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O.O. NECHYPURENKO[1]*, L.V. AVDEEVA[1], D.V. DREVAL[2], I.O. SOBKO[2]

[1] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Akademika Zabolotnogo Str., Kyiv, 03143, Ukraine

[2] Centre of Veterinary Diagnostic,
15a Kaisarova Str., Kyiv, 03022, Ukraine

* Author for correspondence; e-mail: ne4upura@ukr.net

AVIAN PATHOGENIC *ESCHERICHIA COLI* AND ITS ANTIBIOTIC RESISTANCE

Avian pathogenic Escherichia coli (APEC) is a widely distributed pathogen and one of the main death causes in poultry farming. Also, these bacteria are harmful food-born pathogens with multiple virulence factors and pathogenicity that could be dangerous for humans. Moreover, APEC is characterized by a wide range of resistance to antimicrobials which successfully transmit to other microbes. Therefore, the ***aim*** *of the work was to define the frequency of APEC isolation in dead birds during 2015—2022, as well as to investigate its resistance to antibiotics.* ***Methods.*** *During the investigation, the routine autopsy was made on dead birds of different ages from broiler, layer, and broiler-breeder poultry farms in Ukraine. The type and severity of lesions were evaluated as characterized by 0 to 3 points. Then bacteriological examination was provided with further biochemical identification through Api20 E and APi20 NE tests. The susceptibility to antibiotics of identified E. coli was detected by the Kirby-Bauer disk diffusion method regarding CLSI data.* ***Results****. 1427 birds from 113 poultry farms were examined, and as a result, 1806 bacterial isolates were detected, among which 1183 were referred to APEC. The frequency of detection of APEC isolates in dead birds was 82.9%. Most often APEC-induced severe lesions (3 points) such as fibrinous perihepatitis, pericarditis, and peritonitis. The most effective antibiotics were colistin, gentamicin, ceftiofur, florfenicol, and norfloxacin, to which activity 91.6, 85.6, 63.9, 61.0, and 52.1% of isolated APEC strains, respectively, were susceptible, However, more than 50% of detected APEC were resistant to amoxicillin, amoxiclav, doxycycline, oxytetracycline, flumequine, and enrofloxacin. Moreover, 59.4% of isolated bacteria were multi-resistant and avoided negative impact of more than 6 antibiotics.* ***Conclusions****. Avian pathogenic E. coli was considered one of the leading bacteria agents in the poultry industry of Ukraine, because 82.9% of birds were infected with the systemic form of colibacillosis. The largest number of resistant isolates during 2015—2022 was detected to flumequine (78.5%), amoxicillin (78.4%), amoxiclav (69.3%), oxytetracycline (75.5%), and doxycycline (58.3%). The resistance to tetracyclines and quinolones increased dramatically through the years. It is therefore necessary to implement a new strategy for controlling APEC distribution based not only on rational antibiotic treatment but also on complex diagnostic and further immunization.*

Keywords: *avian pathogenic E. coli, antibiotic resistance, lesions.*

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Avian pathogenic *Escherichia coli* (APEC) is a causative agent of extraintestinal infections, known as colibacillosis, which results in significant losses in the poultry industry (Johar et al., 2021). For example, in Indonesia, the annual losses associated with this disease have been estimated near 1000 mil. dollars (Wibisono et al., 2018). APEC constitutes a large group of diverse serotypes, with the O1, O2, and O78 serogroups among the most prevalent globally (Alber et al., 2020). Avian colibacillosis is manifested in diverse ways, such as peritonitis, salpingitis, yolk sac infection, and cellulitis, which are localized infections while colisepticemia, pericarditis, aracialities, coligranuloma, and arthritis are systemic infections leading to high morbidity and mortality.

The pathogenic capability of *E. coli* is mainly due to multiple virulence factors. The presence of at least five or more of eight virulence-associated genes determines the role of APEC. These genes include iron acquisition genes (*iucD*, *iroN*), adhesion genes (*tsh*), toxin genes (*vat*, *hlyF*), serum resistance genes (*iss*), housekeeping proteases (*ompT*), and ColV operon genes (*cvi/cva*) (Johar et al., 2021).

Moreover, APEC is a heterogeneous group closely related to extraintestinal pathogenic *E. coli* (ExPEC), dangerous for humans, which is divided into uropathogenic *E. coli*, newborn meningitis *E. coli*, and septicemia-associated *E. coli* (Joseph et al., 2023). The whole-genome sequencing of *E. coli* strains indicated that the genomic level of human ExPEC strains is clustered with avian isolates. Some *E. coli* strains can acquire a combination of mobile genetic elements via a horizontal exchange to become a highly adapted pathogen capable of survival and causing a range of diseases in humans and animals. Thus, the APEC might be a reservoir of virulence genes and antibiotic-resistant genes for human ExPEC strains with considering the zoonotic potential (Hu et al., 2022).

The epidemiological situation regarding the spread of APEC in Ukraine is currently unresearched, and monitoring of resistance to antimicrobial agents is not provided. Thus, research concerns in preventing the spread of antibiotic-resistant strains of APEC among chickens, as well as in the general population of people who have direct contact with sick birds or infected meat products, is very relevant nowadays. Hence, it is necessary to monitor the spread of APEC (determining the specific impact of these bacteria on the general structure of birds' disease) and identify the levels of sensitivity to antibiotics. Therefore, the aim of the work was to define the frequency of APEC isolation in dead birds during 2015—2022, as well as to investigate its resistance to antibiotics.

