



Research Article

Study of Avian Colibacillosis and Antimicrobial Resistance in *Escherichia coli* from Chickens (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria

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ABSTRACT

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This study investigates avian colibacillosis in chickens (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria, focusing on the isolation, identification, and antibiogram of *Escherichia coli* (*E. coli*). Eosin Methylene Blue (EMB) agar and biochemical tests were used to identify *E. coli*, revealing a prevalence of 28.3% across 300 chicken samples from live bird markets, poultry farms, and veterinary hospitals. Market, farm, and hospital prevalence rates were 9.3%, 5.3%, and 13.7%, respectively. Risk factors such as sex, age, health status, and on-farm biosecurity were significantly associated with colibacillosis. Females (24.0%) and adults (20.3%) showed higher prevalence than males (4.3%) and young chickens (8.0%). Exotic breeds (12.0%) had higher prevalence than noiler (9.3%) and village chickens (7.0%). Clinically sick chickens (23.0%) were more affected than apparently healthy ones (5.3%). Poor on-farm biosecurity (24.0%) measures correlated with higher prevalence. Antimicrobial usage impacted prevalence, with lower rates in chickens from farms with minimal antibiotic use. Clinical and necropsy findings were consistent with *E. coli* infection. Identified serovars O1, O2, and O78 were consistent with Avian Pathogenic *E. coli* strains. Antimicrobial susceptibility testing showed *E. coli* sensitivity to Amoxicillin-Clavulanic acid, Gentamicin, and Ciprofloxacin, but resistance to Ampicillin and Neomycin. Multi-drug resistance emphasized the need for prudent antibiotic use. This study underscores the importance of tailored management practices and judicious antibiotic use in poultry farming to mitigate antibiotic-resistant strains, advocating robust biosecurity and hygiene measures to control colibacillosis in Maiduguri, Borno State, Nigeria.

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INTRODUCTION

Poultry farming involves raising domesticated birds like chickens, ducks, turkeys, and geese for meat, eggs, and feathers (Alders *et al.*, 2018). This practice has been crucial to agriculture and food production for centuries, especially in developing countries such as Nigeria (Attia *et al.*, 2022). It provides income for small-scale farmers and households, with a low initial investment leading to steady income from selling poultry products (Birhanu *et al.*, 2023; Wongnaa *et al.*, 2023). Poultry products, including eggs and chicken meat, are rich in proteins, vitamins, and minerals, improving nutritional security in regions facing malnutrition (de Vries-Ten *et al.*, 2020; Birhanu *et al.*, 2023).

Chickens, part of the *Gallus domesticus* species, are the most common poultry birds globally and crucial for providing protein and livelihoods to millions (Masaki, 2021; Alders *et al.*, 2018). However, chicken farming in developing countries faces challenges such as limited capital, poor infrastructure and menace of infectious diseases, affecting market access and product quality (Hafez and Attia, 2020; Grace *et al.*, 2024).

Infectious disease outbreaks, such as colibacillosis, can cause significant economic losses in poultry farms (de Mesquita *et al.*, 2022). The lack of resources and expertise for disease prevention and control worsens the impact of these outbreaks. Insufficient technical knowledge and training in modern poultry farming practices hinder productivity and limit the adoption of improved methods (Grace *et al.*, 2024).

Colibacillosis is one of the most common and economically significant bacterial infections in chickens, caused by the bacterium *Escherichia coli* (Apostolakos *et al.*, 2021). It primarily affects the digestive and respiratory systems of birds, leading to increased mortality, decreased egg production, and reduced growth rates

(Kathayat *et al.*, 2021). Pathogenic *E. coli* strains, possessing virulence factors, are responsible for this disease (Kathayat *et al.*, 2021; Hu *et al.*, 2022).

Risk factors for colibacillosis include poor biosecurity, overcrowding, inadequate ventilation, contaminated water and feed, and stressful conditions compromising the birds' immune systems (Swelum *et al.*, 2021; Abdel-Rahman *et al.*, 2023). Clinical signs vary but often include diarrhea, dehydration, decreased appetite, respiratory distress, swollen wattles, and a drop in egg production (Gedeno *et al.*, 2022). Colibacillosis affects multiple organs in chickens, including the liver, intestines, and respiratory system. Pathological changes include inflammation, necrosis, and congestion in affected tissues (Kromann *et al.*, 2021).

Diagnosis involves clinical examination, flock history, and laboratory analysis, including post-mortem examinations and bacterial culture (Fancher *et al.*, 2020; Kakooza *et al.*, 2021). Prevention and control require strict biosecurity measures, a clean environment, vaccination, and proper management practices. Antibiotics may be used judiciously due to resistance concerns (Nielsen *et al.*, 2022; Casalino *et al.*, 2023).

The following reasons were identified for the widespread use of antimicrobials in poultry production: (1) farmers aim to lessen disease impact on poultry health, (2) growth promotion to enhance feed efficiency and profitability, (3) limited and costly veterinary services, (4) inadequate or absent antibiotic regulations, and (5) substandard farm management and biosecurity practices (Joshua *et al.*, 2018).

