment just before chicken placement, as well as to screen newly arrived chickens for prevalent bacterial pathogens, thereby clarifying potential early transmission routes within the broiler production cycle.

## MATERIALS AND METHODS

### Ethical approval

All animal procedures were approved by the Animal Ethics Committee at Nong Lam University, Ho Chi Minh City, Vietnam (Approval number: NLU 250429).

### Study locations

Samples were collected from nine broiler houses across nine different farms located in two Southern provinces of Vietnam from 2020 to 2022. Each house had a capacity of 50,000 to 150,000 broiler chickens per rotation and was equipped with evaporative cooling systems. All farms were managed in accordance with comparable commercial biosecurity protocols, including controlled access, routine sanitation procedures, and standardized flock management practices. The houses remained empty for 2 to 3 weeks following depopulation, during which thorough cleaning and disinfection were carried out. A double disinfection process was applied; either formaldehyde or glutaraldehyde was used, followed by a thorough rinse and then dried for a few days. Rice husk bedding was placed, and clean equipment was set up. The houses were then either disinfected with a mixture of formaldehyde and  $\text{KMnO}_4$  or sprayed with glutaraldehyde. The facilities remained sealed until the arrival of the new batch. According to farm records, no major disease outbreaks were reported before the preceding production cycle, and antibiotics were used only for therapeutic purposes rather than routine prophylaxis. Cleaning and disinfection were conducted following the farm's routine protocols and carried out by the farm's staff.

### Environmental and equipment sampling

Sampling was conducted on-site on the day of chicken arrival. Samples from the environment and equipment were collected approximately 2-6 hours before the houses

were refilled with the new broiler chicken stocks. From each house, five pooled swab samples were collected from ceilings and walls, feeders, drinkers, fan plates, and heaters. Each surface was swabbed using sterile medical gauze pre-moistened with a portion of 100 mL of sterile saline (Schott bottle). After sampling, each gauze was placed back into the original bottles, which included 100 mL. This method kept the total bacterial recovery volume consistently at 100 mL. Sampling was standardized across all farms to ensure consistency. Specifically, three 10 cm × 15 cm center areas of the ceiling and the two walls that did not mount fans or cooling pads were swabbed and pooled as a single sample. The surfaces of two random feeders and two drinkers were entirely swabbed inside and outside. Ventilation fans were sampled by swabbing the entire blade surface. Similarly, the entire surface of the heaters was swabbed. The five bottles containing swab samples were placed in an ice box maintained at 4-8°C and transported to the laboratory immediately after sampling. Samples were analyzed within 12 hours upon arrival at the laboratory.

### Sampling newly arrived chickens

Upon arrival at each farm, and prior to placement in the houses, 10 chickens were randomly selected from different transport crates. The selected chickens were divided into two groups of five chickens, placed in separate clean containers, and transported to the laboratory. A total of 90 chickens (10 per farm) were euthanized by cervical dislocation. For every group of five chickens, a pooled feather sample was collected by swabbing the entire body surface of all five chickens using sterile gauze pre-moistened with 10 mL of saline. Following body surface sampling, the abdominal cavity of each chicken was opened to aseptically remove the entire yolk. Five yolks from each group were pooled into a sterile zipper bag. As a result, two pooled replicates for feathers and two for yolk samples were collected from each farm.

### Sample processing and bacterial culture

For the house and equipment surface samples, coliforms (common enteric Gram-negative indicators) and *S. aureus* (common Gram-positive pathogens in young chickens) were determined. Each bottle containing swab samples was vortexed. Serial ten-fold dilutions of each sample were prepared in sterile saline using 1.5-mL sterile Eppendorf tubes. Microbial counts were performed using the drop technique as described by Wang et al. (2002). Four consecutive dilutions ( $10^{-1}$ - $10^{-4}$ ) were prepared for

