Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (2) 10.29079/qjvms.2025.166451.1132

ISSN P: 1818-5746
E: 2313-4429
qjvms@qu.edu.iq

Isolation and Identification of *E. coli* as Secondary Infection with Avian Metapneumo Virus in Broiler Chickens

Dyar Adil Morad[1]✉ Aqeel Mohamed Shareef[2]
[1]Dept. of Microbiology and Pathology College of Veterinary Medicine, University of Duhok, Iraq.
[2] Dept. of Pathology-Poultry Disease, College of Veterinary Medicine, University of Mosul, Iraq.

Submitted: 19-10-2025
Revised: 16-12-2025
Accepted: 16-12-2025

Correspondence
Dyar Adil Morad
Dyar.morad@uod.ac

Abstract Infections with Escherichia coli (E. coli) affect poultry of all ages and types. It is frequently associated with poor hygiene, unmet technology needs, or respiratory problems. The current study was intended to detect the isolation and identification of E. coli as a secondary infection in broiler chickens co-infected with AMPV in Duhok, Iraq. The duration of the study started from September 2024 to March 2025, about 120 nasal and tracheal swabs were collected from infected broilers aged 3-6 weeks from 20 flocks in Duhok city. Furthermore, microbiological culturing and biochemical examinations were done to identify E. coli. The samples were isolated and identified to detect E. coli using MacConckey Agar (MCA), Eosin Methylene blue agar (EMBA), Gram staining, indole test, Methyl Red, Voges Proskauer (MR-VP), citrate, and triple sugar iron agar (TSIA), and antibiotic susceptibility test. Overall (83.3%) of broilers had been infected with E. coli .Out of 100 isolates were examined for the antimicrobial susceptibility test, It was stated that the isolates exhibited antibiotic resistance Amoxicillin, Cephalothin, Tetracyclin, Fosfomycin, Taichamphnicol, Spiramycin, lincomycin, tilocin, doxacillin were 100 % and ciprofloxacin was 78% and neomycin was 96% , Cephalexin was 92% and Erythromycin was 98%, Tylosin was 96%, Levofloxacin 54% Trimethoprime-Sulfamethoxazole was 90% , Azithromycin was 80% ,Gentamycin was 46% and Enrofloxacin was 46%. It is essential to use effective prevention and control strategies to decrease the disease.

Keywords: AMPV, *E. coli*, Broiler chickens, Antibiotics sensitivity test.

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Introduction Avian metapneumovirus (AMPV) is a poultry virus that causes swollen head syndrome and a contagious upper respiratory tract infections in chickens. These conditions result in significant financial losses for the chicken sector in many countries and the first seroprevalence study represented in Duhok province for detection of AMPV infections in broiler chickens (1). The first study in Duhok dedicated to evaluating phenotypic methods for detecting AmpC β-lactamase–producing Escherichia coli isolated from infected broiler chickens with aMPV (2). Since the first recorded case of aMPV, numerous bacteria, such as E. coli, were isolated from developing cases (3Avian colibacillosis is the most prevalent bacterial illness affecting chicken in all ages. This condition is caused by avian pathogenic E. coli (5), also its characterized by facultative anaerobic bacilli as well as gram-negative bacteria (6). Pathogenic bacteria that cause mortalities in animals and death in humans can be transmitted through diseased chickens (7). The increase in the incidence of infectious diseases is mainly because of inappropriate use of antibiotics is the most dominant in poultry farms (8). World Health Organization (WHO) identifies antimicrobial resistance as an important global health issue in the 21st century (9). The mixture treatment of amoxicillin and colistin (60.8%) is extensively used in numerous fields. The belief of owners that administration of antibiotics has not consequences, as well as is an inexpensive approach to avoid infection (10 & 11).This subsequently results in a rise in the rudiments that interfere to the formation of resistance for antibiotics in poultry as an animal-derived diet. This disease causes high mortality, delayed growth rate. It is critical to select suitable antibiotics depend on bacterial resistance forms variables and control the prevalence of resistance from bacteria in animal products to humans (12 & 13). In view of the importance of E. coli infection in broiler chickens, this study evaluate to isolate E. coli from infected samples of broiler flocks to study prevalence of E. coli as secondary bacterial infection

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for aMPV and to determine the present status of E. coli in broiler.

Material and Methods

Ethical Considerations

This study did not require ethical approval as it did not involve human participants, animal subjects, or sensitive personal data. Only swabs were taken from infected birds

Sample Collection and Bacterial Identification

The duration of the study from September 2024 to March 2025, overall, 120 nasal and tracheal swabs were taken from twenty flocks of broilers in Duhok the age of broilers were between three and six weeks old. The broilers showed upper portion of respiratory passages especially sinuses, trachea infections. Swabs inoculated in MacConky broth. Then a loop full was cultured on MCA agar and plates were incubated at 37 ℃ for 24 hour. The macroscopic appearance of bacterial colonies for E. coli identification (14 &15). Suspected colonies of E. coli cultured on EMBA media were stained with gram stain to confirming microscopic appearance of bacterial structures. Then biochemical IMViC tests (Indole, MR, VP, and citrate) and TSIA (12 & 16).

Antibiotic Sensitivity Test

Muller-Hinton agar was used in process of examination for One hundred positive *E. coli* isolates and according to Kirby-Bauer disk-diffusion method based on CLSI's recommendation. After that plates were incubated for 24-hour period at 37˚C, finally . reading of results by using of electronic calibrator and inhibition zone were illustrated depended on CLSI guidelines (17).