Antimicrobial drugs are also readily available over the counter without prescription because access to veterinary drugs is presently not being regulated in the country, thereby encouraging the use of these drugs by poultry farmers indiscriminately in production (Adebowale *et al.*, 2022). Poultry farmers continue to use antibiotics in poultry feed or water for

prophylaxis, treatment of diseases and as growth promoters in Nigeria (Chah *et al.*, 2022). Commonly used antibiotics in poultry production in Nigeria include; oxytetracycline, neomycin, enrofloxacin, doxycycline, gentamicin, colistin, streptomycin, tylosin, ciprofloxacin, nitrofurans and chloramphenicol. This is similar to the practice in most countries (Adebowale *et al.*, 2022). In 2018, Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) issued a ban on the use of antibiotics as growth promoters in animal feeds.

Antimicrobial-resistant (AMR) *E. coli* are frequently detected in food-producing animals, particularly in the gastrointestinal tracts of chickens and the environments where these chickens are kept (Hegde *et al.*, 2016; Jochum *et al.*, 2021). The presence and persistence of resistance in commensal *E. coli* serve as a crucial biomarker for the selective pressure imposed by antibiotic use, indicating potential resistance in other pathogenic bacteria (Subramanya *et al.*, 2021). Numerous studies have assessed and reported the high prevalence of multidrug-resistant (MDR) *E. coli* in poultry across several countries, including Nigeria (Igwe *et al.*, 2016; Aworh *et al.*, 2019; Aworh *et al.*, 2021; Ibrahim *et al.*, 2021; Adebowale *et al.*, 2022)

Resistance to commonly used antibiotics has major socioeconomic and public health implications. The socioeconomic implications of AMR include increased cost and duration of treatment while the public health implications include decreased ability to treat common infections resulting in increased human suffering and ultimately death (Dadgostar, 2019)

In Nigeria, colibacillosis significantly threatens the poultry industry (Aworh *et al.*, 2020; Nwankwo *et al.*, 2021; Onuoha *et al.*, 2023). However, comprehensive data on its prevalence, distribution, and impact, especially in Maiduguri, is limited. This study aims to isolate

and identify multi-drug resistant (MDR) *E. coli* in chickens in Maiduguri, determine the prevalence and distribution of colibacillosis, identify risk factors, assess antibiogram profiles, and identify serotypes of *E. coli* isolates.

MATERIALS AND METHODS

Study Area

The study was conducted in Maiduguri, the capital and largest city of Borno State in northeastern Nigeria. Maiduguri is a major urban center with many poultry farms and a significant chicken population. The region has a tropical savanna climate with distinct dry and rainy seasons. The study period was from February, 2023 to November, 2023.

Selection of Poultry Farms

A representative sample of poultry farms from different locations within Maiduguri was selected. Farms with a history of avian colibacillosis and those in different ecological zones were included. Twelve poultry farms were randomly chosen to ensure diverse representation.

Sampling Procedure

Systematic random sampling was used to collect samples from various age groups, sexes, breeds, and management systems within each live birds' market, poultry farm and veterinary hospital. Cloacal swabs were collected from healthy chickens and those showing clinical signs, while tissue samples from fresh carcasses during necropsy. A total of 300 chickens were sampled during the study period, this include 100 samples from live bird's markets, 150 samples from poultry farms and 50 samples from veterinary hospitals within the study area.

Sample Collection

Cloacal swabs were collected from each chicken following aseptic protocols, including proper hand washing and use of personal protective equipment. Sterile cotton swabs were labeled with sample information and used to collect swabs aseptically from both healthy and clinically sick chickens. Samples were labeled,

placed in sample bags, and transported on ice packs to the Microbiology research laboratory at the University of Maiduguri.

Isolation and Identification of *Escherichia coli*

E. coli was isolated and identified using standard bacteriological methods (ISO, 2001). Using aseptic technique, swabs were streaked onto nutrient agar plates with an inoculation loop. Samples were cultured on MacConkey, EMB, and blood agar plates, and incubated at 37°C for 24-48 hours. *E. coli* colonies were identified by their pink color on MacConkey agar and green metallic sheen on EMB agar. After incubation, plates were examined for bacterial colonies, and colonies resembling *E. coli* were selected for further testing. Presumptive *E. coli* colonies were sub-cultured to obtain pure cultures. Identification involved Gram staining and biochemical tests, including catalase, oxidase, indole, methyl red, Voges-Proskauer, and sugar fermentation tests using triple sugar iron agar.

Serotyping of *E. coli* Isolates

E. coli isolates were tested using specific antisera targeting O (somatic) and H (flagellar) antigens. The slide agglutination test involved mixing colonies with saline and latex reagents, and observing for clumping within 60 seconds. Positive agglutination indicated the presence of specific *E. coli* serotypes. All procedures were carried out following standard serotyping protocol for *E. coli* as previously described by Momtaz *et al.* (2013) and Tegegne *et al.*, (2024).

Antibiotic Sensitivity Testing:

Eighty-five isolates were tested for susceptibility to various antibiotics used in Nigerian poultry. These antibiotics included Amoxicillin-Clavulanic acid (Augmentin), Ampicillin, Amoxicillin, Penicillin G, Gentamicin, Neomycin, Ciprofloxacin, Ofloxacin, Levofloxacin, Ceftriaxone, Nitrofurantoin, Trimethoprim/Sulfamethoxazole (Cotrimoxazole), Enrofloxacin, Tylosin, Erythromycin, Oxytetracycline, Doxycycline,

and Ceftazidime. The Kirby-Bauer disc diffusion method was employed to determine the zone of inhibition for each antibiotic. Results were categorized as sensitive, intermediate or resistant based on Clinical and Laboratory Standards Institute (2019) guidelines.