each sample. Each dilution was inoculated in triplicate with 10- $\mu$ L drops onto selective agar plates. After incubation, identified colonies were counted from the highest countable dilution. The average count from the three replicates was used to calculate the bacterial loads, with results expressed as  $\log_{10}$  CFU (Colony-Forming Unit; Maturin and Peeler, 2001). MacConkey agar (CM007, Oxoid Ltd., UK) and mannitol salt agar (CM0085, Oxoid Ltd., UK) were used for the detection of coliforms and *S. aureus*, respectively. The plates were incubated aerobically for 24 hours, and colony morphology was assessed according to the standard microbiological criteria described by Maturin and Peeler (2001). On MacConkey agar, coliform colonies were typically pink to red. *Staphylococcus aureus* was presumptively identified on Mannitol salt agar by the formation of yellow colonies with a yellow halo. Bacterial counts for the ceiling and wall samples were calculated and reported as  $\log_{10}$  CFU/cm<sup>2</sup>. Bacterial counts of fans, feeders, and drinkers were expressed as  $\log_{10}$  CFUs per item. Additionally, the presence of *C. perfringens* in the surface samples was examined by plate-plating 10 microliters of each sample onto tryptose sulfite cycloserine (TSC) agar (M8371, Himedia Laboratories, India) containing TSC supplement (FD014, Himedia Laboratories, India). The plates were anaerobically incubated at 37°C for 48 hours.

Detection of *Salmonella* colonies was carried out according to Vietnamese National Standard 10780-1 (2017), with minor modifications. Briefly, yolk samples (a pool of five) were homogenized, and 25 g was transferred into 225 mL of sterile buffered peptone water (RM001, HiMedia Laboratories, India) for pre-enrichment. Similarly, feather samples were diluted 1:9 (v/v) in buffered peptone water, homogenized, and incubated at 37°C for 18 hours. One milliliter of each pre-enriched culture was transferred into 10 mL of tetrathionate broth (Oxoid, CM0029) and 0.1 mL into 10 mL of Rappaport-Vassiliadis enrichment broth (CM0669, Oxoid Ltd., UK). The inoculated tetrathionate broth and Rappaport-Vassiliadis enrichment broth cultures were incubated for 24 hours at 37°C and 41.5°C, respectively. Each enrichment was then streaked onto a xylose-lysine-desoxycholate agar plate (CM0469, Oxoid Ltd., UK) and incubated at 37°C for 24 hours. Two red colonies with black centers from each plate were inoculated into triple sugar iron (TSI) agar slants (103915, Merck KGaA, Darmstadt, Germany) at 37°C for 24 hours. Typical *Salmonella* spp. positive TSI reactions were characterized by a red slant, a yellow butt, and blackening due to H<sub>2</sub>S

gas production. For *E. coli* detection, yolk and feather samples underwent pre-enrichment in peptone water. Following pre-enrichment, the samples were streaked onto eosin methylene blue agar (101347, Merck KGaA, Darmstadt, Germany) and incubated aerobically at 37°C for 24 hours (Lal and Cheeptham, 2007). Typical dark purple colonies with a green metallic sheen were subsequently tested with the IMViC biochemical test series for *E. coli* (Powers and Latt, 1977; MacWilliams, 2009; McDevitt, 2009).

### Statistical analysis

Data were analyzed descriptively, and no statistical comparisons between farms or surface types were conducted due to the observational nature of the study and heterogeneous sampling units.

## RESULTS

### Detection of bacteria from environmental and equipment

The results of bacterial recovery from the house environment and the equipment of each farm were summarized in Table 1. High numbers of coliforms and *S. aureus* were detected across all samples and farms. In the ceiling and wall samples, coliform counts ranged from 4.4 to 6.8  $\log_{10}$  CFUs/cm<sup>2</sup>, while *S. aureus* levels ranged from 4.6 to 6.6  $\log_{10}$  CFUs/cm<sup>2</sup>. Median coliform and *S. aureus* count on feeders reached 8.2 and 8.4  $\log_{10}$  CFU/item, respectively. Samples from the drinkers yielded similar results, with coliforms ranging from 6.6 to 9.8  $\log_{10}$  CFU/item and staphylococcal counts ranging from 6.7 to 11.1  $\log_{10}$  CFU/item. High bacterial loads were recovered from ventilation and heating equipment samples. On fan blades, coliform and *S. aureus* counts ranged from 5.8 to 10.9  $\log_{10}$  CFU/item, with a median of 8.1-8.2. Samples from heaters indicated counts between 6.6 and 11.1  $\log_{10}$  CFU/item for *S. aureus* and 6.8 to 9.3  $\log_{10}$  CFU/item for coliforms, with a median value of 8.1 for both bacterial groups. *Clostridium perfringens* was not detected in samples from farms 1, 3, and 5. However, *C. perfringens* was detected in all samples from farm 7 and was recovered from multiple equipment surfaces in five additional farms (2, 4, 6, 8, and 9), while farms 1, 3, and 5 were negative, among a total of nine farms examined.

