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Bacterial Detection and Antimicrobial Susceptibility Profiling in a Broiler Breeder Flock

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ABSTRACT

Background: The health of breeders is essential for the next generation of chicks. Bacterial agents, such as *Escherichia coli*, *Salmonella enterica* and *Mycoplasma gallisepticum* or *Mycoplasma synoviae*, are the leading causes of disease and mortality in breeder poultry. The widespread usage of antimicrobials in poultry, has raised concerns about the potential for spread of antimicrobial resistance. The present study aimed to identify bacterial infections and antibiotic resistance patterns in the case of breeder's mortality.

Methods: Samples were collected from tissues and bone marrow of sixteen broiler breeders. The specimens were cultured onto blood agar, MacConkey agar and confirmatory biochemical media for the bacteriological examination. Sabouraud dextrose agar was used for fungal isolation. The specimens were cultured on Mueller-Hinton agar to assess their antibiotic resistance to gentamicin, ceftriaxone, tetracycline, penicillin, erythromycin, streptomycin, lincomycin, and amoxicillin

Results: The bacteria including *Escherichia coli* (93.8%), *Staphylococcus* (31.3%), *Streptococcus* (12.5%), *Bacillus* (12.5%) and *Clostridium* (6.3%) were isolated. Co-infection was observed in 50% of carcasses, most frequently involving *E. coli* and *Staphylococcus*. Isolates from 62.5% were resistant to all the antibiotics tested. Gentamicin was the most effective antibiotic.

Conclusion: *Escherichia coli* was the dominant isolate, and its detection in the bone marrow of 31.3% of cases confirms systemic dissemination. Consequently, enhanced biosecurity protocols are essential to prevent the spread of pathogens such as *E. coli*. Antimicrobial susceptibility testing revealed a multidrug-resistant pattern. Therefore, poultry farms should base antibiotic selection on specific laboratory results rather than relying on empirical broad-spectrum therapy.

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Introduction

The health condition and nutritional status of breeder poultry are essential for the next generation of chicks, which are reared for human consumption. Consequently, ensuring the absence of bacterial infections in breeders becomes critically important (1). Bacterial infectious agents, such as *Escherichia coli*, *Salmonella enterica*, and *Mycoplasma gallisepticum* or *Mycoplasma synoviae*, are the leading etiological agents of disease and mortality in poultry. Notably, Avian pathogenic *E. coli* (APEC), which causes salpingitis–peritonitis syndrome; an inflammation of the reproductive tract and abdominal cavity, can progress from asymptomatic infection to systemic septicemia, often resulting in death (2). In addition, *E. coli* can cause histopathological damages in pulmonary and hepatic tissue, with destruction of lymphoid tissue in the spleen, thymus and bursa of broilers (3).

Several studies have evaluated the role of infectious pathogens in health status of breeders (4-6). A study which evaluated the surveillance of Ontario's breeder flocks revealed a 25.3% period prevalence of *S. enterica*, and the most prevalent serovars were Kentucky, Heidelberg, and Typhimurium from 2009 to 2018 (4). Moreover, *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are broadly distributed in breeder flocks, with seroprevalence rates in breeders reported at approximately 60% (MG) and 50% (MS), substantially higher than in commercial broiler chickens (5). An investigation in 2024 in Austria, highlighted that co-infection of *Staphylococcus lentus* and *Staphylococcus aureus* led to a surge of arthritis, synovitis, and osteomyelitis among broiler breeder chickens in Austria (6). Furthermore, another research conducted in Iran in 2020, reported that some of specific *Salmonella* serovars, including *Salmonella enteritidis*, can be established residence in the oviducts and infect them throughout egg formation and affect the reproductive performance of breeder chickens (7).

When breeder chickens are infected with pathogenic bacteria, one of the conventional therapeutic approaches is the use of antibiotics. Fluoroquinolones such as enrofloxacin are commonly recommended for treatment of salmonellosis in poultry (8). Moreover, penicillin, erythromycin and tetracycline are used as therapeutic administrations against Staphylococcal infections in poultry (9). However, the widespread improper usage of antimicrobials in poultry industry, both for growth and disease management, has raised concerns about the potential for spread of antimicrobial resistance in bacterial pathogens including APEC (10). Recent studies have highlighted this emerging threat globally and locally. High levels of multidrug resistance have been reported in *E. coli* isolates from broiler breeder farms in Korea in 2022 (11). Similarly, a research conducted in Iran in 2014, demonstrated that APEC strains exhibit considerable resistance to a wide range of commonly used antibiotics (12).

According to the available data in Iran, limited research has been done on the prevalence of bacterial infections in breeders. In addition, their antimicrobial resistance has been poorly assessed. The objective of this study was to investigate bacterial etiologies of mortality in breeder flocks and to determine the antimicrobial susceptibility profiles of the isolates.