Materials and Methods. The objects of the study were isolates of *E. coli* obtained from dead birds of different ages (1 to 600 days old) from 213 industrial poultry farms (broilers, layers, broiler-breeders). The research was conducted in 2015—2022 years. The analysis of the pathological condition of the animals selected for research was carried out in the laboratory of pathological anatomy of the Centre of Veterinary Diagnostics using standard autopsy techniques. Autopsy was performed and described, and pathological lesions of organs were photographed (Swyane et al., 2013). The degree of lesions was evaluated from 0 to 3 points: 0 — absent, 1 — mild, 2 — moderate, and 3 — severe based on the presence of fibrin deposition, hemorrhages, and inflammation in internal organs (Hussein et al., 2018).

In the case of fibrinous pericarditis, perihepatitis, peritonitis, ovaritis, omphalitis, and spleen enlargement, pathological material was selected for further bacteriological investigation. Bacterial isolation was made in aseptic conditions from the inner content of the liver, heart, spleen, or yolk of affected birds to tryptone-soy broth (TSB, Himedia, India) and chocolate agar (Himedia, India), considering clinical signs and then the isolates were cultivated at 37 °C for 18—24 hours. After that bacteria from TSB were isolated to MacConkey and mannitol salt medium (Himedia, India) for further pure culture isolation and identification.

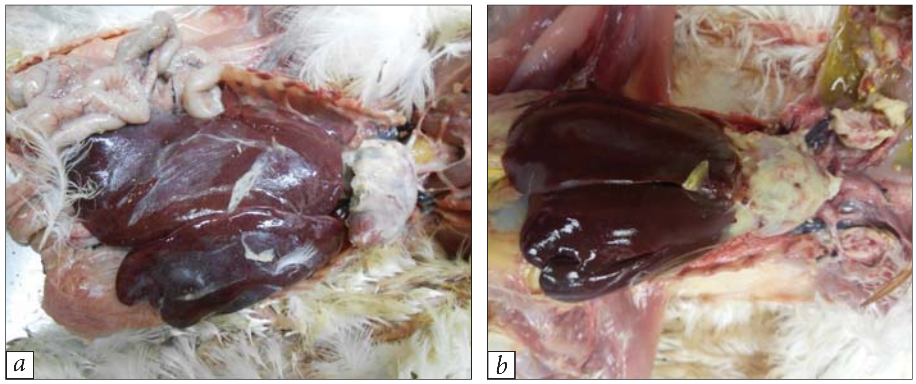

Fig. 1. Lesions associated with colibacillosis in birds: *a* — fibrinous pericarditis and perihepatitis; *b* — fibrinous airsacculitis and pericarditis

The bacterial cultures were referred to pathogenic and identified if they had been isolated from heart, spleen or from more than one internal organ (Afayibo et al., 2022).

Identification of obtained isolates on MacConkey medium was provided with the APi 20 test-system *Enterobacteriaceae* (BioMe'rieux, France), which helps analyze such biochemical features as the ability to synthesize β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, deaminase, and gelatin hydrolysis and the ability to form hydrogen sulfide, indole, acetone, ferment glucose, mannitol, sorbitol, inositol, rhamnose, sucrose, melibiose, amygdalin, and arabinose. To identify non-enterobacteria species, the APi 20 NE and APi 20 NH test systems were used, following the standard protocol (Maina et al., 2014).

The susceptibility of pathogenic *E. coli* isolates to antimicrobials was determined by the Kirby—Bauer disk-diffusion method on plates with Muller-Hinton agar (Himedia, India) (Fritsche et al., 2007). The following antimicrobial compounds (Oxoid, Holland) were used, μg/disk: aminoglycosides — spectinomycin (SPE, 25), gentamycin (GEN, 10); β-lactams — amoxicillin (AMO, 10), amoxiclav (AMC, 30); tetracyclines — doxycycline (DOX, 30), oxytetracycline (OXY, 30); polypeptide — colistin (COL, 10); phenicol's — florfenicol (FLO, 30); cephalosporins — ceftiofur (30); quinolones — enrofloxacin (ENR, 10), flumequine (FLU, 10), norfloxacin (NOR, 10), ciprofloxacin (CIP, 5), and diaminopyrimidines — trimethoprim (TRI, 25) (Kohanski, et al., 2010). Norfloxacin and ciprofloxacin were added to the list of tested antimicrobials after 2016. Isolated multiresistant pathogenic *E. coli* cultures were stored on a semi-solid thioglycolate medium at 2 °C for further study.

Data entry, initial analysis, and figure design were performed using Microsoft Office Excel 2010 (Microsoft Corporation, New York, NY, USA) to generate figures and run initial analysis. The chi-square test was calculated using Pearson's probability value (*p*-value) to compare the number of isolates resistant to aminoglycosides, β-lactams, tetracyclines, polypeptides, phenicol's, cephalosporins, quinolones, and diaminopyrimidines between different years. A *p*-value less than 0.05 was considered statistically significant (Johar et al., 2021).

Results. Since every infection process induces certain specific signs and particular lesions of organs, we conducted a planned autopsy of 1427 dead birds of different ages and types from 113 poultry farms in 21 regions of Ukraine except Crimea, Donetsk, and Lugansk regions for bacteriological confirmation of APEC affection. It was determined that the most common lesions were associated with pericarditis and perihepatitis in 81.1% and 70.6% of cases, respectively (Fig. 1).