Questionnaire Survey

A structured questionnaire was administered to poultry farm owners and managers to collect data on poultry management, biosecurity, vaccination history, and experiences with avian colibacillosis.

Observational Records

Clinical signs of colibacillosis in sick birds were documented during farm visits and from birds presented to veterinary hospitals. Necropsies were performed on suspected cases, and pathological lesions were recorded. Data on mortality, growth performance, and egg production were also collected.

Data Analysis

Data were entered into Microsoft Excel, and statistical analyses were performed using Chi-Square or Fisher's exact test to calculate Risk Ratios (RR) and 95% Confidence Intervals (CI). Prevalence rates were calculated using GraphPad Prism® version 5.01, with p-values ≤ 0.05 considered significant

Prevalence and Distribution of Colibacillosis in Chickens in Maiduguri, Borno State, Nigeria

Table 1 summarizes the prevalence and distribution of colibacillosis in chicken flocks in Maiduguri, Borno State, Nigeria. The study collected 300 cloacal swab samples from both healthy and clinically ill chickens at various locations: live bird markets (Monday, Custom, Tashan Bama), poultry farms (GRA, Mairi area, UniMaid staff quarters), and veterinary hospitals (UniMaid VTH, SASUMVH). From 100 samples at live bird markets, 28 (28.0%) were *E. coli* positive, the isolation frequency of *E. coli* and market-wise prevalence revealed an overall value of 9.3% (95% CI: 6.5 – 13.2), with

varying values from Monday (5.3%; 95% CI: 3.3 – 8.5) followed Custom (3.0%; 1.6 – 5.6) and Tashan Bama (1.0%; 0.3 – 2.9) in a descending order of prevalence rates, while out of 150 samples from poultry farms, 16 (10.7%) tested positive, poultry farm – wise overall prevalence was 5.3% (95% CI: 3.3 – 8.5), with higher prevalence from Mairi area (2.3%; 95% CI: 1.1 – 4.7) followed by Unimaid staff quarters (2.0%; 95% CI: 0.9 – 4.3) and GRA (1.0%; 95% CI: 0.3 – 2.9). Moreover, from 50 samples at veterinary hospitals, 41 (82.0%) were positive for *E. coli*, veterinary hospital-wise overall prevalence was 13.7% (95% CI: 10.2 – 18.0), the prevalence of the bacterium was found to be higher in chickens sampled from Unimaid VTH (7.0%, 95% CI: 4.6 – 10.5) compared to those from SASUMVH (6.5%; 95% CI: 4.4 – 10.1). Bacteriological analysis showed that 85 samples (28.3%) were positive for *E. coli*, with an overall prevalence rate of 28.3% (95% CI: 23.5 – 33.7%).

Isolation and Identification of *Escherichia coli* (*E. coli*) from Chicken in Maiduguri, Borno State, Nigeria

E. coli grown on EMB agar appeared as smooth, circular, black colonies with a metallic sheen. Table 2 presents the biochemical test results confirming *E. coli* in chicken flocks from Maiduguri, Borno State, Nigeria. On EMB agar, *E. coli* showed distinctive greenish-black colonies with a metallic sheen, while on MacConkey agar, colonies were bright pink, transparent, smooth, and rose. Gram staining revealed pink, rod-shaped, short-chain, single, or paired Gram-negative bacilli.

E. coli fermented five sugars—dextrose, sucrose, lactose, maltose, and mannitol—producing both acid and gas. The isolates were positive for citrate, nitrate, xylose, and maltose tests but negative for oxidase, inositol, and urease tests. Additionally, *E. coli* fermented galactose, sorbitol, fructose, and glucose, with acid production indicated by a color change

from reddish to yellow and gas production by bubbles in Durham tubes.

All *E. coli* isolates tested positive for catalase, indole, and Methyl Red (M-R) tests, but negative for the Voges-Proskauer (V-P) test.

Risk Factors Associated with Colibacillosis in Chicken (*Gallus domesticus*) Flocks from Maiduguri, Borno State, Nigeria

The results of the risk factors associated with colibacillosis in chicken (*Gallus domesticus*) flocks from Maiduguri, Borno State, Nigeria were summarized on Table 3.

The frequency of *E. coli* isolate and prevalence of colibacillosis in chickens, categorized by sex, age, breed, health status, husbandry system, level of on-farm biosecurity measures and frequency of antimicrobial usage by farmers in poultry production were presented in Table 3. The frequency of isolate was lower in male 8.2% (13/158) compared to female 50.7% (72/142) chickens. The prevalence is higher in female (24.0%; 95% CI: 19.5 – 29.1) compared to the male (4.3%; 95% CI: 2.6 – 7.3) chickens. The difference in the prevalence rates between the sexes of chickens was statistically (p -value <0.0001; $\chi^2 = 46.56$; RR = 0.1770; OR = 0.1526) significant.