Materials and Methods

Necropsy was performed on sixteen broiler breeders, and samples were collected from the heart, liver, spleen, lungs, bone marrow, and kidneys. The specimens were cultured onto blood agar and MacConkey agar and incubated aerobically and anaerobically at 37 °C for 48 h. Additional biochemical tests were performed to determine the type of bacteria, based on the characteristics of the isolates and the outcomes of culture tests. Sabouraud dextrose agar was used for fungal isolation and incubated at room temperature for 5 days. The specimens were also cultured on Mueller-Hinton agar to assess their antibiotic resistance to gentamicin, ceftriaxone, tetracycline, penicillin, erythromycin, streptomycin, lincomycin, and amoxicillin.

Results

The bacteria including *Escherichia coli*, *Staphylococcus* spp, *Streptococcus* spp, *Bacillus* spp and *Clostridium* spp were isolated from different specimens of all the sixteen carcasses. *E. coli* was isolated from 15 (93.8%) birds, recovered from heart, liver, spleen and bone marrow. Five birds (31.3%) were positive for *Staphylococcus* spp., which was isolated from both cardiac and hepatic tissues. *Streptococcus* spp. were isolated from bone marrow and liver of two cases (12.5%). *Bacillus* spp. were identified in liver and heart of two cases (12.5%). *Clostridium* spp. were the sole isolate from heart of case number 3. Yeasts were identified in three samples from tissues including heart, liver and lung (18.8%). Co-infection was observed in 8 of 16 cases (50%), most frequently involving *E. coli* and *Staphylococcus* (31%). Case number 10 exhibited systemic infection across all organs. Four cases demonstrated bone marrow infection with *E. coli*, two with *Streptococcus*, and one with the both pathogens. The results of antibiotic susceptibility test are available in Table 2. The specimens belonged to case number 12, were sensitive to all the antibiotics and gentamicin was the most effective antibiotic for the cultured samples.

Participants (19), Healthy Adult Subjects (20), Healthy Subjects(21), Healthy Male Participants (22), Healthy People (23), Healthy Men and Women (24), Healthy Female Participants (25), Healthy Males (26), Healthy Adult Male Subjects (27), or simply Healthy Participants (28).

In some studies, race and nationality are taken into account, such as Healthy Male Japanese Study Participants (29) or Healthy Chinese Volunteers (30). However, in some Phase I studies, participants are not healthy. For example, they may have mental illness, AIDS, tumors, cancer, etc., such as A Study of GFH018 in Patients with Advanced Solid Tumors(31), where intervention was conducted on 50 individuals with advanced solid tumors.

Table 1. Isolated agents from different tissues of the poultry carcasses.

Case number	**Isolated agents**						**Infected tissues**				
	Escherichia coli	Staphylococcus	Streptococcus	Bacillus	Clostridium	Yeast	Heart	Liver	Spleen	Bone marrow	Lung
1	+	-	-	+	-	-	+	+	-	-	
2	+	-	-	-	-	-	+	-	-	-	
3	+	-	-	-	+	+	+	+	-	-	+
4	+	+	-	-	-	-	+	-	-	+	-
5	+	-	-	-	-	-	+	+	-	-	-
6	+	+	-	-	-	-	+	+	-	-	-
7	+	-	-	-	-	-	+	+	+	+	-
8	+	-	-	-	-	-	+	+	-	+	-
9	+	+	-	-	-	+	+	+	-	-	+
10	+	-	+	-	-	-	+	+	+	+	+
11	-	+	+	-	-	-	-	+	-	+	-
12	+	+	-	+	-	+	+	+	-	-	+
13	+	-	-	-	-	-	+	+	-	-	-
14	+	-	-	-	-	-	+	+	+	-	-
15	+	-	-	-	-	-	+	+	+	-	-
16	+	-	-	-	-	-	+	+	+	-	-

Table 2. Antibiotic susceptibility test using a single swab for each bird carcass (R: resistant, S: sensitive).

Antibiotic Cases	Gentamycin	Ceftriaxone	Tetracycline	Penicillin	Erythromycin	Streptomycin	Lincomycin	Amoxicillin
1	S	R	R	R	R	R	R	R
2	R	R	R	R	R	R	R	R
3	R	R	R	R	R	R	R	R
4	R	R	R	R	R	R	R	R
5	R	R	R	R	R	R	R	R
6	R	R	R	R	R	R	R	R
7	R	R	R	R	R	R	R	R
8	R	R	R	R	R	R	R	R
9	R	R	R	R	R	R	R	R
10	R	R	R	R	R	R	R	R
11	R	R	R	R	R	S	R	S
12	S	S	S	S	S	S	S	S
13	S	R	R	R	R	R	R	R
14	R	S	R	R	R	R	S	S
15	S	S	R	R	R	R	R	R
16	S	R	R	R	R	S	S	S

Discussion

In the current study, post-mortem culture of internal organs (heart, liver, spleen, lungs, bone marrow, and kidneys) from sixteen broiler breeders yielded a variety of bacterial isolates and yeasts. Among the agents, *E. coli* was the dominant isolate (93.8%). The heart and liver were the most frequently affected organs, respectively (Table 1). In agreement with the present results, Joseph et al. identified avian pathogenic *E. coli* (APEC) as principle cause of systemic infections and mortality in breeders (2). Recovery of *E. coli* strains from internal organs of poultry with colisepticemia or localized extra-intestinal disease demonstrates its capacity for systemic infection, which can lead to substantial mortality in commercial flocks (13).

