

Colibacillosis caused by multi-drug resistant *Escherichia coli* in nineteen-weeks-old layer chicken flock at Nsukka, Enugu State, Nigeria: A case report

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Abstract

Colibacillosis is an acute, infectious and mostly systemic disease that has been reported to cause significant economic losses in poultry flocks worldwide. Rearing laying chickens, which is increasingly recognized for its economic potential in the poultry sector is greatly constrained by the occurrence of diseases, including colibacillosis. The present case report is on colibacillosis caused by multi-drug resistant *Escherichia coli* in nineteen-weeks-old layer chickens (n = 1,200) that were intensively managed on a battery cage system in Nsukka, Enugu State, Nigeria. The birds showed reduced feed intake, torticollis, staggering gait, well-formed greenish feces with white portions, weakness, poor body condition, huddling and 2% mortality. Necropsy of some of the dead chickens and euthanized living ones showed that the carcasses were emaciated, with fibrin deposits in the thoracic cavity, enlarged heart, congested liver, a focal region of congestion on the spleen and petechial hemorrhage on the intestines. Microscopically, there was nephrosis, hepatitis, erosion of the intestinal villi and depletion of lymphocytes in the spleen. Pure cultures of *E. coli* were isolated from the spleen, liver and heart (one dead bird) and liver (two live birds that were euthanized) and identified using both cultural and microscopic features as well as biochemical characteristics. The isolates exhibited multi-drug resistance to several antibiotics, including Tobramycin, Gentamicin, Ciprofloxacin, Trimetoprim-Sulphamethoxazole and Tetracycline but were susceptible to only Cefotaxime, which was used to successfully treat the flock. This case highlights the critical issue of antimicrobial resistance in animal husbandry. It reaffirms the need for better management practices in poultry production to mitigate transmission of pathogenic microorganisms, as well as the need to develop alternative strategies for controlling antibiotic-resistant bacterial organisms in avian species. This case further highlights the need for alternative treatments and strict antimicrobial stewardship to combat multidrug-resistant *E. coli* in poultry.

Keywords: Colibacillosis; *Escherichia coli*; Layer chickens; Antimicrobial resistance; Multidrug resistance.

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Introduction

The poultry industry is a leading sector in contemporary livestock production, and is a key component of the agricultural economy both in developed and developing countries. Its benefits extend beyond food production, as it also provides employment opportunities. Worldwide, poultry production is constrained by occurrence of diseases caused by bacteria, fungi, viruses, parasites and toxic agents (Mammo and Wude, 2022). Diseases caused by the bacteria *Escherichia coli* (*E. coli*) are of economic importance in the poultry industry in Nigeria, being reportedly responsible for 30 - 40% mortality (Raji, 2014).

Escherichia coli, a Gram-negative bacterium often referred to as avian pathogenic *E. coli* (APEC) in birds, is the primary etiological agent of colibacillosis, and is known to be responsible for respiratory disorders, septicemia and systemic disease in poultry (Dho-Moulin and Fairbrother, 1999; Panth, 2019). Key virulence factors, including adhesins, serum resistance and toxin production, enhance host colonization and immune evasion (Khairullah *et al.*, 2024; Abdulkhaleq *et al.*, 2025).

Colibacillosis can manifest in diverse ways, including acute fatal septicemia, salpingitis, peritonitis, and cellulitis. In laying hens, peritonitis and salpingitis are common, whereas the disease in young chicks may include omphalitis (yolk sac infection) or swollen head syndrome. Birds that survive septicemia can develop sub-acute fibrinopurulent air sacculitis, peri-hepatitis, and lymphocytic depletion of the bursa and thymus. (Nolan and Logue, 2025). Beyond *Escherichia coli*, other pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Mycoplasma gallisepticum*, *Pasteurella multocida* (fowl cholera), and viruses like Newcastle disease or infectious bronchitis have been reported to

produce similar clinical signs and lesion in affected chickens. (Hassan *et al.*, 2010; Miller, 2014)

Despite ongoing research, effective and broadly protective vaccines are still under development, leaving antimicrobial treatments as the primary control measures for colibacillosis. However, the widespread use of antibiotics has raised concerns about antimicrobial resistance, and there are heightened consumer demands for antibiotic-free poultry products, necessitating alternative control strategies (Zhang *et al.*, 2021; Soares *et al.*, 2021; Yousef *et al.*, 2023 and Zaki *et al.*, 2024).