Also, the frequency of isolate was lower in young 17.6% (24/136) compared to adult 37.2% (61/164) chickens. The prevalence is higher in adult (20.3%; 95% CI: 16.2 – 25.3) than in young (8.0%; 95% CI: 5.4 – 11.6) chickens. And difference in the prevalence rates between the ages of chickens was also found to be statistically (p -value = 0.0002; $\chi^2 = 13.99$; RR = 0.4744; OR = 0.3618) significant.

Moreover, the frequency of *E. coli* isolate was found to be higher exotic 36.0% (36/100) followed by noiler 28.0% (28/100) and village 21.0% (21/100) chickens. The prevalence is lower in village (7.0%; 4.6 – 10.5) compared to noilers (9.3%; 95% CI: 6.5 – 13.2) and exotic (12.0%; 95% CI: 8.8 – 16.2) chickens, in an ascending order of the prevalence rates. The

difference in the prevalence rates between the breed of chickens was not statistically (p -value = 0.0624; $\chi^2 = 5.549$) significant.

The frequency of isolate was higher in sick 46.0% (69/150) compared to healthy 10.7% (16/150) chickens. The prevalence rate is higher in clinically sick (23.0%; 95% CI: 18.6 – 28.1) compared to apparently healthy (5.3%; 95% CI: 3.3 – 8.5) chickens. The difference in the prevalence rates between the health status of chickens was statistically (p -value < 0.0001; $\chi^2 = 46.11$; RR = 0.2319; OR = 0.1402) significant.

The frequency of *E. coli* isolate was found to be higher in intensively reared 28.4% (52/183) compared to extensively reared 28.2% (33/117) chickens. The prevalence rate was found to be higher in chickens reared under the intensive (17.3%; 95% CI: 13.5 – 22.0) compared to those reared under extensive (11.0%; 7.9 – 15.1) management system. Although, the difference in the prevalence rates of *E. coli* between the husbandry systems practice by farmers was not statistically (p -value = 0.9686; $\chi^2 = 0.0016$; RR = 1.007; OR = 1.010) significant.

The frequency of *E. coli* isolate was higher in chickens sampled from farms with grossly inadequate 34.4% (72/209) compared to those from farms with apparently adequate 14.3% (13/91) levels of on-farm biosecurity measures. The prevalence was higher in chickens sampled from farms with grossly inadequate on-farm (24.0%; 95% CI: 19.5 – 29.1) compared to those sampled from farms with apparently adequate (4.3%; 2.6 – 7.3) on-farm biosecurity measures. The difference in the prevalence rates between the level of On-farm biosecurity was statistically (p -value = 0.0004; $\chi^2 = 12.69$; RR = 0.4147; OR = 0.3171) significant.

The frequency of *E. coli* isolates was found higher in chickens sampled from farms with seldom usage 57.1% (72/126) compared to those sampled from farms with frequent use 7.5% (13/174) of antibiotics. The prevalence is higher in chickens sampled from farm that seldom

(24.0%; 95% CI: 19.5 – 29.1) compared to those farms that frequently (4.3%; 95% CI: 2.6 – 7.3) administer antimicrobial to the chickens. The difference in the prevalence rates of colibacillosis between the degree and or frequency of antimicrobial usage by farmers in poultry production was found to be statistically (p -value < 0.0001; $\chi^2 = 88.80$; RR = 0.1307; OR = 0.0606) significant.

Table 4 presents the antimicrobial susceptibility and resistance profiles of *E. coli* isolates from Chickens in Maiduguri, Borno State, Nigeria. The results show notable susceptibility to Amoxicillin-Clavulanic acid, Gentamicin, Ciprofloxacin, Ceftriaxone, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. Moderate susceptibility was observed for Enrofloxacin, and fair susceptibility for Tylosin.

The *E. coli* isolates exhibited complete resistance to Ampicillin, Amoxicillin, Penicillin G, Neomycin, Erythromycin, Oxytetracycline, Doxycycline, and Ceftazidime.

Table 5 shows multi-drug resistance profile of 12 *E. coli* isolates from chicken tested against 18 antimicrobial agents. One (1) isolate showed resistance to two (2) of the antimicrobial compounds, one (1) also isolate showed resistance to three (3) of the antimicrobial compounds, one (1) also isolate showed resistance to four (4) of the antimicrobial compounds, two (2) isolates showed resistance to five (5) of the antimicrobial compounds, one (1) isolate showed resistance to six (6) of the antimicrobial compounds, three (3) isolates showed resistance to seven (7) of the antimicrobial compounds, one (1) isolate showed resistance to eight (8) of the antimicrobial compounds, two (2) isolates showed resistance to nine (9) of the antimicrobial compounds.