### Bacterial detection from new flocks

*Salmonella* spp. were found in at least one sample type across six farms. The analysis of 18 feather pools indicated that *E. coli* was present in all samples (18/18),

while *S. aureus* was detected in 94.4% (17/18) of samples (Table 2). Staphylococcal loads on feathers ranged from 4.0 to 6.0 log<sub>10</sub> CFU/sample. A single pooled feather sample tested positive for *C. perfringens*. Notably, eight feather samples from six farms contained three different bacterial groups at the same time. Regarding internal bacterial colonization, *E. coli* was isolated from 100%

(18/18) of the pooled yolk samples. Furthermore, *E. coli* and *Salmonella* spp. were detected in 61.1% (11/18) of yolk pools originating from six different farms. These findings demonstrated that broiler chickens were significant carriers of opportunistic pathogens upon arrival, with contamination localized both externally on feathers and internally within the yolk samples.

**Table 1.** Bacterial load recovered from facility and equipment surfaces across nine broiler chicken farms in two Southern provinces in Vietnam from 2020 to 2022

Samples/ Bacteria	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Min	Max	Median
<b>Ceiling and wall samples (log<sub>10</sub> CFUs/cm<sup>2</sup>)</b>												
Coliforms	4.4	6.5	4.5	6.8	4.9	6.1	6.3	5.6	6.5	4.4	6.8	6.2
<i>S. aureus</i>	5.5	6.5	4.6	6.2	6.6	6.4	6.6	5.4	6.4	4.6	6.6	6.4
<i>C. perfringens</i>	-	-	-	-	-	Yes	Yes	Yes	Yes			
<b>Feeders (log<sub>10</sub> CFU/item)</b>												
Coliforms	7.8	9.0	9.2	7.0	7.3	9.5	6.7	9	7.0	6.7	9.5	8.2
<i>S. aureus</i>	6.8	8.1	9.4	10.5	8.0	8.5	8.0	9.1	8.2	6.8	10.5	8.4
<i>C. perfringens</i>	-	-	-	Yes	-	Yes	Yes	Yes	Yes			
<b>Drinkers (log<sub>10</sub> CFU/item)</b>												
Coliforms	6.6	8.2	7.2	7.8	9.1	8.3	9.8	8.3	9.1	6.6	9.8	8.3
<i>S. aureus</i>	8.0	7.7	6.8	6.7	7.2	7.6	8.4	11.1	7.9	6.7	11.1	7.7
<i>C. perfringens</i>	-	-	-	Yes	-	-	Yes	Yes	-			
<b>Fans (log<sub>10</sub> CFU/item)</b>												
Coliforms	5.8	8.4	8.3	7.2	9.4	6.8	10.5	6.7	8.0	5.8	10.5	8.2
<i>S. aureus</i>	6.8	8.2	9.0	7.2	10.9	6.3	10.5	7.5	8.0	6.3	10.9	8.1
<i>C. perfringens</i>	-	Yes	-	Yes	-	-	Yes	-	Yes			
<b>Heaters (log<sub>10</sub> CFU/item)</b>												
Coliforms	7.7	8.7	8.1	6.8	6.9	8.2	8.0	9.3	8.0	6.8	9.3	8.1
<i>S. aureus</i>	7.7	8.3	8.0	6.6	8.9	7.8	7.1	11.1	8.2	6.6	11.1	8.1
<i>C. perfringens</i>	-	Yes	-	Yes	-	Yes	Yes	Yes	-			

*C. perfringens*: *Clostridium perfringens*, *S. aureus*: *Staphylococcus aureus*, Min: Minimum, Max: Maximum

**Table 2.** Bacterial detection from yolk and feather wash from day-old chickens across nine broiler chicken farms in two Southern provinces in Vietnam from 2020 to 2022

