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Avian Pathogenic *Escherichia coli* Resistance Patterns According to Poultry Species in Albania, a Two Years Study

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Abstract

Avian pathogenic *Escherichia coli* (APEC) strains cause colibacillosis, an acute and systemic poultry disease. Poultry infections with *E. coli* result in substantial economic losses in the poultry sector worldwide, by reducing levels of meat and egg production. To investigate resistance patterns of avian *Eschericia coli,* 77 tissue samples were collected from 6 poultry different species found-dead in Albania, during the years 2021 and 2022. This present study co-examines colibacillosis manifestation in different poultry species regarding the prevalence of the multidrug resistant patterns in such species. A total of 62 (93, 50%) *E. coli* strains were isolated according to their species as (19, 35%) in layers, (35, 49%) in broilers, (19, 35%) turkeys, (16, 13%) backyard poultry, (4, 84%) ducks and (4, 84%) pigeons. Multidrug resistance (MDR) was encountered in 100 % of the *E. coli* isolates, with an absolute treatment resistance in ducks and pigeon's species. Absolute drug-resistance towards Oxytetracycline (100%) and Amoxicillin (100%), was detected. Antibiotic-resistance rate was extremely high against Neomycin (94, 91%), Doxycycline (86, 44%), Trimethoprim-Sulfamethoxazole (71, 19%) and Florfenicol (61, 01%). While quinolones were significantly resistant in rates of 50, 85% for Enrofloxacin and 42, 37% for Norfloxacin. The incidence of resistance to Quinolones, Tetracyclines, Aminoglycosides, Amphenicols, Penicillin-like and Sulfonamides drug classes in cases of avian colibacillosis remains high. Due to the development of AMR not only in poultry production poultry chain, but also in backyard, waterfowl and game-birds this study is an evidence to assess the antibiotic resistance profile linked with different poultry species, which could be a useful data reporting to prevent the overuse or misuse of antibiotics as a potential food safety and veterinary public health risk.

Keywords: Multidrug-resistance, Colibacillosis, Poultry, Alban

1. Introduction

Avian pathogenic *E. coli* strains cause primary and secondary diseases in chickens and other poultry breeds [6]. Poultry can act as an Avian Pathogenic Escherichia Coli reservoir which is spread through eggs and meat of chickens and other poultry [15]. The pathological manifestation of avian colibacillosis include septicaemia (colisepticaemia), pneumonia, yolk-sac infection, omphalitis, cellulitis, [23], airsacculitis, osteomyelitis and peritonitis [3], oftentimes leading to death, due to virulence gene content and antibacterial drug resistance [12]. Infection of poultry with *E. coli* causes economic losses by reducing productivity, egg production, and decreases hatching rates and meat condemnation during processing, in addition to costs of antibiotics use in treatment and prophylaxis [8], [9].

Avian *E. coli* are divided into two main groups, namely, commensal (nonpathogenic) and pathogenic *E. coli*. Commensal *E. coli* exists as normal flora in the digestive tract of humans, mammals, and birds and

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helps the host digest food. Commensal *E. coli* does not possess any virulence factors [4], never the less there are findings of several virulence genes in previous studies, its role remains to be elucidated [20]. The APEC strains of *E. coli* produce verotoxin, also known as Shiga toxin [13]. Some APEC strains possess more than one virulence genes [20] their gross pathology findings are characterized by pericarditis, air sacculitis, perihepatitis, peritonitis and other extraintestinal diseases, referred as colibacillosis [14].
The main control way of poultry collibacillosis treatment still remains the antibiotic administration. The use of antibiotics in compound feeds is considered as an integral part of poultry production costs and veterinary measures, in order to prevent infectious bacterial diseases, but also at the subtherapeutic level as growth promoters, which leads to bacterial resistance in poultry [2]. Antimicrobial resistance limits the therapeutic possibilities of treatment associated with bacterial diseases in domestic animal species and poultry in particular [17] and it has become a worldwide problem in animal and public health. Inadvertent uses of antibiotics in poultry have led to the emergence of multidrug-resistant organisms causing possible threat to human health [18].

2. Material and Methods

2.1 Postmortem Examination

Postmortem examination was carried out on seventy seven (77) poultry carcasses comprising of commercial layer chickens 17), broilers (25), turkeys (15), backyard poultry (11), ducks (5), and pigeons (4). These carcasses were sampled from ninety- nine (99) different poultry flocks of combined flock size, area distribution and breeding type. On necropsy, hepatomegaly, fibrinous layer on the serous layers of liver and heart were mostly evident. Infected birds also had swollen spleens. These findings agreed the observations of Cătană et al. [5] in E. coli infected chickens due to colibacilosis polyserositits.