The serovars of *E. coli* from chickens in Maiduguri are outlined in Table 5. Poultry farm isolates were serovar O1, while veterinary hospital isolates were O2 and O78, showing

typical avian pathogenic characteristics. *E. coli* isolates from exotic breeds included serovars O1, O2, and O78, all displaying avian pathogenic traits. Clinically sick chickens had isolates of serovars O2 and O78, showing similar pathogenic traits. Isolates from live bird markets and local/noiler chicken breeds, representing healthy chickens, were identified as commensal *E. coli* serotypes, non-reactive and classified as avian non-pathogenic

DISCUSSION

Current data on Multi-drug resistant (MDR) *E. coli* in chickens in Northeastern Nigeria is scarce. This study aims to explore the prevalence and risk factors associated with MDR *E. coli* isolates in both healthy and sick chickens. Our findings indicate that *Escherichia coli* was successfully isolated and identified from cloacal swabs and tissues of chickens, regardless of their health status, in Maiduguri, Borno State, Nigeria. The identification was carried out using Eosin Methylene Blue (EMB) agar, various biochemical tests, and agglutination testing. This finding aligns with the common biological understanding that this bacterium exists in the gastrointestinal tract of warm-blooded animals, including chickens, as harmless commensal symbionts, normal flora, opportunistic pathogens, or pathogenic strains. Chickens, in particular, are considered one of the major reservoirs of avian pathogenic *Escherichia coli* (APEC), as described by Tenailon *et al.* (2010) and Nguyen *et al.* (2021). The findings of this study indicate a significant prevalence of *Escherichia coli* infection in chickens, consistent with similar research conducted in Central Ethiopia and Addis Ababa by Sarba *et al.* (2019), Mandal *et al.* (2022) and Tegegne *et al.* (2024). In these studies, *E. coli* was isolated from both sick and apparently healthy chickens.

The *E. coli* colonies displayed distinctive greenish-black appearances with a metallic sheen on EMB agar and tested positive in

various biochemical assays, including sugar fermentation, citrate, nitrate, xylose, and maltose tests, as well as catalase, indole, and Methyl Red tests. These results confirm the presence of *E. coli* in the chicken flocks in Maiduguri, Borno State, Nigeria, suggesting colonization by microbiota, avian non-pathogenic, or avian pathogenic strains of the bacterium. These findings are consistent with previous reports by Ibrahim and Habibu (2021) from Kano State, who isolated *E. coli* from poultry litter.

Our study indicated an overall prevalence rate of 28.3% of *E. coli* in chickens in Maiduguri. The overall prevalence of *E. coli* was 28.3% in the present study is lower than 76% from previous reports of Mandal *et al.* (2022) in Bangladesh. Several factors can contribute to variations in the prevalence rates such as regional differences, sample collection techniques, season, and bacterial identification methods. The presence of *E. coli* in chicken flocks is not uncommon, as it is a normal inhabitant of the avian gastrointestinal tract. The findings of this study affirmed that isolating *E. coli* from chickens is expected due to its ubiquitous nature, supporting the results of Aworh *et al.* (2019) and Maganga (2019).

The prevalence rate in the present study varied among different locations, with the highest prevalence found at live bird markets, followed by poultry farms and veterinary hospitals. These findings confirmed that *E. coli* may be associated with other pathogens found in chickens, as observed by Thapa (2021). Various factors might have influenced the distribution of *E. coli* which includes inadequate sanitary measures and contaminations from infected sources. The high prevalence of *E. coli* in live bird markets can be attributed to overcrowding, stress, and the mixing of chickens with other poultry species from various sources. This observation aligns with the findings of Fancher *et al.* (2020) and Swelum *et al.* (2021), who reported a moderately high prevalence of *E. coli*

in stressed chickens in their studies. Saif (2009) previously reported that high human and bird traffic in markets can facilitate the spread of disease agents, including *E. coli*.

The prevalence of *E. coli* on poultry farms, as observed in this study, may be influenced by the level of biosecurity measures in place. The Mairi area, which had the highest prevalence, might have inadequate biosecurity practices, making chickens more susceptible to infection. This finding is likely due to poor sanitary practices among poultry farmers in the study location, consistent with the report by King (2017), Zou *et al.* (2021) and Adebowale *et al.* (2022). The prevalence of *E. coli* in veterinary hospitals suggests that the bacterium is a common issue among chickens brought in for medical care. This could be because the severity of the disease makes it more likely for sick chickens to be taken to a hospital. This finding supports the reports of Kunert Filho *et al.* (2015), who identified the bacterium in clinically sick birds.

Our study identified several risk factors associated with colibacillosis, including sex, age, breed, health status, husbandry practices, on-farm biosecurity and antibiotic usage. These findings align with those of Gray *et al.* (2021) and Moffo *et al.* (2022), who highlighted similar parameters as significant risk factors for colibacillosis in chicken flocks in their respective studies.

The higher prevalence of colibacillosis in female chickens may be due to hormonal and physiological differences. Female chickens might be more susceptible to infection, possibly related to their reproductive processes. This aligns with the findings of Joseph *et al.* (2023), who stated that the occurrence of colibacillosis in female chickens is related to their reproductive processes.

This study found an increased prevalence in adult chickens, which is expected since older birds have more exposure to potential sources of infection over time. This finding supports the

results of Hofmann *et al.* (2020), who reported a higher prevalence of colibacillosis in adult chickens compared to younger ones.

This study recorded a higher prevalence of colibacillosis among exotic breeds of chickens compared to local ones. The variation in prevalence among chicken breeds might be influenced by genetic factors and susceptibility to infections. Exotic chickens may have different immunity and management practices, consistent with the findings of Fallata (2023).

The present study also revealed a higher prevalence of colibacillosis among clinically sick chickens compared to apparently healthy ones. The higher prevalence in clinically sick chickens underscores the importance of clinical monitoring and early treatment, as sick chickens are likely to be carriers of the disease. This finding supports the reports by Dos Santos *et al.* (2013), who observed a higher prevalence of the disease in sick birds. Additionally, the present study aligns with the findings of Tawyabur *et al.* (2020), who isolated the bacterium in both sick and healthy turkeys.