Samples/Bacteria	Number of positive samples (N: 18)	Number of positive farms* (N: 9)
<b>Feather wash</b>		
<i>Salmonella</i> spp.	7 (38.9%)	5 (55.6%)
<i>E. coli</i>	18 (100.0%)	9 (100.0%)
<i>C. perfringens</i>	1 (5.6%)	1 (11.1%)
<i>S. aureus</i>	17 (94.4%)	9 (100%)
<i>E. coli</i> and <i>S. aureus</i>	10 (55.6%)	3 (33.3%)
<b>Three groups of bacteria</b>		
<i>Salmonella</i> , <i>E. coli</i> , <i>S. aureus</i>	8(44.4%)	6(67.7%)
<i>E. coli</i> , <i>C. perfringens</i> , <i>S. aureus</i>	7 (38.9%)	5 (55.6%)
<i>E. coli</i> , <i>C. perfringens</i> , <i>S. aureus</i>	1 (5.6%)	1 (11.1%)
All four groups	0 (0.0%)	0 (0.0%)
<b>Yolk samples</b>		
<i>Salmonella</i> spp.	11 (61.1%)	6 (66.7%)
<i>E. coli</i>	18 (100%)	9 (100.0%)
<i>Salmonella</i> and <i>E. coli</i>	11 (61.1%)	6 (66.7%)

\* Each farm had two pooled samples of five chickens. *C. perfringens*: *Clostridium perfringens*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

## DISCUSSION

Coliforms and *E. coli* have been used as microbial indicators for assessing the potential presence of fecal pathogens (Li et al., 2021). The coliform group includes lactose-fermenting members of the *Enterobacteriaceae*. Although coliforms are not inherently pathogenic, elevated coliform counts indicate inadequate hygiene practices (Center for Food Safety, 2017). *Staphylococcus aureus* is prevalent in poultry facilities and hatcheries and is also a common commensal of the skin and upper respiratory tract mucosa. Additionally, *S. aureus* can be found in litter, dust, drinking water, air, and on farm equipment (Matos et al., 2024). *Staphylococcus aureus* can cause infection by entering through damaged skin or mucous membranes, or by infecting the vulnerable, unhealed navels of recently hatched chickens (Shaheen et al., 2024). Staphylococcosis in young chickens is typically characterized by localized infections, including arthritis, tenosynovitis, osteomyelitis, and omphalitis (Matos et al., 2024). In poultry, infections are often caused by opportunistic bacteria, including Gram-negative species such as *Pseudomonas* spp., *Proteus* spp., and different coliforms, as well as Gram-positive species such as *Staphylococcus* and *Enterococcus* (Crespo, 2024).

The high levels of coliforms and *S. aureus* found in all surface samples during the present study, particularly on feeders and drinkers, suggested inadequate cleaning and disinfection. Additionally, the present findings indicated the probable presence of other pathogens from the previous contaminated batch. An environment and equipment that were not properly cleaned might serve as a major source of pathogens from the previous batch, allowing bacteria and fungi to proliferate and increasing the risk of disease exposure to the new flock (Liu et al., 2025). Bacteria readily form biofilms on internal surfaces and equipment, complicating cleaning and disinfection efforts and leading to persistent contamination and a higher risk of pathogen transmission to farms (Donlan, 2002). *Campylobacter* spp., *Salmonella* spp., *E. coli* and coliforms, *Staphylococcus* spp., *Enterococcus* spp., and *Clostridium* spp. are among the major biofilm-producing pathogenic bacteria in poultry farms (Butucel et al., 2022). *Clostridium perfringens* is a spore-forming bacterium that can survive for long periods in different environmental conditions. Van Immerseel et al. (2004) noted that chickens can be infected by *C. perfringens* from a contaminated environment in the house, and it is recognized as the primary etiological agent of necrotic

enteritis, a disease that can lead to higher mortality and significant subclinical production losses in poultry.

Day-old chickens have been identified as the primary vectors for carrying pathogens into production facilities (Moreno et al., 2019). In the present study, the recovery rates of *E. coli* and *Salmonella* spp. from yolk samples were remarkably high. *Echerchia coli* was detected from the yolk samples of all nine farms, and *Salmonella* spp. was found from the samples of six farms. *Staphylococcus aureus* was present in nearly all feather samples (17/18). Infected chickens can carry microorganisms that rapidly transmit these pathogens to other members of the same flock, thereby contaminating the housing environment and equipment.