However, in 75.6% of 1-day-old chicks, the absence of omphalitis or lesions was detected. Also, in 45% of investigated productive layers, ovaritis and peritonitis were identified, which means the

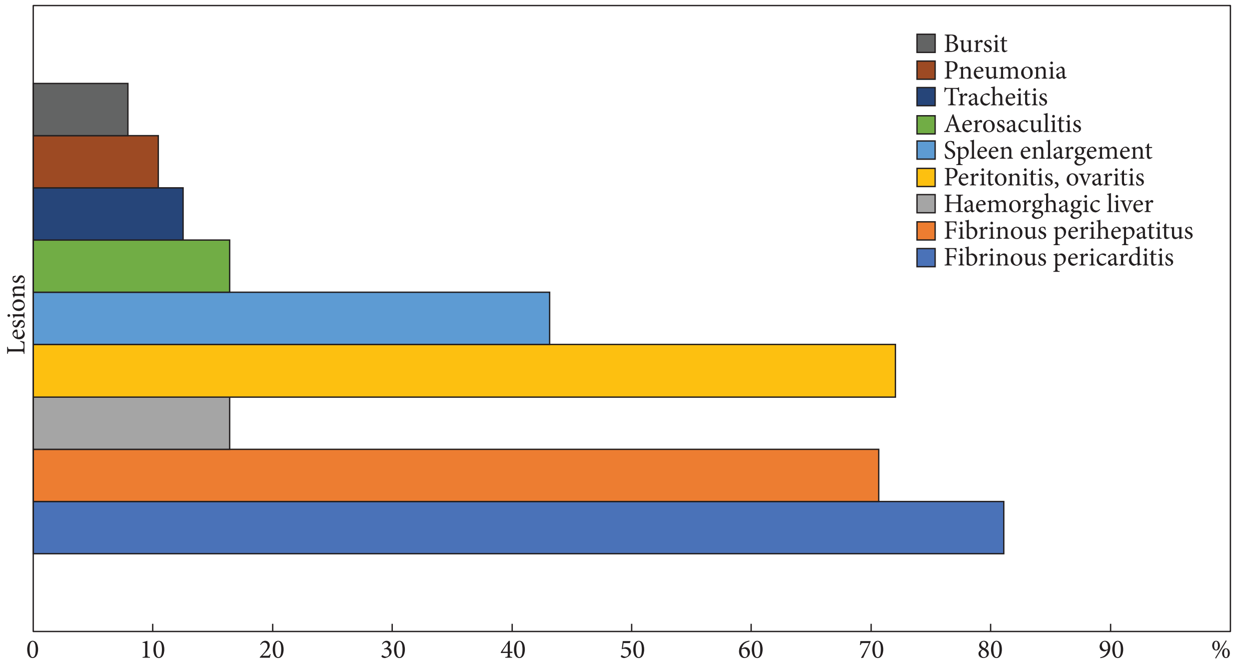

Fig. 2. The frequency of main lesions detected in investigated birds

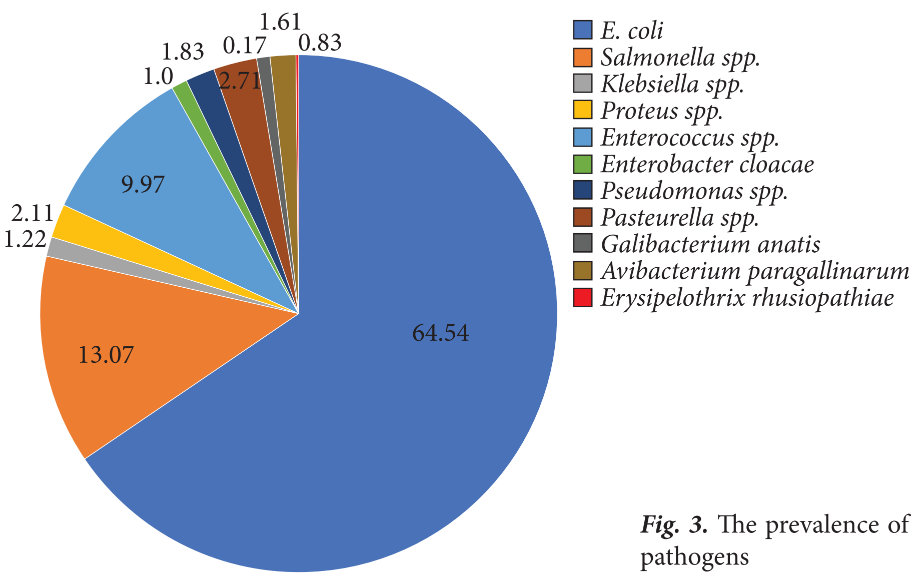

Fig. 3. The prevalence of APEC among other bacterial pathogens

affection of reproductive and digestive systems by probable enterobacteria.

Nevertheless, visual signs of bacterial pathogens among which were also pneumonia (10.4%) and aerosaculitis (16.4%). The signs of viral infection of the immune and respiratory systems, such as hemorrhagic bursitis (7.9%) and hemorrhagic tracheitis (12.5%), respectively, were seen as well. Viral infection could negatively affect the work of the immune system and provoke the development of a secondary bacterial infection, which may ultimately kill the bird (Fig. 2).

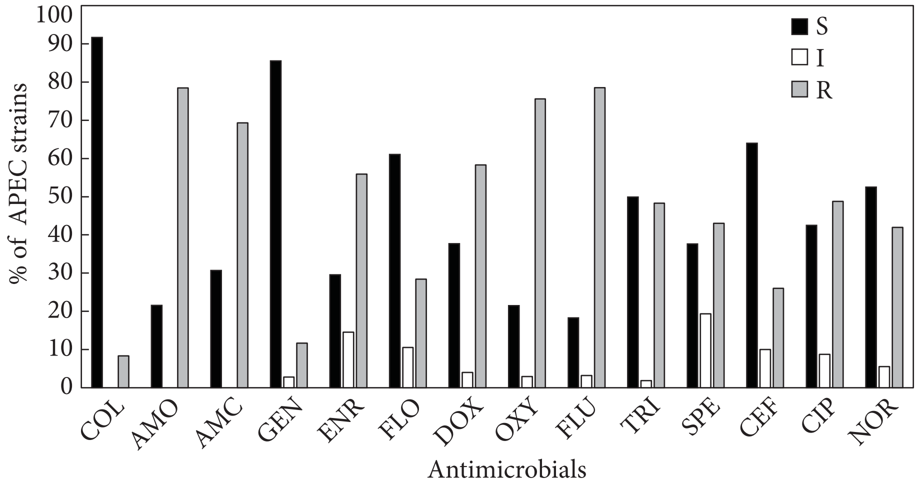

Fig. 4. The number (%) of susceptible (S), intermediate (I), and resistant (R) isolates of APEC isolated in 2015—2022: COL — colistin, AMO — amoxicillin, AMC — amoxiclav, GEN — gentamicin, ENR — enrofloxacin, FLO — florfenicol, DOX — doxycycline, OXY — oxytetracycline, FLU — flumequine, TRI — trimethoprim, SPE — spectinomycin, CEF — ceftiofur, CIP — ciprofloxacin, NOR — norfloxacin

