Evaluation of phenotypic tests for the detection of AmpC beta-lactamases in Swabs isolates of *Escherichia coli* from Broiler Chickens infected with Avian Metapneumovirus

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***Abstract*— The emergence of AmpC β-lactamases-producing *Escherichia coli* (*E. coli*) strains in poultry, especially in broiler chickens suffering from Avian Metapneumovirus (AMPV) infections, is a growing concern due to the potential for antibiotic resistance. Current study purposes to assess the prevalence of AmpC β-lactamase-producing *E. coli* isolates in broiler chickens in Duhok, a region in northern Iraq, and to evaluate the effectiveness of phenotypic screening methods for detecting these resistant strains. A total of 100 samples from clinically affected broiler chickens were collected and cultured for the isolation of *E. coli*. The phenotypic screening was achieved using three methods, acidimetric, ESBL and ESBL-*ampC*, were collected from September 2024 to March 2025. The results revealed that a significant proportion of *E. coli* isolates (68% acidimetric), (22% ESBL) and (38% ESBL-*ampC*) respectively were positive. The phenotypic tests showed varying sensitivity and specificity, with the combined disc test demonstrating the highest accuracy in identifying AmpC-producing strains. This study highlights the prevalence of AmpC β-lactamases in *E. coli* isolates from poultry infected with AMPV and emphasizes the need for regular surveillance and the application of effective antimicrobial programs to manage the spread of resistance in veterinary settings.**

***Keywords* — AMPV, *E. coli*, acidimetric, ESBL, ESBL-*ampC*.**

INTRODUCTION

Avian Metapneumovirus (AMPV) is a significant poultry virus that causes swollen head syndrome and acute, highly contagious upper respiratory tract infections in chickens. These conditions result in significant financial losses for the chicken sector in the majority of countries (1).Since the primary infection of the sickness was described, numerous bacteria for instance *E. coli* was isolated from progressive infection of aMPV (2), due to insufficient biosecurity measures and potential close contact with wild birds can resist many infection agents as well as a carrier for many microbial kinds and transmitting to many kinds of birds mainly poultry and unclear showing gross pathological changes and clinical symptoms of illness (3).

Avian colibacillosis is represented as important bacterial illness effecting poultry industry in any ages. *Escherichia coli* , is a member of the Enterobacteriaceae family, can infect mainly the intestine and many portions of the body in both animals and persons. Since the 2000s, the management of these infections has been difficult due to the advent of extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae that are resistant to third and fourth-generation cephalosporins (4). There was suggestion that *E. coli* initiating from infected animals or animal-by products containing zoonotic pathogens. The number of cases of ESBL-producing *E. coli* has significantly increased during the past two decades, posing an important threat to the general population (5,6).

In response to the worldwide spread of antibiotic-resistant bacteria, organizations including the The World Organisation for Animal Health, Food and Agriculture Organization, and World Health Organization have adopted the "One Health" plan. This technique involves joint efforts among ecological, animal welfare, and health departments to diminish the spread of antibiotic resistance (7). This study aims to determine the prevalence of AmpC β-lactamase–producing *E. coli* isolates recovered from broiler chickens suffering from Avian Metapneumovirus infection using phenotypic screening methods by evaluating the efficacy of these detection methods, the study contributes to the broader understanding of antimicrobial resistance patterns in poultry pathogens and the role of co-infections in exacerbating resistance dissemination.

MATERIALS AND METHODS

Sample Collection and Bacterial Detection

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 duration of the study from September 2024 to March 2025. 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 hours. The macroscopic appearance of bacterial colonies showed for *E. coli* identification (8,9).

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 depend on CLSI's recommend-dation. Then plates were incubated at 37 ℃ for 24 hour, finally reading of results by using of digital calibrate and inhibition zone were illustrated depended on CLSI guidelines (10).

Detection of β-Lactamase Enzymes by Acidimetric Method

The bacterial isolates were inoculated in to 0.1ml test tube containing acidimetric reagent to provide heavy bacterial suspensions. After that suspensions were left to settle and suspension stain (violet) altered to yellow was represented as positive, at room temperature (11).

ESBL Produce Detection

Overall, 100 positive *E.coli* isolates were examined utilizing of disk diffusion method, based on (10) recommendation. Through applying the 5 antibiotics β lactam -Cephalothin (KF30µg), Ceftriaxone (CRO10µg), Aztreonam (ATM30µg), Ceftazidim (CAZ30µg) and cefopodoxime (CPD10 µg) on Muller-Hinton agar, for 15 minutes then plates were incubated at 37 ℃ for 24 hour. Finally reading of results by using of electronic calibrator and inhibition zone were illustrated depended on CLSI guidelines, absence of inhibition zone around any antibiotic considered primary producers of extended spectrum β -Lactamase (10).

Phenotypic Screening Test

The disk diffusion method (Kirby-Bauer) was used for the cefoxitin sensitivity test. 100 positive *E. coli* isolates were screened through utilizing of cefoxitin (30µg) for detection of AmpC βlactamase. Isolates with a zone diameter of <18 mm to cefoxitin that were recorded as intermediate or resistant, as recommended by the CLSI standards, were selected for further processing by phenotypic assenting examining and were believed to be possible manufacturers of potential AmpC.