Although mortality and causes depend on multiple factors, colibacillosis, aspergillosis, and salmonellosis were identified as the main causes of early chickens' death (Yadav et al., 2024). Crespo (2024) reported that yolk infection, known as omphalitis, is a frequent cause of death in newly hatched chickens, with mixed infections often observed. *Escherichia perfringens* and *Enterococcus faecalis* account for approximately 50% of omphalitis-related mortality during the first week of life in chickens. *Salmonella enteritidis* and *E. coli* strains can be transmitted vertically from infected or carrier hens and are present in egg yolk, the vitelline membrane, and the albumen (Oikarainen et al., 2019; Shaji et al., 2023). Fecal contaminants on the egg surface can penetrate through the eggshell pores (Trudeau et al., 2020). An investigation in Nigeria reported the isolation of *E. coli* and *Salmonella* spp. from 54.4% and 10.3% of yolk samples, respectively (Okorie-Kanu et al., 2016). *Salmonella* spp. was isolated from the yolks of 5.2% of DOCs in a study conducted in Korea (Im et al., 2015) and from 5.3% of DOCs in Kosovo (Hulaj et al., 2016).

Hatcheries are known to contribute to the spread of *Salmonella* spp., other pathogens, and antibiotic-resistant bacteria across farms (Wales and Davies, 2020). Congenitally infected chickens can readily spread the organisms to other chickens in the same hatch, contaminate the hatchery environment, and transportation containers (Khan et al., 2022). Moosavy et al. (2015) reported cross-contamination between contaminated and non-contaminated eggs in hatcheries. Additionally, Khan et al. (2022) reported the detection of *Salmonella* spp. in sources such as eggshell fragments and chicken fluff from hatcheries. Furthermore, hatching facilities are considered a reservoir for transmission of antimicrobial-resistant bacteria (ARM) and resistance genes (Osman et

al., 2018). Marin et al. (2011) reported that delivery-box liners and feces were the leading sources of *Salmonella* spp. in Spanish broiler houses, with contamination rates of 32.0% and 31.2%, respectively. Marin et al. (2011) indicated that the house environment following cleaning and disinfection, the arrival of infected DOCs, and feed contamination within feeders were the main risk factors associated with *Salmonella* presence in flocks at the end of the production cycle.

The combination of transport-related stress, adaptation to new housing, and the possibility of congenital or post-hatch infection may explain the high level of anxiety surrounding disease transmission in poultry operations. Thus, preventive use of antibiotics in young chickens is a widespread practice in commercial poultry farming, especially in developing countries (Liang et al., 2023). However, early antibiotic administration has been shown to disrupt gut microbiota development and promote antimicrobial resistance (Liang et al., 2023; WHO, 2023). In a One Health approach, reducing antimicrobial reliance in poultry production depends on preventive strategies such as improved hygiene, biosecurity, vaccination, and the use of alternatives to antibiotics (WHO, 2023; Ibeagha-Awemu et al., 2025). It has been indicated that enhanced farm biosecurity was associated with reduced antimicrobial use. Furthermore, enhancing farm biosecurity measures, especially in lower- and middle-income countries, was emphasized. Effective cleaning and disinfection are critical measures to reduce the incidence of infections and reduce antibiotic use (Dhaka et al., 2023; Pinto Jimenez et al., 2023).

## CONCLUSION

The current results demonstrated that, despite the implementation of comprehensive cleaning and disinfection protocols before the arrival of the new flocks, substantial populations of coliforms and opportunistic pathogens, such as *Staphylococcus* spp. and *C. perfringens*, persisted in the housing environment and on equipment surfaces. Additionally, day-old chickens were identified as carriers of *Salmonella* spp. and other opportunistic pathogens upon their arrival at the farms. Mitigating early-life mortality and improving performance in poultry production requires a comprehensive biosecurity approach across breeder farms, hatcheries, and commercial facilities. Future studies should focus on long-term monitoring of pathogen persistence over multiple production cycles. Additionally, assessing improved sanitation practices and hatchery-level interventions,

combined with molecular epidemiological tools, would be crucial for gaining a clearer understanding of contamination sources and transmission pathways.