This study found a higher prevalence of colibacillosis among intensively reared chickens compared to free-range chickens. The higher prevalence in intensively reared chickens could be attributed to factors such as overcrowding and stress, which are common in intensive systems. This agrees with the findings of Yensuk (2019), who indicated that overcrowding and stress might predispose chickens to colibacillosis in intensive management systems.

The present study discovered a lower prevalence of colibacillosis among chickens reared under apparently adequate on-farm biosecurity measures compared to those reared under grossly inadequate on-farm biosecurity measures. The lower prevalence in chickens from farms with apparently adequate on-farm biosecurity practices highlights the importance of these measures in preventing the spread of

colibacillosis. This agrees with the findings of Awawdeh *et al.* (2022), who reported the influence of biosecurity measures on the outbreak of colibacillosis among chicken flocks in similar studies

The results of the present study reveal a higher prevalence of colibacillosis in chicken flocks with frequent antibiotic administration. This suggests that frequent antibiotic use may lead to the development of antibiotic-resistant *E. coli* strains. These findings emphasize the need for responsible antibiotic use in poultry production. Our results align with those of Ibrahim *et al.* (2019), who reported the negative impact of indiscriminate antibiotic use on the incidence of colibacillosis in chicken flocks.

Based on the findings of the present study, the antibiotic susceptibility and resistance profiles of *E. coli* isolates highlight the necessity for responsible antibiotic use in poultry production. The isolates demonstrated susceptibility to several antibiotics, but resistance to others, including those critical for human medicine. The isolates were susceptible to Amoxicillin-Clavulanic acid, Gentamicin, Ciprofloxacin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole, which might be considered as the drugs of choice for treating colibacillosis in infected chickens. These antimicrobials have previously been reported to be very effective in treating colibacillosis in infected flocks (Rafique *et al.*, 2020).

The resistance of *E. coli* isolates to commonly used antibiotics is concerning. This resistance can limit treatment options and increase the risk of treatment failure in both chickens and humans (Ndokui, 2021). The findings emphasize the importance of responsible antibiotic use in poultry farming to mitigate the development of antibiotic-resistant strains. It also underscores the need for the prudent use of antibiotics important for human health (Wallinga *et al.*, 2022).

Several studies have explored the microbiological characteristics of *E. coli* in poultry production in Nigeria. While most of these studies have concentrated on examining the virulence attributes and antibiotic resistance of *E. coli* strains obtained from both poultry hosts and their environments (Ibrahim and Habibu, 2021; Jesumirhewe *et al.*, 2023), information regarding the distribution of serotypes of such *E. coli* strains remains scarce.

The findings of the current study have identified *E. coli* isolates belonging to serovars O1, O2, and O78, which exhibit somatic antigenic characteristics consistent with Avian Pathogenic *E. coli* (APEC) strains. This suggests a potential association between these strains and avian hosts. These results align with a previous study highlighting APEC serotypes O1, O2, O18, and O78 as targets for serotyping, as these are the most common APEC pathotypes causing colibacillosis in animals and humans (Kathayat *et al.*, 2021; Nguyen *et al.*, 2021). These serotypes were found in exotic breed chickens sampled from poultry farms and clinically ill ones from veterinary hospitals, which is consistent with earlier studies indicating that *E. coli* can be confirmed in all groups of infected chickens regardless of location (Jesumirhewe *et al.*, 2023). Notably, serotypes O2 and O78 were more frequently predominant in the infected samples, followed by O1. Contrary to the findings of Nguyen *et al.* (2021), serotype O18 was not detected in the samples, suggesting that certain *E. coli* serovars are more commonly associated with avian infections.

The presence of these specific serovars in avian hosts may reflect the evolutionary adaptation of *E. coli* to different host environments. APEC strains are known to possess virulence factors that enable them to colonize and cause disease in birds (Kathayat *et al.*, 2021). The identification of these avian pathogenic serovars in *E. coli* isolates raises concerns about their potential impact on poultry health and food safety in the

study area. APEC strains are known to cause diseases such as colibacillosis in poultry, leading to significant economic losses in the poultry industry (Hu *et al.*, 2022; Joseph *et al.*, 2023). Additionally, if these strains are present in poultry products destined for human consumption, there could be implications for public health.

However, the present study has some limitations, including the inability to verify the virulence of the isolates and the fact that the pathotype characteristics of the obtained *E. coli* were not examined, nor was a large proportion of *E. coli* serotyped. Despite these limitations, this study has provided valuable information on the biological characteristics and epidemiological dynamics of *E. coli*, which are essential for better control of avian colibacillosis and the prevention of zoonotic diseases caused by APEC.

While the primary focus of this study was on avian colibacillosis, it is crucial to consider the potential for zoonotic transmission of these *E. coli* strains. Some APEC strains have been implicated in human infections, particularly in individuals with close contact with poultry or their environment (Kathayat *et al.*, 2021; Hu *et al.*, 2022). Therefore, further investigation into the zoonotic potential of these strains is warranted.

