



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

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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 MacConkey 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% Trimethoprim-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 (3) Avian 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 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 °C 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.

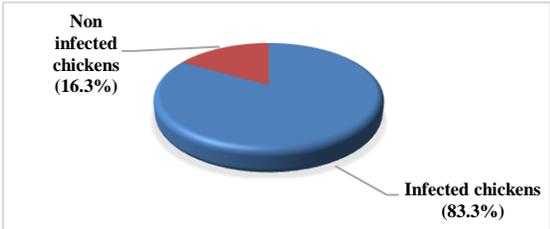


Figure 1: Prevalence of *E.coli* among infected and non infected chickens

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%.

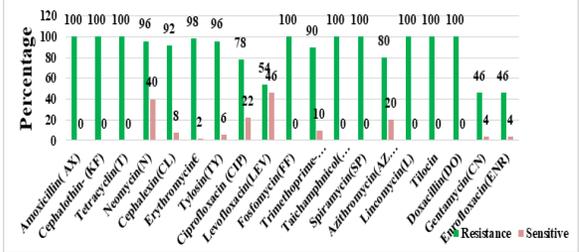


Figure 2: Frequency of antibiotic sensitivity test.

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 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 gentamycin resistance percentage (10%) (31) and (32). Nevertheless, (29) showed (100%) of resistance to gentamycin.

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.

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References

1. Morad DA, Sharef AM. Seroprevalence avian metapneumovirus in broiler chickens by indirect elisa in Duhok province, Kurdistan, Iraq. Egyptian Journal of

Veterinary Sciences, (2023); Jul 1;54(4):589-92. DOI: 10.21608/ejvs.2023.197075.1456.

2. Morad DA, Sharef AM. Evaluation of phenotypic tests for the detection of AmpC beta-lactamases in swabs isolates of *Escherichia coli* from broiler chickens infected with Avian Metapneumovirus. Kerbala Journal of Veterinary Medical Sciences, (2025) Vol.1 Issue 4 Dec.

3. Abdelmoez NS, Shawky MM, Abdelhady HA, Lebda MA, Salama SS. Isolation and identification of some possible causative agents of swollen head syndrome (SHS) in broiler chickens in Egypt. Slovenian Veterinary Research, (2019); 56: 781-788. DOI: 10.26873/SVR-819-2019.

4. AL-Barwary DA. Detection of newcastle disease virus in wild pigeon by serological test in Sumel region. Journal of University of Thi-Qar Vol, (2015); Mar;10(1).

5. Barnes, H. J., Saif, Y. M., Glisson, J. R., Fadly, A. M., McDougald, L. R., & Swayne, D. E. (2003). Diseases of poultry 11th ed. Iowa State University Press, 56, 137-149

6. Fotadar U, Zaveloff P, Terracio L. Growth of *Escherichia coli* at elevated temperatures. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms. (2005); Oct;45(5):403-4. <https://doi.org/10.1002/jobm.200410542>

7. Suardana IW, Utama IH, Ayu P, Putriningsih S, Rudyanto D. Antibiotic sensitivity test of *Escherichia coli* O157: H7 isolate from chicken feces. Bul Vet Udayana. (2014);6(1).

8. Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. The presence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* on layer chicken farms in Blitar Area, Indonesia. Biodiversitas. (2020);21(6):2667-71. <https://repository.unair.ac.id/101307>.

9. World Health Organization. Interagency Coordination Group on Antimicrobial Resistance. No time to wait: Securing the future from drug-resistant infections, Report to the secretary-general of the united nations. (2019); Apr;1:28.

10. Niasono AB, Latif H, Purnawarman T. Antibiotic Resistance to *Escherichia coli* Bacteria Isolated from Broiler Farms in Subang Regency, West Java. J Vet (2019);20 (2): 187-195.

11. Masruroh CA, Sudarwanto MB, Latif H. The Occurrence of extended spectrum B-Lactamase-producing *Escherichia coli* from broiler feces in Bogor.