The emergence of multidrug-resistant (MDR) bacteria represents a significant public health threat, as highlighted by the World Health Organization. Multidrug resistance is typically characterized by resistance to antibiotics from at least three distinct classes (Falagas *et al.*, 2006; Laxminarayan *et al.*, 2013; Wei *et al.*, 2020; Javed *et al.*, 2023). The increasing prevalence of infections caused by MDR pathogens, coupled with the limited availability of effective treatments, is expected to lead to higher mortality rates among both humans and animals affected by infectious diseases (Murray *et al.*, 2022)

Environmental conditions in poultry farming play a pivotal role in pathogen transmission and disease development. Poor hygiene in hatcheries, microbial contamination of hatching eggs and inadequate housing conditions contribute to the spread of pathogens, including *E. coli*. Furthermore, structural factors, such as the flooring and drainage design, significantly influence the microbial load, with direct implications for animal health and product safety (Zhang *et al.*, 2021; Benameur *et al.*, 2023; Niculae *et al.*, 2024).

Inadequate management practices, such as poor biosecurity, improper nutrition and unsanitary living conditions, are significant risk

factors for disease onset. Stress factors, like overcrowding and improper handling, can also weaken the immune system, increasing susceptibility to infections (Verbrugghe *et al.*, 2012). The lack of comprehensive diagnostic tools further complicates disease management, underscoring the importance of improved surveillance and disease prevention strategies. (Niculae *et al.*, 2024).

The present case report is on colibacillosis caused by multidrug-resistant *Escherichia coli* in nineteen-weeks-old layer chickens (n = 1,200) that were intensively managed on a battery cage system in Nsukka, Enugu State, Nigeria.

Case History and Observations

This case report involved a total of 1,200 layer chickens reared on intensive management battery cage system at a poultry farm located in Nsukka, Enugu State, Nigeria. The case was reported to University of Nigeria Veterinary Teaching Hospital (VTH) on August 26, 2025. The farmer brought one dead layer chicken and two moribund ones of nineteen-weeks of age for investigation. The clinical signs the farmer reported were drop in feed intake, torticollis, staggered gait, well-formed greenish feces with white portions, weakness, poor body condition, huddling and mortality of 2%. The farmer further reported having given

the following vaccines and medications to the birds before reporting to the VTH: 3-in-1 vaccine (NDV La Sota, Egg drop syndrome and Infectious bronchitis), Kenflo[®] (Enrofloxacin) and Centre Neoflor[®] (Neomycin sulphate, Florfenicol, Oxytetracycline hydrochloride).

Necropsy and Histopathology

At necropsy, the birds were observed to be in poor body condition. The gross lesions observed were emaciated pectoral muscle with prominent keel bone, enlarged heart, dark and congested liver, and spleen with focal region of congestion (Figure 1). Also, there were fibrin deposits within the thoracic cavity (Figure 1).

Sections of the kidney, liver, spleen and small intestine were collected for histopathology. They were routinely processed and stained with hematoxylin and eosin (H & E) (Slaoui and Fiette, 2011). Microscopic evaluation of the stained tissue sections showed that there was severe degeneration and necrosis of the renal tubules (Figure 2). Sections of the liver showed degeneration and necrosis of the hepatocytes with severe infiltration of inflammatory cells at the peri-portal zone and haemorrhages (Figure 2). In the spleen, there was severe necrosis of the lymphocytes, while the small intestine had severe desquamation of the villi (Figure 2).



Figure 1. Gross lesions associated in colibacillosis in the layer chickens: 1A: dark and congested liver (yellow arrow), enlarged heart (white arrow) and fibrin deposits in the pleura (blue arrow). 1B: focal area of congestion on the spleen (yellow arrow).