The spleen enlargement (43.2%) and edematous lesions can be associated with the reaction of the immune system to the pathogen presence and the development of the systemic form of colibacillosis (Wang et al., 2023).

In general, the severity of lesions varies from 0 to 3 points, which can be explained by different stages of infection and the influence of additional factors such as immune suppression, timeline of the disease, coinfection, and other possible causes. In 77% of cases, the degree of lesions in internal organs was more than 2 points, which indicates tissue dysfunction and inability to recover.

After the autopsy, we provided routine bacteriology of birds with specific lesions. 1806 isolates of pathogenic bacteria were detected, among which 65.5% were identified as avian pathogenic *E. coli* (Fig. 3). We observed that 82.9% of birds were infected with APEC, which was identified in 65.6% and 17.3% of cases with mono-infection or bacteria co-infection, respectively. Also, other most often isolated bacteria were referred to *Salmonella* spp. (13.1%) and *Enterococcus* spp. (9.9%). It should be noted that in 2.3% of birds' pathology, lesions were absent, but APEC was isolated from internal organs, which indicates the presence of the subacute systemic form of colibacillosis.

Thus, APEC is widely distributed in Ukraine and plays a crucial role in the pathological process of poultry farming as one of the main causes of chicken death.

Nowadays in the veterinary field, more and more antibiotics are used irrationally from the medicine sphere, which provokes the appearance of multiresistant bacteria harmful even to humans (Palma et al, 2020). Therefore, the second part of our work was dedicated to the identification of antibiotic susceptibility of all isolated APEC cultures and the tendency of resistance development in 2015—2022 years.

It was found that the highest number of isolated APEC was susceptible to colistin (91.6%), gentamicin (85.6%), ceftiofur (63.9%), and florfenicol (61.0%). However, such antimicrobials as amoxicillin, amoxiclav, oxytetracycline, doxycycline, enrofloxacin, ciprofloxacin, and flumequine were ineffective to more than 50% of isolated APEC. This indicates the high potential of these bacteria for resistance development and adaptation in the technogenic environment (Fig. 4). Also, about

59.4% of APEC isolates were resistant to more than 6 different antibiotics simultaneously, which features their multi-resistance. Moreover, during 8 years of research, we identified 1.4% (n=17) of *E. coli* isolates without any susceptibility.

The largest number of intermediate isolates were detected as resistant to enrofloxacin (14.5%) and spectinomycin (19.4%). That indicates a possible decreasing rate of susceptibility to mentioned antimicrobials.

Another means that can help in understanding the prevalence of antibiotic- resistant APEC is an analysis of their profiles, which are a combination of antibiotic resistance determinants. In 2022, we detected 39 (40.2%) isolates that were resistant simultaneously to more than 10 antibiotics from the polypeptide, aminoglycoside, tetracycline, beta lactams, quinolones, cephalosporin, and diaminopyrimidine groups (Table 1), although in 2015 there were only 16 isolates with mentioned resistant profile. This indicates the process of bacteria evolution. Concerning these findings, the treatment of such adopted pathogens with standard compounds would be ineffective. Moreover, 59.4% of all isolated avian pathogenic *E. coli* were multi-resistant and avoided the negative impact of more than 6 antibiotics, which could be linked to the presence of ColV plasmid.

It was found that resistance to oxytetracycline, doxycycline, and flumequine could be linked together because of its presence in about 34% of isolated APEC. The same tendency was observed concerning amoxicillin and amoxiclav.

Regarding susceptibility to polypeptide antibiotics, such as colistin, the periodical variation in the resistance to it was detected during 2015—2022. The number of resistant isolates was decreased in 2017—2018 to 4% (n=12), but in 2021—2022 grew to 9.3% (n=25). This number did not change significantly comparing to the obtained data in 2015—2016 years.

It should be noted that beta lactams antibiotics were rather ineffective. For example, 78.4% (n=928) and 69.3% (n=820) of the isolates were

Table 1. **The antibiotic profile of multi-resistant *E. coli* isolates in 2022**

Antibiotic profile	N
GEN, AMO, AMC, ENR, CIP, NOR, DOX, OXY, FLU, CEF	1
SPE, AMO, AMC, ENR, CIP, NOR, TRI ,DOX, OXY, FLU	7
SPE, AMO, AMC, ENR, CIP, TRI ,DOX, OXY, FLU, FLO	2
SPE, AMO, AMC, ENR, CIP, NOR, FLO, DOX, OXY, FLU	2
COL, SPE, AMO, AMC, DOX, OXY, FLU, ENR, NOR, CIP	2
SPE, AMO, AMC, ENR, CIP, NOR, FLU, TRI, OXY , CEF	1
AMO, AMC, ENR, CIP, NOR, TRI, DOX, OXY, FLU, CEF	4
GEN, AMO, AMC, ENR, CIP, NOR, DOX, OXY, FLU, FLO	1
AMO, AMC, ENR, CIP, NOR, FLU, FLO, DOX, OXY, TRI	3
SPE, AMO, AMC, ENR, CIP, NOR, FLU, DOX, OXY, TRI, CEF	2
SPE, AMO, AMC, ENR, CIP, NOR, TRI, FLO, OXY, FLU, DOX	8
GEN, SPE, AMO, AMC, ENR, CIP, NOR, TRI, FLO, OXY, FLU	3
GEN, SPE, AMO, AMC, ENR, CIP, NOR, TRI, FLO, OXY, FLU, DOX	2
COL, GEN, SPE, AMO, AMC, ENR, CIP, NOR, TRI, DOX, OXY, FLU, FLO	1