- JSV. (2016);34:42-9.<https://doi.org/10.1155/2014/195189>
12. Putra AR, Effendi MH, Koedarto S, Tyasningsih W. Molecular identification of extended spectrum betalactamase (ESBL) producing *Escherichia coli* isolated from dairy cows in East Java Province, Indonesia. *The Indian Veterinary Journal*. (2019);96(10):26-30.
<https://repository.unair.ac.id/101276>.
13. Vasilakopoulou A, Karakosta P, Vourli S, Tarpatzi A, Varda P, Kostoula M, Antoniadou A, Pournaras S. Gastrointestinal carriage of vancomycin-resistant enterococci and carbapenem-resistant gram-negative bacteria in an endemic setting: prevalence, risk factors, and outcomes. *Frontiers in public health*. (2020)Mar ;18;8:55.. DOI: 10.3389/fpubh.2020.00055.
14. Effendi MH, Harijani N, Yanestria SM, Hastutiek P. Identification Of Shiga Toxin-Producing *Escherichia coli* In Raw Milk Samples From Dairy Cows In Surabaya, Indonesia. *Jurnal International The Philippine Journal of Veterinary Medicine*. (2018);55:109-14.
15. Putra AR, Effendi MH, Koedarto S, Suwarno S, Tyasningsih W, Estoe pangestie AT. Detection of the extended spectrum β -lactamase produced by *Escherichia coli* from dairy cows by using the vitek-2 method in tulungagung regency, Indonesia. *Iraqi J Vet Sci* (2020); 34 (1): 203-207
16. Kristianingtyas L, Effendi MH, Tyasningsih W, Kurniawan F. Genetic identification of blactx-M gene and blatem gene on extended spectrum beta lactamase (ESBL) producing *Escherichia coli* from Dogs. *The Indian Veterinary Journal*. (2020);97(1):17-21.<https://repository.unair.ac.id/113170>.
17. Badger S, Abraham S, Stryhn H, Trott DJ, Jordan D, Caraguel CG. Intra-and inter-laboratory agreement of the disc diffusion assay for assessing antimicrobial susceptibility of porcine *Escherichia coli*. *Preventive Veterinary Medicine*. (2019) Nov 15;172:104782.
18. Saif, Y. M., Barnes, H. J., Glisson, J. R., Fadly, A. M., McDougald, L. R., & Swayne, D. E. (2003). *Diseases of poultry 11th ed.* Iowa State University Press, 56, 137-149.
19. Shawki MM, Lebdah MA, Shahin AM, Nassif SA. Some studies on swollen head syndrome in broiler chickens in Egypt. *Zagazig Veterinary Journal*. (2017) Oct; 1;45(Supplementary 1):133-41. DOI: 10.21608/zvjz.2019.28658.
20. AL-Barwary DA, Goreal AA. Diagnosis of infectious bronchitis disease in broiler chickens by serological test (ELISA) and RT-PCR in Duhok. *International Journal of Animal and Veterinary Advances*. (2012) Feb; 15;4(1):71-5.
21. Al-Ankari AR, Al-Ramadan MA, El-Demerdash MM. Risk factors associated with prevalence of Swollen Head Syndrome (SHS) in broiler chickens in Eastern Province–Saudi Arabia. *International Journal of Poultry Science*. (2004);3(10):646-50.
22. Rashid M, Kotwal SK, Malik MA, Singh M. Prevalence, genetic profile of virulence determinants and multidrug resistance of *Escherichia coli* isolates from foods of animal origin. *World*. (2013) Mar; 1;6(3):139-42.. doi:10.5455/vetworld.2013.139-142.
23. Enany ME, Algammal AM, Nasef SA, Abo-Eillil SA, Bin-Jumah M, Taha AE, Allam AA. The occurrence of the multidrug resistance (MDR) and the prevalence of virulence genes and QACs resistance genes in *E. coli* isolated from environmental and avian sources. *AMB Express*. (2019) Dec;9:1-9.<https://amb-express.springeropen.com/articles/10.1186/s13568-019-0920-4>.
24. Suandy, I. (2011). Antimicrobial resistance in *Escherichia coli* isolated from commercial broiler farms in bogor district, west JAVA, Indonesia (Doctoral dissertation, Chiang Mai: Graduate School, Chiang Mai University and Freie Universitat Berlin, 2011).
25. Akmal M, Rastina HA, Ismail D, Masyita D. *Escherichia coli* resistance to antibiotics from broiler chicken meat in Rukoh Market. *JIMVET*. (2017);1(3):492-8..
26. Ibrahim MS, Hussein AH, Eid AA, Lebdah MA. Molecular characterization of *Escherichia coli* strains causing respiratory signs in broiler chickens in Egypt. *Zagazig Veterinary Journal*. (2019) Oct; 3;47(2):168-82. DOI: 10.21608/zvjz.2019.10214.1026
27. Eid S, M ERFAN AH. Characterization of *E. coli* associated with high mortality in poultry flocks. *Assiut Veterinary Medical Journal*. (2013) Oct; 23;59(139):51-61. DOI: 10.21608/avmj.2013.171926.
28. Yaqoob M, Wang LP, Wang S, Hussain S, Memon J, Kashif J, Lu CP. Associations between anti-microbial resistance phenotypes, anti-microbial resistance genotypes and virulence genes of *Escherichia coli* isolates from Pakistan and China. *Transboundary and Emerging Diseases*. (2013) Oct;60(5):416-24. <https://doi.org/10.1111/j.1865-1682.2012.01360.x>
29. Mohamed MA, Shehata MA, Rafeek E. Virulence genes content and antimicrobial resistance in *Escherichia coli* from broiler chickens. *Veterinary Medicine International*. (2014);2014 (1):195189..



30. Tong P, Sun Y, Ji X, Du X, Guo X, Liu J, Zhu L, Zhou B, Zhou W, Liu G, Feng S. Characterization of antimicrobial resistance and extended-spectrum β -lactamase genes in *Escherichia coli* isolated from chickens. *Food borne Pathogens and Disease*. 2015 Apr 1;12(4):345-52.<https://doi.org/10.1089/fpd.2014.1857>
31. Awad A, Arafat N, Elhadidy M. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. *Annals of clinical microbiology and antimicrobials*. (2016) Dec;15:1-8.<https://link.springer.com/article/10.1186/s12941-016-0174-9>.
32. Majhi M, Pamia J, Panda SK, Samal L, Mishra R. Effect of age and season on enteritis and antibiotic sensitivity test of *E. coli* isolated from infected chickens in Odisha, India. *International Journal of Current Microbiology and Applied Sciences*. (2018);7(3):2037-45.
33. Literak I, Reitschmied T, Bujnakova D, Dolejska M, Cizek A, Bardon J, Pokludova L, Alexa P, Halova D, Jamborova I. Broilers as a source of quinolone-resistant and extraintestinal pathogenic *Escherichia coli* in the Czech Republic. *Microbial drug resistance*. (2013) Feb; 1;19(1):57-63..<https://doi.org/10.1089/mdr.2012.0124>