In the current study, *E. coli* was isolated from multiple tissues of each carcass. Its presence in the bone marrow in 5 cases (31.3%) confirms systemic dissemination. Furthermore, bone marrow serves as an excellent source for genomic-scale expression profiling in APEC infection, as it harbors primordial cells unaffected by the developmental cytokines and other factors present in established lymphoid organs (14). Alfifi et al. demonstrated a high prevalence (48%) of bacterial contamination in broiler bone marrow samples from a Danish abattoir, confirming systemic infection. Among the isolated bacteria, *E. coli* was the most prevalent pathogen (15). In addition to *E. coli*, we isolated *Streptococcus* spp. from bone marrow. This finding is significant as streptococcal species are established etiological agents of septicemia and endocarditis in poultry (16).

Staphylococcus was detected as the second most frequent pathogen in the breeder's carcasses (Table 1). Poultry flocks harbor a diverse population of *Staphylococcus* species, encompassing both coagulase-positive and coagulase-negative types (17). *Staphylococcus aureus* is a recognized etiological agent of several poultry diseases, such as omphalitis, pneumonia, arthritis and may cause acute and subacute septicemia (18). In the present investigation, *Bacillus* spp. were isolated from only two birds (12.5%). While most *Bacillus* species are environmental commensals, specific pathogens within the genus, including B. cereus and *B. licheniformis*, can cause respiratory disease, septicemia, and endocarditis in immunocompromised or stressed birds (19, 20).

The least frequent pathogen was *Clostridium* which was only detected in the heart of case number 3 (Table 1). Although *Clostridium* is normal flora, isolating it from heart is significant and shows its role in the bird death. *Clostridium* perfringens and *Clostridium sordellii* may be associated with mortality in poultry (21, 22). The detection of yeast in poultry is an indicator of compromised hygiene particularly when established in the lungs (Table 1) and can lead to opportunistic infection. Yeasts like *Candida* species have been documented as avian pathogens. Furthermore, their metabolic activity is a known causative agent of food spoilage and associated diseases (23).

Concurrent infection with the isolated agents was seen in 50% of carcasses and most frequently involving *E. coli* and *Staphylococcus* (31%). One of the birds (case number 13) showed simultaneous infection with 5 agents including *E. coli*, *Staphylococcus* spp, *Streptococcus* spp, *Bacillus* spp and yeast (Table 1). Several studies have documented that concurrent infections in poultry act synergistically, leading to more severe disease, amplified inflammatory responses, increased tissue damage, and higher mortality (6, 24, 25).

Antimicrobial susceptibility testing revealed a multidrug-resistant pattern in the isolates. Specifically, isolates from 10 carcasses (62.5%) were resistant to all the antibiotics tested (Table 2). Notably, gentamicin was the only antibiotic with effectiveness in five cases which demonstrated a partial susceptibility of *E. coli* to this antibiotic since most of the carcasses showed infection with the bacterium (Table 1). The present results, aligned closely with the report of Talebiyan et al. that indicated high resistance among Iranian APEC isolates, including 88.7% resistance to tylosin, 71.7% to erythromycin, and around 40% resistance to several tetracyclines and sulfonamides (12). Similarly, Kim et al. reported high resistance to tetracycline, nalidixic acid, ampicillin, and cephalothin in breeder farms in Korea in 2022 (11). Moreover Aberkane et al. in 2023, demonstrated high levels of resistance to ampicillin and tetracycline (100%), nalidixic acid (95%), ofloxacin (93.75%), doxycycline (91.87%), ciprofloxacin (87.50%), trimethoprim/sulfamethoxazole (62.50%) among Algerian APEC isolates (26-28).

Conclsuion

Based on the present findings, a few practical changes can make a big difference in controlling infections and resistance in breeder flocks. First, biosecurity needs to be stricter. Simple steps like better cleaning between flocks and limiting farm traffic help to prevent pathogens such as *E. coli* directly. It is also important to improve the treatment protocols. Instead of just using broad-spectrum antibiotics, poultry farms should rely on laboratory tests to pick the correct antibiotic for the specific infection. Using available vaccines and reduce the stress through better ventilation and nutrition will naturally lower the need for drugs. Ultimately, the success of these measures hinges on education; staff must be trained to understand the rationale behind the protocols to ensure consistent compliance.

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Ethics approval and consent to participate

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Conflict of interest

The authors report no conflict of interest.

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