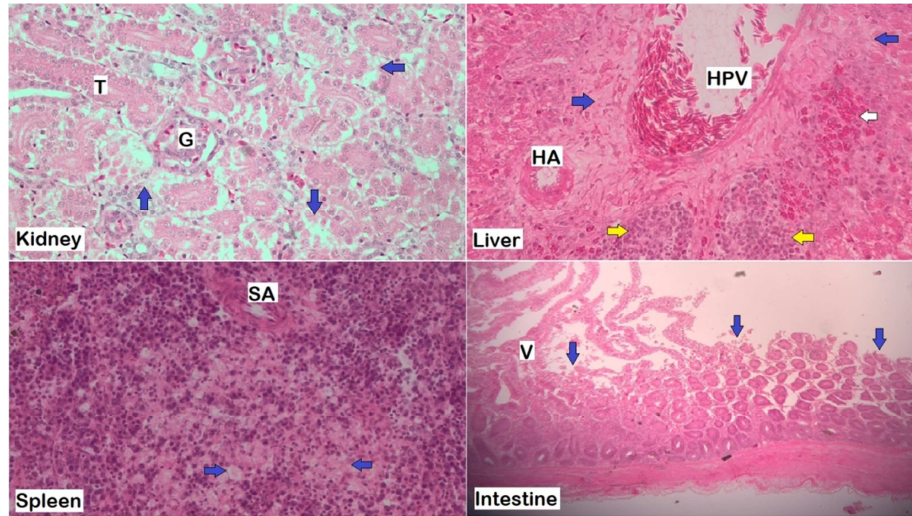


Figure 2. Photomicrograph of tissue sections from the layer chickens that had colibacillosis, showing the kidney, with severe degeneration and necrosis of the tubules (purple arrow); liver, with degeneration and necrosis of the hepatocytes (purple arrow), infiltration of inflammatory cells (yellow arrow), haemorrhage (white arrow); spleen, with necrosis of lymphocytes (purple arrow); and intestine, with desquamation of the villi. [H & E; ×400] Key: G – glomerulus, T – renal tubules, HPV – hepatic portal vein, HA – hepatic artery, SA – sheathed arteriole, V – villi.



Figure 3. Bacterial growth from liver, spleen and heart inoculum of layer chickens with colibacillosis, streaked on MacConkey agar, showing dry pinkish colonies.

Following the post mortem examination, the spleen, liver and heart were aseptically collected from the carcass of two live chickens (designated as L1 and L2) and one dead chicken (designated as D). The intestine of the dead chicken was also harvested (D Int). The tissue samples were collected for bacterial culture, isolation and anti-microbial susceptibility testing.

Microbial Evaluation

Inoculum was obtained from the harvested organs using sterile wire loop and were streaked on MacConkey agar and incubated for 24 hours at 37 °C. Bacteria growth were observed as dry discrete pinkish colonies from the liver, spleen and heart of the dead birds and liver of L1 bird, but no growth was observed in the rest of the cultures (Figure 3). The pinkish coloration of the colonies on MacConkey agar was indicative of lactose

fermentation, characteristic of *E. coli*, *Klebsiella* and *Enterobacter*.

The colonies from each analyzed organ were Gram stained, and Gram-negative rod-shaped organisms were observed for all the isolated colonies (Figure 4).

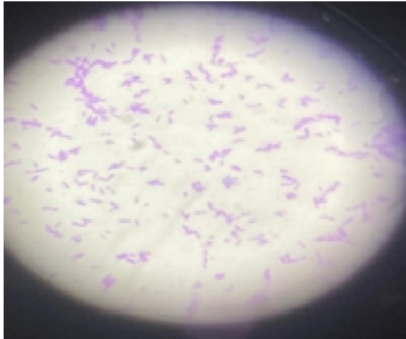


Figure 4. Microscopy showing Gram-negative bacilli (pinkish short rods) after Gram-staining of colonies obtained from liver, spleen and heart inoculum of layer chickens with colibacillosis.

Furthermore, the isolated colonies were inoculated in Triple sugar iron (TSI) agar. The result showed yellow slant/yellow butt (acidic/acidic), gas production positive and hydrogen sulphide (H₂S) negative for all the isolates (Figure 5); suggestive of either of *E. coli*, *Klebsiella* or *Enterobacter*.

In order to confirm the organisms, colonies were sub-cultured on eosin methylene blue (EMB) agar and incubated for 24 hours at 37 °C. All the colonies isolated from dead chicken organs showed greenish metallic sheen appearance on EMB agar and were considered to be *E. coli* (Figure 6).

The organism was further subjected to some biochemical tests – indole, urease and citrate tests, for confirmation of *E. coli*. All the isolated organisms tested positive for indole test and negative for urease and citrate utilization test (Figure 7). These results confirmed the organism as *E. coli*.