N — number of isolates; titles of antibiotics are abbreviated as given in Fig. 4

characterized by resistance to amoxicillin and amoxiclav, respectively. Moreover, the number of APEC isolates resistant to amoxiclav increased during 2019—2022 to the maximum quantity of 88.7% in 2022.

Also, the group of tetracyclines characterized by low level of susceptibility was detected in 75.6% (n=894) and 58.3% (n=690) of isolates resistant to oxytetracycline and doxycycline, respectively. Over the last 6 years of investigation, we observed the statistically significant growth of the number of doxycycline-resistant cultures, which was described by the equation y = 4.7787x + 42.456 (Fig. 5).

Among aminoglycosides, the most effective of them was gentamycin: about 85.5% (n = 1012), 2.8% (n = 33), and 11.7% (n = 138) of isolated *E. coli* cultures were susceptible, intermediate, and resistant, respectively. However, the difference between the start and end points of the investigated period was not significant. It should be noted that another antimicrobial compound from aminoglycosides, namely spectinomycin, is characterized by a high number of intermediate strains (n=229, 19.4%). That indicates the possibility of rapid development of APEC's resistance of to this antibiotic, which was shown through the years (Fig. 6).

Florfenicol's mechanism of action is directed at disrupting bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome and is generally considered bacteriostatic (Somogyi et al., 2023). Despite this fact, we isolated only 28.4% (n = 336) and 10.6% (n = 125) of resistant and intermediate APEC isolates. However, the number of these isolates did not increase significantly during the investigation period.

Ceftiofur is a third-generation cephalosporin antibiotic that is widely used in the veterinary field (Hornish et al., 2002). However, we isolated 26.0% (n = 308) and 10% (n = 118) of avian pathogenic *E. coli* isolates that were resistant and intermediate to ceftiofur, respectively. Despite its wide usage, we did not detect any growth in the resistance rate to this antibiotic in 2015—2022.

The next group of antibiotics tested belongs to

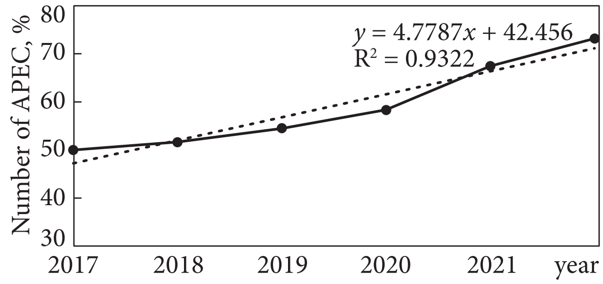

Fig. 5. The tendency of APEC's resistance to doxycycline in 2017—2022 (p<0.01)

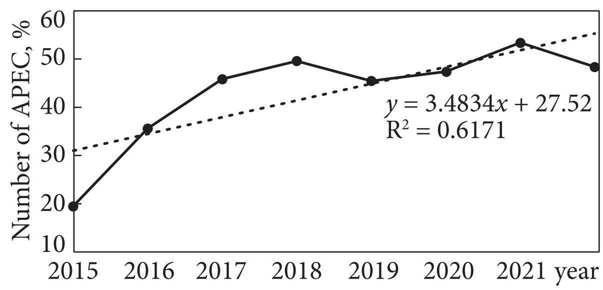

Fig. 6. The tendency of APEC's resistance to spectinomycin in 2015—2022 (p<0.01)

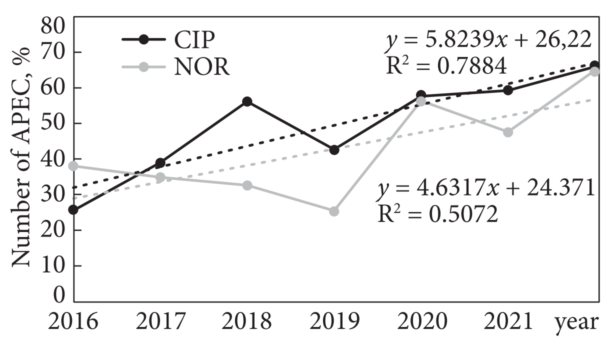

Fig. 7. The tendency of APEC's resistance to ciprofloxacin and norfloxacin in 2015—2022

fluoroquinolones. Among APEC, 18.3%, 29.6%, 42.3%, and 5.5% of isolates were susceptible to flumequine, enrofloxacin, ciprofloxacin, and norfloxacin, respectively. The highest rate of intermediate bacteria was observed for enrofloxacin (14.5%). However, isolated pathogenic *E. coli* was characterized by the highest resistance rate to flumequine (78.5%). There was observed positive dynamics in resistance development to ciprofloxacin, norfloxacin, and enrofloxacin. For example, the number of APEC isolates that were not vulnerable to ciprofloxacin increased from 25.6% (n=41) to 66.0% (n=66) in 2016 and 2022. A similar tendency was observed for norfloxacin (Fig. 7).