STATISTICAL ANALYSIS

Statistical analysis was performed using Chi square. The level of P-value < 0.0001was considered as a significant difference.

RESULT

Acidimetric Test

The result detected that the highest percentage of positive isolates (68%), suggesting high sensitivity or possibly low specificity.

ESBL Phenotypic Test

The result showed that the lowest number of positive isolates (22%), likely specific for extended-spectrum β-lactamase producers only.

ESBL + AmpC Phenotypic Test

The result represented as intermediate detection (38%). This is likely a more specific approach for detecting multiple resistance mechanisms. There is a highly significant difference ($p < 0.0001$) in the detection rates between the Acidimetric, ESBL, and ESBL-ampC methods. The results are shown in Table 1.

Table 1. The results of *E. coli* isolates, which produce the β -lactamases

Method	No. Positive (%)	No. Negative (%)	Total Tested	Chi square	(df)
Acidimetric	68 (68%)	32 (32%)	100	χ^2=44.32	df=2
ESBL	22 (22%)	78 (78%)	100		
ESBL-*ampC*	38 (38%)	62 (62%)	100		
highly significant difference among studied tests P-value < 0.0001					

DISCUSSION

The detection of AmpC β-lactamase–producing *Escherichia coli* in broiler chickens infected with Avian Metapneumovirus (AMPV) highlights a critical intersection between viral-induced immunosuppression and the rising challenge of antimicrobial resistance (AMR) in poultry production. This study's phenotypic screening revealed a significant prevalence of AmpC-producing *E. coli* in AMPV-infected chickens, suggesting that respiratory viral infections may facilitate the emergence or proliferation of resistant bacterial strains. Viral infections like AMPV can compromise the respiratory tract's defenses, creating an environment conducive to secondary bacterial colonization and potentially promoting horizontal gene transfer among bacteria. These findings emphasize the importance of integrated disease management strategies that address both viral infections and antibiotic resistance to mitigate economic losses and safeguard poultry health.

Infection of respiratory tract has a significant economic impact on poultry production worldwide. Various pathogens have been known to cause respiratory disease acting either in a primary or a secondary role. aMPV is a respiratory virus that infects a range of avian hosts including chickens and turkey. Swollen head syndrome is a disease of upper respiratory tract and is considered as one of these problems in last few years. Moreover, there are several studies showed that viral and bacterial factors are involved in occurrence of this condition. So, clinicians have used the term respiratory complex to describe this syndrome (12), Other respiratory viral diseases, such as Infectious Bronchitis (IB), have also been reported in Duhok, highlighting the presence of multiple respiratory pathogens circulating in poultry populations in the region (13). According to recent research, ESBL-producing *E. coli* have

been isolated from tracheal swab samples, suggesting that these bacteria may be distributed through the environment and inhaled by poultry, indicating atmospheric transmission within chicken farms (14).

The high prevalence of phenotypically confirmed AmpC-producing *E. coli* isolates in AMPV-infected flocks aligns with previous reports from poultry environments, although most earlier studies did not assess the influence of concurrent viral infections. The immunosuppress- sive effects of AMPV could facilitate increased colonization and proliferation of multidrug-resistant *E. coli* strains, thereby exacerbating clinical outcomes and complicating treatment protocols. A large number of microbes in the atmosphere are common in cage environments during the manufacturing of food (15,16). The quantity of microbes is strongly correlated with the cleanliness of the environment. A contaminated atmosphere can facilitate the transmission of ESBL-producing *E. coli* , these microorganisms can persist in the air for lengthy periods of time as aerosols and transfer by airflow. Previous research has demonstrated the widespread dissemination of ESBL-producing *E. coli* from (17).

Previous studies have revealed that distribute of ESBL-producing *E. coli* from the surrounding region (18). The increasing prescribing of β-lactam antibiotics in veterinary medicine has contributed to the rise of ESBL antibiotic-resistant bacteria in recent years (19).In a study in Egypt showed that detection of *E. coli* in about 70% of cases in broiler chickens ,this high prevalence suggests that *E. coli* implay a significant role as a secondary invader in cases involving primary viral infections, such as avian metapneumovirus (AMPV), which compromises the respiratory epithelium and predisposes birds to opportunistic bacterial infections (20).

CONCLUSION

The present study underscores the potential co-occurrence of *E. coli* infections producing AmpC β-lactamases in broiler chickens affected by Avian Metapneumovirus (AMPV) infection. While AMPV is primarily associated with respiratory illness and immunosuppression in poultry, our findings suggest that secondary bacterial infections, particularly by multidrug-resistant *E. coli*, may be more prevalent in such immunocompromised hosts. This emphasizes the need for routine surveillance using reliable molecular confirmation where possible. Furthermore, the interplay between viral infections like AMPV and bacterial resistance evolution warrants deeper investigation to guide responsible antimicrobial use and integrated disease management strategies in broiler farms.

Conflict of Interest

The authors declare there is no conflict of interest.

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