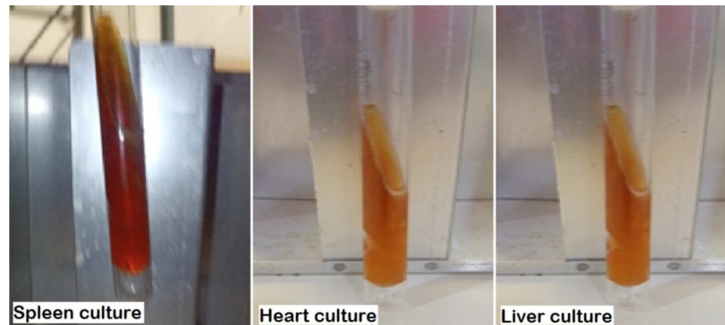


Figure 5. Colonies obtained from liver, spleen and heart inoculum of layer chickens with colibacillosis on triple sugar iron (TSI) agar showing yellow slant/yellow butt (acidic/acidic) and gas production.

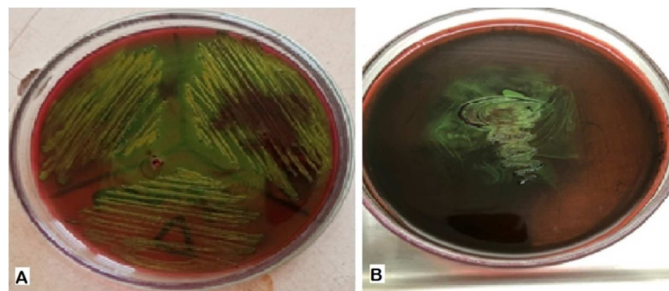


Figure 6. Growth of cultures obtained from liver, spleen and heart inoculum of layer chickens with colibacillosis on eosin methylene blue (EMB) agar showing greenish metallic sheen colonies for the spleen, liver and heart of the dead chicken (A) and liver of the living euthanized chicken (B).

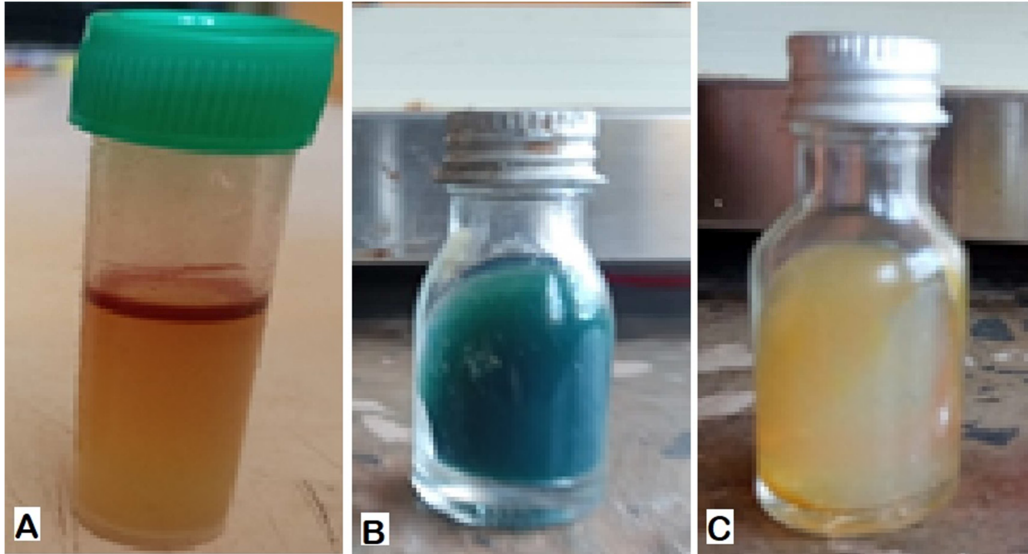


Figure 7. Confirmatory biochemical tests for *E. coli* cultures obtained from liver, spleen and heart inoculum of layer chickens with colibacillosis: Indole positive (A), Citrate (B) and Urease (C) negative.

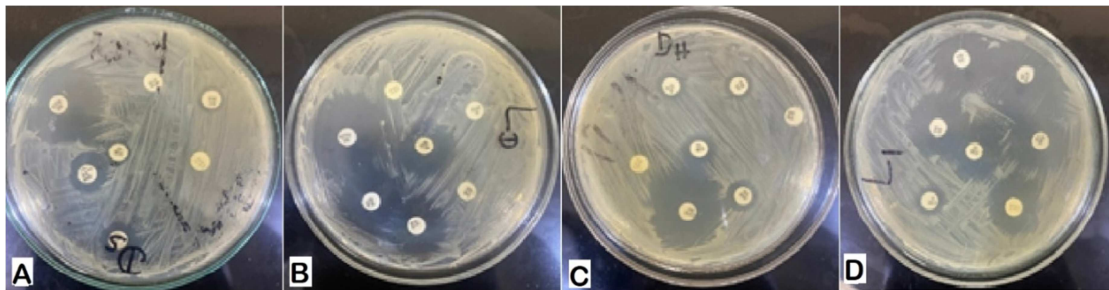


Figure 8. Antimicrobial resistance testing (Kirby-Bauer Disc Diffusion Method) of *E. coli* isolates obtained from organs of layer chickens with colibacillosis.