Another group of antimicrobials that were used in the research included diaminopyrimidines. Despite the wide-range activity of trimethoprim, about 48.3% of *E. coli* isolates were not killed by its action. Moreover, there were about 1.9% intermediate isolates, which could serve as the source of resistance in the near future. However, during 8 years of investigation, we did not find a significant difference in the susceptibility of isolated APEC to trimethoprim, to which were resistant 47.4% (n = 73) and 56.7% (n = 55) in 2015 and 2022 years, respectively.

Discussion. The poultry industry is one of the growing fields of the agricultural sphere not only in Ukraine but all over the world. However, there are a lot of viral and bacterial infections that negatively influence the productive parameters of broiler, layer, breeder, and turkey husbandry. Among all pathogens, *E. coli* plays a crucial role in poultry farming despite its commensal nature. Avian pathogenic *E. coli* is widely distributed in Europe, Asia, America, Africa, and even in Australia. There is no connection between these bacteria distribution and climate zones (Swayne et al., 2013). Taken together, along with the treatment expenses, APEC costs the poultry industry hundreds of millions of dollars. For example, in the United States, as estimated, economic losses can be as high as $40 million annually only due to carcass condemnation (Kathayat et al., 2021). APEC often serves as a secondary pathogen, but at the same time, *E. coli* could be the main cause of birds' death due to the harboring of many virulence factors. Moreover, they are the source of resistance genes and can be dangerous even for humans (Delago et al, 2023).

During eight years of our research, we identified avian pathogenic *E. coli* in 82.9% of birds, which shows a great role of colibacillosis in poultry pathology. Besides, the frequency of APEC among other isolated bacteria such as *Salmonella* spp., *Enterococcus* spp., *Proteus* spp., *Klebsiella* spp., *Pasteurella* spp., and others was 65.5%. That indicates the high prevalence and variety of APEC in Ukraine.

Regarding the results of recently written scientific works, APEC is widely distributed in Spain, the United Kingdom, Italy, Germany, China, India, and other countries despite the improved vaccination strategies against viral diseases and high biosecurity measures (Afayibo et al., 2022; Ha et al., 2023; Gordoni et al., 2016; Ginés et al., 2015; Krishnegowda et al., 2022). These data coincide with ours and showthe necessity of finding new ways to control colibacillosis (Christensen et al., 2020).

Coinfection of APEC with other bacterial agents was reported in 17.3% of cases, mostly with *Salmonella* spp. and *Enterococcus* spp., which increased the mortality rate of affected birds. This corresponds to the results obtained by Walker G. et al., who have shown that *Enterococcus faecalis* augmented APEC survival and growth under iron-limiting conditions, possibly translating to the increased virulence of APEC in broiler embryos (Walker et al., 2020). The presence of coinfection with viral agents was not studied. However, after autopsy results, we detected that secondary bacterial infection was the main cause of chicken death due to heart failure or respiratory disorder.

The pathogenicity of *E. coli* depends on many virulence factors, such as the presence of hemolysins, enterotoxins, adhesins, invasins, protectins, iron acquisition systems, type 3 and 4 secretion systems, and other effectors molecules (Kathayat et al., 2021). As known, APEC strains differ from swine pathogenic *E. coli* and refer to extraintestinal ExPEC. Due to the similarity between APEC and human ExPEC, such as uropathogenic *E. coli* and neonatal meningitis *E. coli*, the zoonotic risks of APEC need to be addressed (Ha et al., 2023).

In our research, we did not identify virulence factors but detected pathogenic properties of *E. coli* regarding organ invasion. It should be noted that all *E. coli* strains were isolated from parenchymal organs or the heart, which indicates a systemic form of the disease and they belong to the APEC group. These data correlate with Kathayat D. et al.'s, who have identified a high prevalence of systemic colibacillosis in poultry in contrast with swine farming (Kathayat et al., 2021). The difference was explained by the upregulation of genes involved in metabolism, cell envelope and integrity, transport systems, and virulence (*metH, lysA, pntA, purL, serS, ybjE, ycdK (rutC), wcaJ, gspL, sdsR, irp2, eitD, ylbE, yjiY, tkt1*, and phage-related genes) (Dozois et.al., 2003). Moreover, genes involved in adherence (*pilN, pilQ, tsh, hpb, TcfD, Z5222*), LPS synthesis (*waaO, waaY*), iron acquisition (*iutA, iucA, iucD, iroC*), plasmid function (*ColE2, traK, traG, traT, SopA, psiA*), phage-related (*hkaG, hkbV, hkbQ, Z3370, Int*), and unknown functions (*CC0532, TM0427, YPO3000, rhsH, RSp0733*) were highly expressed in APEC-infected tissues (Zhang et al., 2019).

As well as swine pathogenic *E. coli*, APEC was able to produce putrescine and cadaverine due to the function of ornithine and lysine decarboxylase enzymes, respectively. The ability to produce the mentioned biogenic amines by *E. coli* was also reported in the work of Nurten Yilmaz (Nurten et al., 2022). Furthermore, putrescine and cadaverine can react with nitrites to form carcinogenic nitrosamines and cause dilatation of peripheral blood vessels, capillaries, and arteries, thus resulting in hypotension, flushing, and systemic disease development (Kamil et al., 2020).

Regarding pathological lesions that we visualized, the main changes were observed in the liver and heart of infected birds and were associated with fibrinous deposition such as pericarditis and perihepatitis. Although omphalitis and brown yolk sack were the most prevalent abnormalities in one-day-old chicks. Therefore, avian colibacillosis is not an age-dependent disease, which was reported by Fancher A. (Fancher et al., 2022). Other remarked lesions such as tracheitis and bursitis could be associated with respiratory viruses and infectious bursal disease viruses, respectively (Swayne et al., 2013).