2.2 Isolation and identification of Escherichia coli

Seventy-seven (77) liver and spleen samples collected from dead poultry during the years 2021 and 2022, were plated on MacConkey Agar (Oxoid) at 37°C for 24h. The cultured plates were examined for the presence of typical colonies of E. coli based on cultural and morphological characteristics, that is, metallic sheen colonies on EMB and pinkish lactose fermenting colonies on MCA.
Suspected as positive - E. coli colonies were isolated on TBX agar and confirmed as E. coli by the API 20E Test (BioMerieux).

2.3 Antibiotic resistance evaluation

Six (6) classes of antibiotics were used for the research, i. Quinolones and Fluoroquinolones (Enrofloxacine and Norfloxacine), ii. Penicillin-like antibiotics Beta lactam antibiotics (Amoxicillin), iii. Antimicrobial protein synthesis inhibitor (Florfenicol), iv. Tetracyclines (Chlortetracycline and Doxycicline), v. Sulfonamide antibiotics (Trimethoprim Sulfamethoxazoli, and vi. Aminoglycoside antibiotics (Neomycin), were used for the antimicrobial susceptibility test.
Antibiotic susceptibility testing (AST) was determined using a modification of the Kirby-Bauer (Bauer et al., 1966) in accordance disk diffusion method (CLSI, 2012). The following panel of antimicrobial agents was used: 35 µg Amoxicillin (AMX), 50 µg Enrofloxacin (ENR 5), 30 µg Oxytetracycline (OTC 30), 25 µg Trimethroprim sulphamethoxazole (STX), 30 µg Florfenicol (FFC), 30 µg Norfloxacin (NOR), 30 µg Doxycycline (DO), 30 µg Neomycin (N30).
These antibiotics are frequently employed in poultry feeds and in treating colibacillosis and other avian infectious diseases [11], [19].

3. Results and Discussion

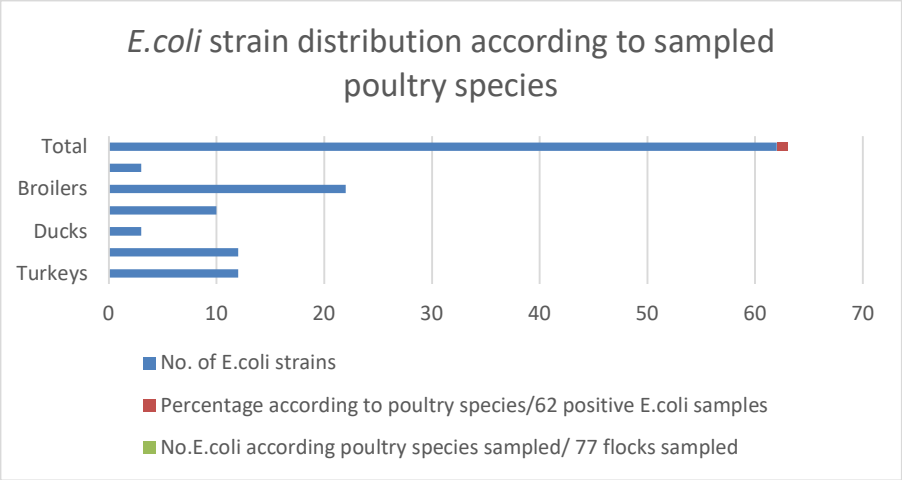
Colibacillosis gross lesions in sampled poultry was characterized by acute form of septicemia resulting in death and in its subacute and or cronic forms of peri-hepatitis, spleen-megalia, and aerosacculitis. 95,50 % of the sampled poultry resulted positive for *E. coli* and all of the 62 *E. coli* strains were considered pathogenic due to their isolation from poultry visceral organs such as liver and or spleen, also other clinical findings.
In the study it was relevant that the total of Avian Pathogenic *Escherichia Coli* strains in tested poultry, waterfowl and game bird species were isolated from liver and spleen tissues and field cases of perihepatis ans spleen-megalia were already observed in all of the

poultry specimens from where the systemic infection was spread. Colibacillos was suspected based on the clinical features and the typical macroscopic lesions. The diagnosis is obtained by *E. coli* isolation from cardiac blood and affected tissues, like liver, spleen, pericard or bone marrow [23].

The risk for colibacillosis increases with increasing infection pressure in the environment. A good housing hygiene and avoiding overcrowding are very important. Other principal risk factors are the duration of exposure, virulence of the strain, breed, and immune status of the bird [23], as in the the present study the tested poultries originated not only from intensive or high density flocks, but also from small and individual rearing type (backyard poultry, ducks and pigeons).

The distribution of the reported and tested dead poultries was from 99 flocks located in different regions of Albania and the range of flock or breeding type was various.

Table 1. *E. coli* strain distribution according to sampled poultry species.



The isolated strains were evaluated toward a panel of six different drug classes and eight antibiotic substances to determine the resistance levels according to drug class, antibiotic substance and respective poultry specia. The present study reveals very hight levels of antibiotic-resistant in E.coli strains, collected in Albania, during the years 2021-2022.