CONCLUSIONS

The study successfully identified *E. coli* in chicken flocks in Maiduguri, Borno State, Nigeria, using cultural and biochemical tests. The overall prevalence of colibacillosis was 28.3%, with the Monday market and poultry

farms in the Mairi area showing the highest prevalence. Risk factors included female gender, adult age, clinical illness, and inadequate biosecurity and hygiene measures. Antibiotic susceptibility testing revealed varied susceptibility, with resistance to Ampicillin and Amoxicillin. Multi-drug resistance was also observed. Serovars O1, O2, and O78 were identified, consistent with Avian Pathogenic *E. coli* strains. These findings underscore the severity of colibacillosis and highlight the importance of targeted interventions and antibiotic stewardship.

Recommendations

To combat colibacillosis in chicken flocks, the following key recommendations are proposed based on our study:

Improve on-farm biosecurity and hygiene practices to reduce disease prevalence. Promote responsible antibiotic use, considering observed resistance patterns among *E. coli* isolates. Recognize demographic factors such as gender and age to guide vaccination and management strategies. Educate poultry farmers on clinical signs and implement regular monitoring programs. Raise public awareness about disease risks and proper food safety practices. Monitor exotic chicken populations closely and select antibiotics based on resistance profiles. Equip veterinary hospitals and clinics to diagnose and manage colibacillosis effectively. Conduct additional research into the genetic diversity of *E. coli* strains and transmission dynamics for targeted control measures.

Table 1: Prevalence and Distribution of Colibacillosis in Chicken (*Gallus domesticus*) Flocks from Maiduguri, Borno State, Nigeria

Sample origin/ Study location	Study Units	Number of chickens sampled	Number (%) of chickens Infected	Prevalence (%) (95% CI: LL – UL)
Live birds' markets	Monday	35	16 (45.7)	5.3 (3.3 – 8.5)
	Custom	35	9 (25.7)	3.0 (1.6 – 5.6)
	Tasha Bama	30	3 (10.0)	1.0 (0.3 – 2.9)
Total		100	28 (28.0)	9.3 (6.5 – 13.2)
Poultry Farms	GRA	50	3 (6.0)	1.0 (0.3 – 2.9)
	Mairi area	50	7 (14.0)	2.3 (1.1 – 4.7)
	UniMaid Staff quarters	50	6 (12.0)	2.0 (0.9 – 4.3)
Total		150	16 (10.7)	5.3 (3.3 – 8.5)
Veterinary Hospitals	UniMaid VTH	25	21 (84.0)	7.0 (4.6 – 10.5)
	SASUMVH	25	20 (80.0)	6.7 (4.4 – 10.1)
Total		50	41 (82.0)	13.7 (10.2 – 18.0)
Overall		300	85 (28.3)	28.3 (23.5 – 33.7)

Key: UniMaid VTH = University of Maiduguri, Veterinary Teaching Hospital,
SASUMVH = Senator Ali Modu Sheriff Ultra-Modern Veterinary Hospital,
GRA = Government Residential Area,
CI = Confidence Interval; LL – UL = Lower Limit – Upper Limit

Table 2: Biochemical identification of *Escherichia coli* (*E. coli*) Isolated from Cloacal Swabs Collected from Chickens (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria

Biochemical test	Number of isolates tested	Number of isolates positive
Oxidase	85	0
Citrate	85	85
Nitrate	85	85
Xylose	85	85
Inositol	85	0
Maltose	85	85
Urease	85	0
Indole	85	85
Catalase	85	85
Methyl Red	85	85
Voges-Proskauer	85	0
Dextrose	85	85
Lactose	85	0
Galactose	85	85
D – glucose	85	85
Fructose	85	85
D – mannitol	85	85
Sucrose	85	85
Sorbitol	85	85

Table 3: Risk Factors Associated with Colibacillosis in Chicken (*Gallus domesticus*) Flocks from Maiduguri, Borno State, Nigeria

Parameter	Risk factor	No. of samples collected	No. (%) of samples positive for <i>E. coli</i>	Prevalence (%) 95% CL (LL – UL)	χ^2	P -value	RR	OR
Sex	Male	158	13 (8.2) ^a	4.3 (2.6 – 7.3)	46.58	<0.0001	0.1770	0.1526
	Female	142	72 (50.7) ^b	24.0 (19.5 – 29.1)				
Age (months)	Young (3 – 4)	136	24 (17.6) ^a	8.0 (5.4 – 11.6)	13.99	0.0002	0.4744	0.3618
	Adult (> 5)	164	61 (37.2) ^b	20.3 (16.2 – 25.3)				
Breed	Village	100	21 (21.0) ^a	7.0 (4.6 – 10.5)	5.549	0.0624	–	–
	Exotic	100	36 (36.0) ^a	12.0 (8.8 – 16.2)				
Health status	Noilers	100	28 (28.0) ^a	9.3 (6.5 – 13.2)	46.11	<0.0001	0.2319	0.1402
	Apparently healthy	150	16 (10.7) ^a	5.3 (3.3 – 8.5)				
Husbandry system	Clinically sick	150	69 (46.0) ^b	23.0 (18.6 – 28.1)	0.0016	0.9686	1.007	1.010
	Intensive	183	52 (28.4) ^a	17.3 (13.5 – 22.0)				
On-Farm biosecurity	Extensive	117	33 (28.2) ^a	11.0 (7.9 – 15.1)	12.69	0.0004	0.4147	0.3171
	Apparently adequate	91	13 (14.3) ^a	4.3 (2.6 – 7.3)				
Antibiotic usage	Grossly inadequate	209	72 (34.4) ^b	24.0 (19.5 – 29.1)	88.80	<0.0001	0.1307	0.0606
	Frequently	174	13 (7.5)	4.3 (2.6 – 7.3)				
	Seldom	126	72 (57.1)	24.0 (19.5 – 29.1)				