Antibiotic resistance development is one of the irreversibly occurring evolutional processes during the bacterial life. Nevertheless, in conditions of poultry farming intensification, it is impossible to avoid antibiotic usage, which almost always improves the results of the resistance pattern. Nowadays, many antibiotics from human medicine are widely used in the veterinary sphere, which is dangerous and fraught with the appearance of superbugs worldwide (Castro et al., 2022). Moreover, the situation with susceptibility to antibiotics in Ukraine is rather incomprehensive, the actual data are absent, and antimicrobials are used not only as a growth promotor in feed but during different periods of birds' life for prophylaxis treatment.

After the research on APEC susceptibility to antibiotics, it was found that colistin is characterized by the lowest number of resistant isolates (8.4%). However, number is bigger than that in Senegal, Sweden, Denmark, France, USA, and other countries where less than 8.0% of resistant isolates have been found (Joseph et al., 2023). Moreover, all colistin-resistant isolates exhibited multidrug-resistant phenotypes. It should be noted that there was no significant difference in theincrease in colistin resistance of isolates throughout the research period. Regarding Hu J. et al., the resistance to colistin is due to the plasmid-encoded *mcr-1* gene (Hu et al., 2022). It is responsible for the expression of a phosphoethanolamine transferase that catalyzes the addition of a phosphoethanolamine moiety to lipid A, which may modify its structure and change membrane permeability (Li et al., 2020). However, even with such a low resistance rate, it is impossible to treat the systemic form of colibacillosis because the intestine does not absorb colistin. Also, this antibiotic is characterized by a high level of kidney toxicity.

The highest levels of resistant isolates were detected to amoxicillin (78.4%), amoxiclav (69.3%), doxycycline (58.3%), oxytetracycline (75.6%), flumequine (78.5%), and enrofloxacin (55.9%) Also, we observed an increase in the resistance to amoxicillin, spectinomycin, and doxycycline in 2015—2022.

We established that the frequency of APEC isolates non-vulnerable to amoxiclav increased from 42.8% to 88.7% during the last four years. The same tendency was detected concerning amoxicillin. However, the lower rate of resistance to amoxiclav compared to amoxicillin can be explained by the hyperproduction of chromosome-encoded class C β-lactamases, acquired plasmid-encoded cephalosporinases (AmpC type), and plasmid-mediated class A β-lactamases (such as TEM-1 and SHV-1 enzymes), the production of class D oxacillinases, inhibitor-resistant TEM (IRTs or CMTs), and SHV mutants (whose β-lactamase activities are poorly inhibited by clavulanate) (Gonza et al., 2014).

The frequency of isolation-resistant cultures to doxycycline increased by1.4 times in 2022 compared to 2015. The data on tetracycline resistance also correlated with antibiotics susceptibility survey in Portugal, France, and USA, which shows a great level of resistance among avian pathogenic *E. coli* (Hu et al., 2022; Fancher et al., 2021). The high level of resistance to tetracyclines in different parts of the world probably developed due to the acquisition of *tetA, tetB* genes and cross-resistance with zinc, a feed growth promotor, which uses the same efflux system for antibiotic inactivation (Castro et al., 2022).

Regarding our results for fluoroquinolones, they became less effective through the uncontrolled usage in the veterinary field, for example, only 29.6% and 18.3% of avian pathogenic *E. coli* isolates were susceptible to enrofloxacin and flumequine. Moreover, the new generation of fluoroquinolones, namely norfloxacin and ciprofloxacin were effective only against 42.5% and 52.8% of isolated APEC. The obtained data correspond to the epidemiology survey of fluoroquinolones resistance in the USA and China, where the number of nonvulnerable strains varied from 19% to 90% (Temmerman et al., 2022). Such a low antibiotic activity can be explained by the application of different fluoroquinolones during the first five days of the chick's life with further repetition. In practice, when mortality occurs, a metaphylactic approach is applied, by which all birds in the poultry houses are treated (Mouiche et al., 2022). Despite the similarity of fluoroquinolone structures, we observed a huge difference in the activity rate and increment of resistance frequency**.** For example, this parameter evolved to ciprofloxacin by 2.5 times from 2015 to 2022. The different rates in resistance among fluoroquinolones could be caused by mutations in genes encoding for DNA gyrase (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC* and *parE*). These enzymes play a crucial role in the remodeling of DNA topology and replication. The regions of DNA sequences where these target-site mutations rise are termed «quinolone resistance determining regions» (QRDR), which can decrease the affinity of the mutant enzyme-DNA complex to fluoroquinolones (Temmerman et al., 2022).

Such aminoglycosides as gentamicin are characterized by lower resistance rate as compared to spectinomycin, which was not active to 43.0% of isolated *E. coli.* Also, 19.4% of investigated bacteria were intermediate to spectinomycin, which might have changed into resistant ones. Moreover, Chalmers et al. detected that the use of spectinomycin-lincomycin may be likely selected for gentamicin resistance through the expression of *aadA* and *aac(3)-VI* genes (Chalmers et al., 2017). Remarked aminoglycosides couldn't be absorbed from the intestine to the blood system, which dramatically reduces their effectivity during systemic colibacillosis. The identified level of antidiotic resistance in Ukraine was much higher than in Australia and the USA, countries with developed poultry farming (Joseph et al., 2023).

Trimethoprim is an aminopyrimidine antibiotic that has a wide range of activity but considering our results — 48.3% (n = 571) of APEC isolates were resistant to this antimicrobial. A similar tendency was observed in Vietnam, Thailand, China, Portugal, Nepal, and Spain where about 50% of investigated strains were non-vulnerable. Moreover, the worldwide resistance rate of *E. coli* to trimethoprim increased every year, which makes it impossible to treat uropathogenic infection (Afayibo et al., 2022; Hu et al., 2022; Sevilla-Navarro et al., 2022; Bhattarai et. al., 2024).