## DECLARATIONS

### Acknowledgments

The authors are grateful to Animaid Company, Vietnam, for their kind support in facilitating introductions and sample collection at the farms.

### Authors' contributions

Thi Kim Hoa Ho and Chi Mai Duong were responsible for the study design, data analysis, and manuscript preparation. Nour Al-Din, K.N. Houbari, and Nguyen Tieu Quynh Du conducted the field sampling and performed the laboratory and data analyses. Huu Ngoc Le was the laboratory supervisor. All authors have read and agreed to the last edition of the manuscript before publication.

### Ethical considerations

All authors affirmed the ethical integrity of this study and its presentation. The authors have thoroughly checked the manuscript to ensure it is original, free from data fabrication, and not under consideration by any other publication. Every author has reviewed and consented to this submission. The authors confirm that the data and article content were not prepared and written with the help of AI tools.

### Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declared that there is no conflict of interest related to the current study.

### Funding

The present study was supported by an institutional-level Science and Technology research project (Code: CS-CB21-CNTY-03) funded by Nong Lam University, Vietnam.

## REFERENCES

- Butucei E, Balta I, McCleery D, Morariu F, Pet I, Popescu CA, Stef L, and Corcionivoschi N (2022). Farm biosecurity measures and interventions with an impact on bacterial biofilms. *Agriculture*, 12(8): 1251. DOI: <https://www.doi.org/10.3390/agriculture12081251>
- Center for food safety (2017). Coliforms – traditional hygienic indicator. Centre for Food Safety, The Government of the Hong Kong Special Administrative Region. Available at:

- [https://www.cfs.gov.hk/english/whatsnew/whatsnew\\_fst/whatsnew\\_fst\\_Coliforms\\_Traditional\\_Hygienic\\_Indicator.html](https://www.cfs.gov.hk/english/whatsnew/whatsnew_fst/whatsnew_fst_Coliforms_Traditional_Hygienic_Indicator.html)
- Crespo R (2024). Omphalitis in poultry. MSD veterinary manual. Merck & Co Inc., Rahway (NJ). Available at: <https://www.msdsmanual.com/poultry/omphalitis/omphalitis-in-poultry>
- Dhaka P, Chantziaras I, Vijay D, Bedi JS, Makovska I, Biebaut E, and Dewulf J (2023). Can improved farm biosecurity reduce the need for antimicrobials in food animals? A scoping review. *Antibiotics*, 12(5): 893. DOI: <https://www.doi.org/10.3390/antibiotics12050893>
- Donlan RM (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, 8(9): 881-890. DOI: <https://www.doi.org/10.3201/eid0809.020063>
- Hulaj B, Çabeli P, Goga I, Taylor N, Hess C, and Hess ML (2016). Survey of the prevalence of *Salmonella* species on laying hen farms in Kosovo. *Poultry Science*, 95(9): 2030-2037. DOI: <https://www.doi.org/10.3382/ps/pew149>
- Ibeagha-Awemu EM, Omonijo FA, Piché LC, and Vincent AT (2025). Alternatives to antibiotics for sustainable livestock production in the context of the One Health approach: Tackling a common foe. *Frontiers Veterinary Science*, 12: 1605215. DOI: <https://www.doi.org/10.3389/fvets.2025.1605215>
- Im MC, Jeong SJ, Kwon YK, Jeong OM, Kang MS, and Lee YJ (2015). Prevalence and characteristics of *Salmonella* spp. isolated from commercial layer farms in Korea. *Poultry Science*, 94(7): 1691-1698. DOI: <https://www.doi.org/10.3382/ps/pev137>
- Khan AS, Georges K, Rahaman S, Abebe W, and Adesiyun AA (2022). Occurrence, risk factors, serotypes, and antimicrobial resistance of *Salmonella* strains isolated from imported fertile hatching eggs, hatcheries, and broiler farms in Trinidad and Tobago. *Journal of Food Protection*, 85(2): 266-277. DOI: <https://www.doi.org/10.4315/JFP-21-236>
- Lal A and Cheeptham N (2007). Eosin-methylene blue agar plates protocol. American Society for Microbiology, pp. 1-7. Available at: <https://asm.org/asm/media/protocol-images/eosin-methylene-blue-agar-plates-protocol.pdf>
- Li E, Saleem F, Edge TA, and Schellhorn HE (2021). Biological indicators for fecal pollution detection and source tracking: A review. *Processes*, 9(11): 2058. DOI: <https://www.doi.org/10.3390/pr9112058>
- Liang X, Zhang Z, Wang H, Lu X, Li W, Lu H, Roy A, Shen X, Irwin DM, and Shen Y (2023). Early-life prophylactic antibiotic treatment disturbs the stability of the gut microbiota and increases susceptibility to H9N2 AIV in chicks. *Microbiome*, 11(1): 163. DOI: <https://www.doi.org/10.1186/s40168-023-01609-8>
- Liu H, Pan S, Wang C, Yang W, Wei X, He Y, Xu T, Shi K, and Si H (2025). Review of respiratory syndromes in poultry: pathogens, prevention, and control measures. *Veterinary Research*, 56(1): 101. DOI: <https://www.doi.org/10.1186/s13567-025-01506-y>
- MacWilliams MP (2009). Indole test protocol. American Society for Microbiology, pp. 1-9. Available at: <https://asm.org/getattachment/200d3f34-c75e-4072-a7e6-df912c792f62/indole-test-protocol-3202.pdf>
- Marin C, Balasch S, Vega S, and Lainez M (2011). Sources of *Salmonella* contamination during broiler production in Eastern Spain. *Preventive Veterinary Medicine*, 98(1): 39-45. DOI: <https://www.doi.org/10.1016/j.prevetmed.2010.09.006>
- Maturin L and Peeler JT (2001). Aerobic plate count. In: *Bacteriological Analytical Manual (BAM)*, 8<sup>th</sup> Edition, Revision A. U. S. Food and Drug Administration. Available at: <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>
- Matos M, Mitsch P, Liebhart D, Hess M, and Hess C (2024). Co-infection of chickens with *Staphylococcus lentus* and *Staphylococcus aureus* from an outbreak of arthritis, synovitis, and osteomyelitis argues for detailed characterisation of isolates. *Animals*, 14(17): 2574. DOI: <https://www.doi.org/10.3390/ani14172574>
- McDevitt S (2009). Methyl red and Voges-Proskauer test protocols. American Society for Microbiology, pp. 1-9. Available at: <https://asm.org/getmedia/40946f85-9357-4563-aa8a-994427efa825/methyl-red-and-voges-proskauer-test-protocols.pdf>
- Moosavy MH, Esmaeili S, Bagheri Amiri F, Mostafavi E, and Zahraei Salehi T (2015). Detection of *Salmonella* spp. in commercial eggs in Iran. *Iran Journal of Microbiology*, 7(1): 50-54. Available at: <https://pubmed.ncbi.nlm.nih.gov/26644874/>
- Moreno MA, García-Soto S, Hernández M, Bárcena C, Rodríguez-Lázaro D, Ugarte-Ruiz M and Domínguez L (2019). Day-old chicks are a source of antimicrobial resistant bacteria for laying hen farms. *Veterinary Microbiology*, 230: 221-227. DOI: <https://www.doi.org/10.1016/j.vetmic.2019.02.007>
- Oikarainen PE, Pohjola LK, Pietola ES, and Heikinheimo A (2019). Direct vertical transmission of ESBL/pAmpC-producing *Escherichia coli* limited in poultry production pyramid. *Veterinary Microbiology*, 231: 100-106. DOI: <https://www.doi.org/10.1016/j.vetmic.2019.03.001>
- Okorie-Kanu OJ, Ezenduka EV, Okorie-Kanu CO, Ugwu LC, and Nnamani UJ (2016). Occurrence and antimicrobial resistance of pathogenic *Escherichia coli* and *Salmonella* spp. in retail raw table eggs sold for human consumption in Enugu state, Nigeria. *Veterinary World*, 9(11): 1312-1319. DOI: <https://www.doi.org/10.14202/vetworld.2016.1312-1319>
- Osman KM, Kappell AD, Elhadidy M, ElMougy F, El-Ghany WAA, Orabi A, Mubarak AS, Dawoud TM, Hemeg HA, Moussa IMI et al. (2018). Poultry hatcheries as potential reservoirs for antimicrobial-resistant *Escherichia coli*: A risk to public health and food safety. *Scientific Reports*, 8(1): 5859. DOI: <https://www.doi.org/10.1038/s41598-018-23962-7>
- Pinto Jimenez EC, Keestra T, Tandon P, Cumming O, Pickering AJ, Moodley A, and Chandler CIR (2023). Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: A One Health systematic review. *The Lancet. Planetary Health*, 7(5): e418-e434. DOI: [https://www.doi.org/10.1016/S2542-5196\(23\)00049-9](https://www.doi.org/10.1016/S2542-5196(23)00049-9)
- Powers EM and Latt TG (1977). Simplified 48-hour IMViC test: An agar plate method. *Applied and Environmental Microbiology*, 34(3): 274-279. DOI: <https://www.doi.org/10.1128/aem.34.3.274-279>
- Shaheen R, Abasy M, Sharkawy H, and Ismail M (2024). Prevalence, molecular characterization and antimicrobial resistance among *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* strains isolated from Egyptian broiler chicken flocks with omphalitis. *Open Veterinary Journal*, 14(1): 284. DOI: <https://www.doi.org/10.5455/ovj.2024.v14.i1.25>
- Shaji S, Selvaraj RK, and Shanmugasundaram R (2023). *Salmonella* infection in poultry: A review on the pathogen and control strategies. *Microorganisms*, 11(11): 2814. DOI: <https://www.doi.org/10.3390/microorganisms11112814>
- Trudeau S, Thibodeau A, Côté JC, Gaucher ML, and Fravallo P (2020). Contribution of the broiler breeders' fecal microbiota to the establishment of the eggshell microbiota. *Frontiers in Microbiology*, 11: 666. DOI: <https://www.doi.org/10.3389/fmicb.2020.00666>
- Van Immerseel F, De Buck J, Pasmans F, Huyghebaert G, Haesebrouck F, and Ducatelle R (2004). *Clostridium perfringens* in poultry: An emerging threat for animal and public health. *Avian Pathology*, 33(6): 537-49. DOI: <https://www.doi.org/10.1080/03079450400013162>
- Vietnamese National Standards 10780-1 (2017). TCVN 10780-1:2017 (ISO 6579-1:2017). Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella*