Key: CI = Confidence Interval; LL – UL = Lower Limit – Upper Limit; RR = Relative Risk;

OR = Odd Ratio

^{a,b} Values with different superscripts indicate significant ($p < 0.05$) difference in prevalence rates

Table 4: Antimicrobial Susceptibility and Resistance Profile of *E. coli* isolates from Chicken (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria

Families	Antimicrobials	Number of <i>E. coli</i> Isolates (%) Susceptibility / Resistant to Antimicrobial Test		
		Sensitive	Intermediate	Resistant
Penicillin (Beta-lactams)	Ampicillin (AMP 10 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
	Amoxicillin (AMO 10 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12; (100.0)
	Amoxicillin-Clavulinate acid (AMO/CLA 30 µg/disc) (Augmentin)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
	Penicillin G (PEN 10 units/disc)	0/12 (0.0)	0/12 (0.0)	12/12; (100.0)
Aminoglycosides	Gentamicin (GEN 10 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
	Neomycin (NEO 10 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
Quinolones	Ciprofloxacin (CIP 10 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
	Enrofloxacin (ENR 10 µg/disc)	6/12 (50.0)	3/12 (25.0)	3/12 (25.0)
	Ofloxacin (OFX 5 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
	Levofloxacin (LVX 5 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
	Erythromycin (ERY 10 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
Macrolide and related drugs	Tylosin (TLY 10 µg/disc)	3/12 (25.0)	3/12 (25.0)	6/12 (50.0)
	Oxytetracycline (OXY 10 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
Tetracyclines	Doxycycline (DOX 30 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
	Ceftriaxone (CEF 30 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
First-generation cephalosporin	Ceftazidime (CET 30 µg/disc)	0/12 (0.0)	0/12 (0.0)	12/12 (100.0)
Third-generation cephalosporin	Nitrofurantoin (NIT 300 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
Nitrofuran derivative	Trimetoprim/Sulfamethoxazole (Cotrimoxazole) (TRI/SUL 30 µg/disc)	12/12 (100.0)	0/12 (0.0)	0/12 (0.0)
Diaminopyrimidine with sulfonamide				

Table 5: Multi-Drug Resistance Profile of 12 *E. coli* Isolates from Chickens (*Gallus domesticus*) in Maiduguri, Borno State, Nigeria: Testing against 11 Antimicrobial Agents

S/No	<i>E. coli</i> isolates	Antibiogram (resistant antimicrobials)	Total number of resisted antimicrobials
1.		NEO, ERY, TLY, PEN, OXY, DOX, CET	7
2.		PEN, NEO, ERY, AMP, AMO	5
3.		AMP, AMO, PEN, NEO, ERY, TLY, OXY, DOX, CET	9
4.		TLY, AMO, PEN, OXY, DOX, CET, AMP	7
5.		CET, AMP	2
6.		OXY, DOX, CET, AMP, AMO, NEO, ERY, TLY	8
7.		DOX, CET, AMP, AMO, TLY,	5
8.		PEN, NEO, ERY, OXY, DOX, CET, AMP, AMO, TLY	9
9.		AMO, NEO, ERY, TLY	4
10.		PEN, ERY, TLY, OXY, DOX, CET, AMP	7
11.		PEN, OXY, DOX,	3
12.		ERY, TLY, OXY, DOX, CET, AMP	6

KEY: Ampicillin (AMP 10 µg), Amoxicillin (AMO 10 µg/disc), Penicillin G (PEN 10 units/disc), Neomycin (NEO 10 µg/disc), Erythromycin (ERY 10 µg/disc), Tylosin (TLY 10 µg/disc), Oxytetracycline (OXY 10 µg/disc), Doxycycline (DOX 30 µg/disc), Ceftazidime (CET 30 µg/disc)

Table 6: Serovars of *E. coli* isolated from chickens in Maiduguri, Borno State, Nigeria

Information		Serotype	Antigenic characteristics	<i>E. coli</i> strain
Sample Origin	Live birds' markets	commensal <i>E. coli</i>	Non-reactive	Avian Non-pathogenic <i>E. coli</i>
	Poultry farms	O1	Somatic	Avian pathogenic <i>E. coli</i>
	Veterinary Hospitals	O2 and O78	Somatic	Avian pathogenic <i>E. coli</i>
Breed	Local	commensal <i>E. coli</i>	Non-reactive	Avian Non-pathogenic <i>E. coli</i>
	Exotic	O1, O2 and O78	Somatic	Avian pathogenic <i>E. coli</i>
	Noiler	commensal <i>E. coli</i>	Non-reactive	Avian Non-pathogenic <i>E. coli</i>
Health status	Apparently Healthy	commensal <i>E. coli</i>	Non-reactive	Avian Non-pathogenic <i>E. coli</i>
	Clinically sick	O2 and O78	Somatic	Avian pathogenic <i>E. coli</i>

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