Antimicrobial Susceptibility Testing

Antibiogram of the isolate was determined by Kirby-Bauer disk diffusion method using the following antibiotics: Tobramycin (Oxoid®) 10 µg (TOB), Gentamicin 10 µg (GM), Cefotaxime 30 µg (CTX), Trimetoprim-Sulphamethoxazole 1.25/23.75 µg (TS), Ciprofloxacin 5 µg (CIP), Tetracycline 30 µg (TE), Tobramycin (Mastdisc®) 10 µg (TN). Results were analyzed based on Clinical and Laboratory Standard Institute guidelines (CLSI, 2021). The recovered *E. coli* isolates showed resistance to majority of the listed antibiotics (Figure 8; Table 2).

Management

Upon visit to the farm, the Veterinarian used hydrogen peroxide solution to wash the drinking lines to remove biofilm from the pipes and dislodge clogs from the nipples. Thereafter, the flock was treated with Cefotaxime at the dosage of 100 g/litre of water for seven days. Multivitamin (Vitamix®; Afrimash, Ibadan, Nigeria), a combination of vitamins, electrolytes and amino acids, were administered afterwards at the dosage of 100 gram to 300 litres of water for five days. The farmer reported great improvement upon commencement of the treatment and return of lay to about 90% after the treatment.

Table 1. Results of antimicrobial susceptibility testing of *E. coli* isolates obtained from organs of layer chickens with colibacillosis.

| Antibiotics | Source of <i>E. coli</i> isolates tested | | | |
|-------------------------------|--|-----------------------------|-----------------------------|-----------------------------|
| | Spleen of Dead layer chicken | Heart of Dead layer chicken | Liver of Dead layer chicken | Liver of Live layer chicken |
| Cefotaxime | S | S | S | S |
| Gentamicin | R | R | S | S |
| Tobramycin (Oxoid®) | R | R | S | S |
| Trimetoprim-Sulphamethoxazole | R | R | R | S |
| Ciprofloxacin | R | R | R | R |
| Tetracycline | R | R | R | S |
| Tobramycin (Mastdisc®) | R | R | S | S |

Key: S – Sensitive, R – Resistant.

Discussion and Conclusion

Colibacillosis is the most common infectious bacterial disease of poultry, and it is responsible for significant economic losses in the poultry industry worldwide due to high morbidity and mortality in chickens, and carcass rejection at slaughter houses (Ragione and Woodward, 2002; Vegad and Katiyar, 2008). The current increasing trends in prevalence and severity of colibacillosis indicates that it is likely to become a greater problem in the poultry industry (Altekruse *et al.*, 2002; Joshi *et al.*, 2012). *Escherichia coli* is responsible for bloodstream infections worldwide (Kern and Rieg, 2020). It resides in the gut and causes no harm, but can cause severe and life-threatening infections when there is weakened immune system.

Confirmatory diagnosis of colibacillosis is usually based on history, clinical signs, necropsy findings, colonial appearance (culture), microscopic appearance (Gram stain) and biochemical tests. The clinical signs reported by the farmer which includes

reduced feed intake, staggered gait, torticollis (due probably to possible infection of the central nervous system), well-formed greenish faeces with white portions, weakness, poor body condition, huddling of chicks and mortality have been reported earlier in layers and breeder flocks, in outbreaks occurring at the onset of egg production, but less frequently at a more advanced age. A rapid onset of severe depression, lameness and diarrhea have been reported (Zanella *et al.*, 2000; Panth, 2019).