The last two groups of antibiotics, such as cephalosporins and phenicols, are characterized by higher effectivity compared to the abovementioned substances, and the resistance level to them was 26.0% (n = 308) and 28.4% (n = 336), respectively. But only florfenicol may be used orally during the systemic form of colibacillosis and ceftiofur and is even forbidden for veterinary use in some countries (Chalmers et al., 2017). However, its wide application in veterinary could increase the resistance rate to cephalosporins and penicillins in medicine, which may be linked with *acrAB* efflux pumps and enzymatic degradation (Kerek et al., 2023). Our data partly confirmed that amoxicillin and amoxiclav were ineffective against florfenicol-resistant *E. coli* isolates.

It should be noted that the majority of multiresistant isolates of *E. coli* were detected in Kyiv, Lviv, and Cherkasy regions with the highest concentration of poultry farms. The long use of different antimicrobials in these regions and the high density of animal husbandry can play a crucial role in selective pressure, virulence genes acquisition, and the appearance of multi-resistance.

Therefore, avian pathogenic *E. coli* is the most often isolated bacteria and the main cause of bird death as a primary or secondary pathogen. An accurate diagnosis of APEC needs an appropriate sampling for the isolation, quantification, and antimicrobial survey. Definitive diagnosis is based on the presence of clinical signs, characteristic lesions, and results of bacteriology along with confirmation of appropriate virulence factors. A new strategy for controlling APEC, including control of antibiotic-resistant APEC, has to focus on limiting vertical transfer from parents to offspring and subsequent horizontal transmission within and between flocks and farms, by using all-in-all-out production systems and implementing a high level of biosecurity. Vaccination and the use of competitive exclusion are important tools to be considered. Strategies to reduce APEC, including antibiotic-resistant APEC, need to be implemented in the whole production pyramid (Pokharel et al., 2023).

Conclusions. Avian pathogenic *E. coli* was considered one of the leading bacteria agents in the poultry industry of Ukraine because 82.9% of birds were infected with a systemic form of colibacillosis. In 2015—2022, the largest number of resistant isolates was detected to flumequine (78.5%), amoxicillin (78.4%), amoxiclav (69.3%), oxytetracycline (75.5%), and doxycycline (58.3%). The resistance to tetracyclines and quinolones increased dramatically through the years. Therefore, it is necessary to implement a new strategy for controlling APEC distribution which could be based not only on rational antibiotic treatment but also on complex diagnostics and further immunization with concurrent specific tools.

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О.О. Нечипуренко[1], Л.В. Авдєєва[1], Д.В. Древаль[2], І.О. Собко[2]*

[1] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143, Україна
[2] ТОВ «Центр Ветеринарної Діагностики»,
вул. Кайсарова, 15а, Київ, 03022, Україна

ПАТОГЕННІ ДЛЯ ПТАХІВ *ESCHERICHIA COLI* ТА ЇХНЯ РЕЗИСТЕНТНІСТЬ ДО АНТИБІОТИКІВ

Патогенні для птахів *Escherichia coli* (APEC) є розповсюдженими бактеріями і однією з основних причин загибелі птахів у птахівництві. Крім того, ці бактерії є шкідливими харчовими патогенами з кількома факторами вірулентності, що може бути небезпечним і для людини. Також APEC характеризується наявністю факторів резистентності до антимікробних препаратів, які успішно передаються іншим патогенам. Тому **метою** роботи було встановити частоту виділення APEC у загиблих птахів протягом 2015—2022 років, а також дослідити їхню резистентність до антибіотиків. **Методи**. Під час дослідження проведено плановий розтин загиблої птиці різного віку з бройлерних, яєчних і племінних птахофабрик України. Оцінено тип і тяжкість уражень від 0 до 3 балів. Потім проведено бактеріологічне дослідження з подальшою біохімічною ідентифікацією за допомогою тестів Api20 E та APi20 NE. Чутливість ідентифікованих ізолятів *E. coli* до антибіотиків визначали диско-дифузійним методом за Kirby-Bauer відповідно до CLSI. **Результати**. Досліджено 1427 голів птиці з 113 птахофабрик України, з яких виділено 1806 ізолятів бактерій, серед них 1183 віднесено до APEC. Частота виявлення APEC у загиблих птахів становила 82,9 %. Найчастіше патогенні *E. coli* спричиняли значні ураження (3 бали), такі як фібринозний перигепатит, перикардит і перитоніт. Найефективнішими антибіотиками були колістин, гентаміцин, цефтіофур, флорфенікол і норфлоксацин, до яких були чутливі відповідно 91,6, 85,6, 63,9, 61,0 і 52,1 % виділених ізолятів APEC. Проте більше 50 % виявлених штамів APEC були резистентними до амоксициліну, амоксиклаву, доксицикліну, окситетрацикліну, флумеквіну та енрофлоксацину. Крім того, 59,4 % ізольованих бактерій виявились полірезистентними та уникали негативної дії більше, ніж до 6 антибіотиків. **Висновки**. Патогенні для птахів *Escherichia coli* віднесено до одного з провідних збудників бактеріальної інфекції у птахівництві України, оскільки 82,9 % досліджених птахів були інфіковані системним колібактеріозом. Найбільшу кількість резистентних ізолятів протягом 2015—2022 рр. виявлено до флумеквіну (78,5 %), амоксициліну (78,4 %), амоксиклаву (69,3 %), окситетрацикліну (75,5 %) та доксицикліну (58,3 %). Стійкість до тетрациклінів і хінолонів різко зросла з роками, тому необхідно запровадити нову стратегію контролю розповсюдження APEC, яка базувалася б не лише на раціональному лікуванні антибіотиками, а й на комплексній діагностиці та подальшій імунізації.

Ключові слова: *патогенні для птахів E. coli, антибіотикорезистентність, патологічні зміни.*