– Part 1: Detection of *Salmonella* spp. Hanoi, Vietnam. Available at: <https://caselaw.vn/van-ban-phap-luat/343653-tieu-chuan-quoc-gia-tcvn-10780-1-2017-iso-6579-1-2017-ve-vi-sinh-vat-trong-chuoi-thuc-pham-phuong-phap-phat-hien-dinh-luong-va-xac-dinh-typ-huyet-thanh-cua-salmonella>

Wales A and Davies R (2020). Review of hatchery transmission of bacteria with focus on *Salmonella*, chick pathogens and antimicrobial resistance. *World's Poultry Science Journal*, 76(3): 517-536. DOI: <https://www.doi.org/10.1080/00439339.2020.1789533>

Wang X, Brown IL, Khaled D, Mahoney MC, Evans AJ, and Conway PL (2002). Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. *Journal of Applied Microbiology*, 93(3): 390-397. DOI: <https://www.doi.org/10.1046/j.1365-2672.2002.01704.x>

World health organization (WHO) (2023). Antimicrobial resistance. World Health Organization. Available at: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>

Yadav PK, Shah SA, Shafi M, Kamil SA, Mir MS, Rather MA, Ganaie AA, and Wani ZA (2024). Etiological and histo-morphological studies on early chick mortality in broiler chicken in Kashmir, India. *OpenVeterinary Journal*, 14(11): 3037-3046. DOI: <https://www.doi.org/10.5455/OVJ.2024.v14.i11.32>

Zhai R, Fu B, Shi X, Sun C, Liu Z, Wang S, Shen Z, Walsh TR, Cai C, Wang Y et al. (2020). Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. *Environment International*, 139: 105715. DOI: <https://www.doi.org/10.1016/j.envint.2020.105715>

**Publisher's note:** [Scienceline Publication](#) Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access:** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026