Torticollis which is a common sign in avian viral disease such as Newcastle disease was observed in this case. Its occurrence could be linked to central nervous system (CNS) involvement, associated with specific scenario such as chronic (systemic or localized) infection affecting the bone (osteomyelitis) or the ear (otitis) and extending to the cranial bones or meninges as earlier reported in avian colibacillosis in young chicks (Panth, 2019; da Rosa *et al.*, 2020). It could also occur in combination with other diseases which may

have weakened the immune system (Linden, 2015), or as a result of non-infectious factors such as post head injuries, ammonia toxicity, nutrient deficiency (magnesium, vitamin E, Selenium, thiamine) and various food poisoning (botulism, heavy metal/lead toxicity, and aflatoxicosis) (Ali *et al.*, 2014).

Gross and histopathology lesions that were recorded at necropsy are similar to what have been severally reported about the disease (Vegad and Katiyar, 2008; Barnes *et al.*, 2013). Polyserositis (perihepatitis, pericarditis) and peritonitis are also reportedly usually present in cases of colibacillosis in birds (Nolan *et al.*, 2013). Fibrinous exudates are reportedly deposited on the serosa surfaces of multiple organs including the oviduct, ovary, intestines, alveoli, heart, lungs and liver following inflammatory response to the disease agent (Barde *et al.*, 2022).

Results of the biochemical tests used for confirmation of *E. coli* were in agreement with a previous report by Quinn *et al.* (2011). Organisms of the genus *Escherichia* are Gram negative rods. They have the ability to ferment different sugars like glucose and lactose; however, some strains do not ferment lactose. They are oxidase negative and indole positive (Barnes *et al.*, 2008; Quinn *et al.*, 2011).

Although some pathogens (*Pseudomonas aeruginosa*, *Salmonella* species, *Staphylococcus aureus*, *Campylobacter jejuni*, *Mycoplasma gallisepticum*, *Pasteurella multocida*) also produce systemic infections in poultry, the possibility of them being the causative agent in this case was ruled out by their microbiological, clinical and biochemical characteristics. Newcastle disease and infectious bronchitis are viral diseases that share same clinico-pathological characteristics with colibacillosis, but these diseases were vaccinated against in the farm.

Various antibiotics have been utilized in controlling and treating colibacillosis, including sulphamethiazole, co-trimoxazole, cephalixin,

chloramphenicol, amikacin, gentamicin, neomycin, enrofloxacin, ciprofloxacin and other fluoroquinolones (Saha *et al.*, 2020; Yehia *et al.*, 2023), however, their use have faced great challenge with increasing antimicrobial resistance (Magiorakos *et al.*, 2012). The *E. coli* isolated from chickens in this case exhibited some level of antimicrobial resistance to all antibiotics tested, except Cefotaxime. This phenomenon may be attributed to abuse of antibiotics use in avian medicine/poultry production. The multidrug resistant *E. coli* isolates reported in this case raises a public health concern because of the contact of the farm workers with these birds and their further interaction with their families and people at other places (market, church, etc.). Children and immunocompromised patients are at a very high risk of infection if they come in contact with secretions or droppings from these infected chickens (Benameur *et al.*, 2023) or while handling or consuming their eggs (Okorie-Kanu *et al.*, 2016; Atoyebi *et al.*, 2018).

In conclusion, this case showcased the substantial challenges presented by multidrug-resistant *Escherichia coli* within the poultry industry, a fast-developing sector that is increasingly grappling with issues related to animal health and food safety. The identification of resistant strains accentuates the pressing necessity for alternative therapeutic strategies to address infections induced by resistant pathogens. It is imperative to enforce more rigorous antimicrobial stewardship and to devise more efficacious management practices, encompassing enhanced farming conditions and superior biosecurity protocols, to alleviate the threats of pathogen transmission and antimicrobial resistance.

Moreover, the potential for zoonotic transmission and interspecies pathogenicity of *E. coli* necessitates continual investigation into the genetic adaptability of avian pathogenic strains and their extensive repercussions for

both animal and human health. In light of the escalating concerns regarding antimicrobial resistance, alternative therapeutic modalities, such as bacteriophages, probiotics and vaccination, ought to be investigated to provide sustainable solutions for the management of *E. coli* infections in avian species.

Recommendation: The farmer was advised to avoid abuse of antibiotics as it is the primary cause of antimicrobial resistance, which was responsible for the birds not recovering following the initial treatment he instituted. Also, he was advised to disinfect the pen with a good disinfectant such as glutaraldehyde. He should also maintain strict biosecurity measures, reduce overstocking and wash the drinking lines regularly.

Conflict of Interest

The authors declare that there is not conflict of interests regarding publication of this paper.

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