Results

Out of 120 swabs were streaked on MCA agar revealed that 100/120(83.3%) have detected of *E. coli,* while the remaining 20/120 (16.7%) were negative, results are shown in figure 1.

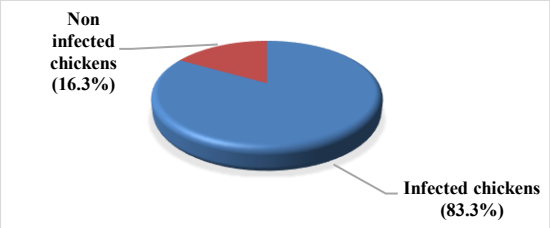

Figure 1: Prevalence of E.coli among infected and non infected chickens

The results of biochemical examinations showed that *E. coli* isolates were positive to catalase, carbohydrate broth, methyl red, indole, carbohydrate (lactose fermentation) and triple sugar iron agar , they were negative to Voges- Proskauer, Simmons citrate and oxidase.The results of antimicrobial susceptibility for the isolates are shown in figure 2.It is clear that *E.coli* recorded the resistance of 100% for Amoxicillin (AX), Cephalothin-(KF), Tetracycline(T), Fosfomycin(FF), Taichamphnicol(TP), Spiramycin(SP), Lincomycin(L) , Tilocin, and Doxacillin(DO).While, the highest sensitivity was found among Levofloxacin(LEV) 46.0%,followed by Ciprofloxacin (CIP) of 22.0%,and Azithromycin(AZM) 20.0%.

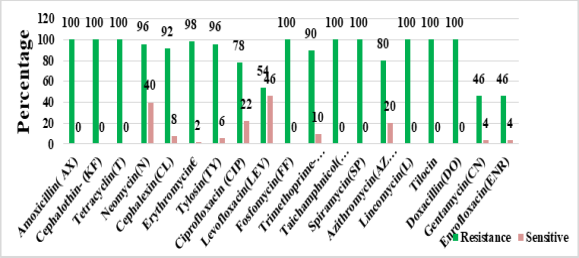

Figure 2: Frequency of antibiotic sensitivity test.

Discussion

Swollen head syndrome is a condition of the upper part of respiratory system that has develop a main concern in current years. There are several studies showed that viral and bacterial factors are involved in manifestation of this condition (18). *E. coli* is the main cause of sickness and mortality in poultry farms, mainly in broiler chicks. Most of scientists suggest that *E. coli* is the main infectious bacterial disease for poultry, causing in enormous financial losses. *E. coli* resistant to antibiotic because of given of antibiotics at sub therapeutic levels to avoid infections and promote of growth. The synthesis and secretion of the β-lactamase enzyme decreases antibiotic efficacy and increases resistance concerns. Present study showed that 83.3% of broiler chicks had *E. coli* infection. Similarly, (19) and (2) investigated the percentage of infection of broiler chicks were 40.0% and 70.0%, respectively. *E. coli* was the most common co-infection associated with aMPV among the 20 affected flocks. Because of great occurrence of disease in the same area which is described by high morbidity and mortality, difficulty to diagnosis and clinical symptoms are same to numerous respiratory sicknesses for example IB infection (20). Pure *E. coli* was isolated from *aMPV* infection (21). From the current outcomes, we could propose a

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initial monitor to reduction the disease through main control of bacterial infection moreover by use of biosecurity processes, decrease of environmental influences, support the reduction of occurrence of infections among broiler flocks. However, another study researchers noticed that samples from the heart, liver, lung, yolk sac, spleen, and air sac of broiler chickens had reduced frequencies of *E. coli* (40%) by (22) and another study by (23) the rate (41.30%). While more than 80 percent of the *E. coli* detects analyzed in present study were showed high resistant to most antibiotics, including amoxicillin and tetracycline. According to a research by (24), *E. coli* isolated from broiler chicken meat had susceptibilities of 80.6% to tetracycline, 14.2% to gentamicin, and 11.4% to chloramphenicol. Due to overuse of several antibiotics, *E. coli* developed a high level of resistance to them. (25) found substantial resistance levels in broiler chicken meat to antibiotics such as tetracycline, ciprofloxacin, and gentamicin. Lincomycin resistance was identified as 100%, which was stable with the results of (26) and (27), who showed 96.4% lincomycin resistance. All tested APEC were amoxicillin resistant (100%) similar results were described (28 & 29). Ciprofloxacin had a significant level of resistance (78%), which is consistent with (30), (31), and (32), who reported ciprofloxacin resistance rates of (81%, 41.4%, and 60%, respectively). Other researches (27) and(33) described minimal resistance of 25% and 26%, respectively. As well as gentamycin showed minimum resistance rate (46%). Lower gentamicin resistance percentage (10%) (31) and (32).Nevertheless, (29) showed (100%) of resistance to gentamicin.

Conclusion

It also assists in guiding appropriate treatment strategies and biosecurity measures, reducing both economic losses in poultry farming and risks to human health. The isolation of *E. coli* from chickens is vital for diagnosing the infection, understanding its pathogenesis, determining transmission routes, and ensuring public health safety.

Conflict of Interest

The authors declare there is no conflict of interest.

Funding

The authors did not receive any funds for this study.

Acknowledgment

We would like to express our gratitude to flocks for their help and kindness

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