No.E.coli strains	Percentages according to poultry specia	Poultry Species	Aminoglycosides/ Neomycin	Amphenicol / Flofenicol	Penicillin like / Amoxicillin	Tetracycline Oxytetracycline	Tetracycline Doxycicline	Quinolones Norfloxacine	Quinolones Enrofloxacin	Sulfonamides / Trimethoprim Sulfamethoxazole	Resistance according poultry specia
12	19,35%	Turkeys	100%	100%	100%	100%	100%	66, 67%	66,67%	66,67%	88,89%
12	19,35%	Layers	100%	66,67%	100%	100%	91, 67%	33,33%	33,33%	50%	90,27%
3	4,84%	Ducks	100%	100%	100%	100%	100%	100%	100%	100%	100%
10	16,13%	Backyard poultry	100%	70%	100%	100%	80%	90%	80%	90%	89, 17%
22	35,49%	Broilers	86,36%	27,27%	100%	100%	31,81%	4,5 %	31,81%	72,72%	61, 73%
3	4,84%	Pigeons	100%	100%	100%	100%	100%	100%	100%	100%	100%
62 total	100%	6 species	94,91%	61,01%	100%	100%	86, 44%	42,37%	50,85%	71,19%	77,82%
						93, 22%		46, 61%			

Figure 1. Distribution of antimicrobial resistance level, according to tested drug classes, antibiotics and poultry species.

There was evidented a link and no difference among the antibiotic-resistance from diffrent poultry species toward the six drug classes tested. The high level of antibiotic resistance in avian pathogenic *Escherichia coli* in Albania is increasing with the passing of the years for the same antimicrobial drug classes (19) and is considered as emergently high, which indicates that widespread use of antibiotics as feed additives for growth promotion and disease prevention could have negative implications for human and animal health and the environment [22].

The total 62 isolated *E. coli* strains showed high resistance rates against Amoxicillin (100%), Oxytetracycline (100%), Neomycin (94, 91%), Doxycycline (86, 44%), Trimethoprim-Sulfamethoxazole (71, 19%), Florfenicol (61, 01%), Enrofloxacin (50, 85%) and Norfloxacine (42, 37%). These antibiotic resistance patterns against *E. coli* for egg and meat production poultry species are similar to those found in previous investigations in Albania [19] and other studies [1], [22], [23]. Also, previous studies have revealed a substantial increase in antibiotic-resistance in water, wild and game-birds [16], [21] not only due to inappropriate and irrational use of antimicrobial molecules in the poultry sector , but because of spreading of new gained multidrug-resistant pathogens in the environment.

E. coli isolated from ducks and pigeons, resulted 100% resistant against all of the antibiotic substances. Ducks and pigeons are avian species which have different habitats than other poultries, due to rarely sharing environment these absolute multi-resistance rates constitute an worrisome relevation fot the veterinary public health, and indicate suggest that pigeons may contribute to the transfer and spread of microorganisms, as well as antibiotic-resistant bacteria.The fact that MDR strains were found in these birds could be explained by their eating habits. Pigeons can acquire pathogens through food and/or water contaminated with human feces and farm waste. This indicates the possible transmission of *E. coli* between birds and humans [16]. Also, veterinary controls and measures are often taken and applied mainly in production poultry species: layers, broilers and turkeys, resulting in lack of information and appropriate actions in other bird species: backyard poultry, ducks and pigeons. This gap, may later result in creating some species in reservoirs of altered, specific and different pathogenic and drug resistant abilities in zoonotic bacteria.

Antibiotics are the main control method of *E. coli* infection in poultry, their effectiviness reduces the avian colibacillosis incidence and the disease mortality. However, the increase of antibiotic-resistance in pathogenic and or commensal *E. coli* strains often leads to therapy failure and potential economic losses for the farmers. Poultry and poultry products are one of the most important sources of protein for humans, especially in developing countries. Antibiotic resistance in veterinary public health is a serious problem, and transfer of resistance from animal to potential human pathogenic bacteria is possible [22]. Similar phenotypes of multidrug resistance in bacteria from animals, birds, and humans indicate that transfer of resistance genes can occur between sources [22].

4. Conclusions

Avian colibacillosis is considered to be the major bacterial disease problems in the poultry industry world-wide and these diseases constitute a major public health burden and represent a significant cost in many countries [23].

The level of the multidrug resistant *E. coli* results according to poultry species suggest of an important impact of the environment as a source of resistant bacteria for poultry farming.

This study revealed the presence in similar levels of antibiotic- resistant *E. coli* strains in different poultry species, including ducks and pigeons.

Multiresistance was observed in the total *E.coli* tested strains, collected in a two years period. The resistance significance level among the layer's chickens, broilers and turkeys flocks show the importance of increased antibiotic-resistant bacteria spread in the food chain production. In the other hand pigeons as game or pet birds are remarkably very close to human and therefore, they may pose potential threats for veterinary public health not only in rural but also in urban areas in carrying and spreading different zoonotic pathogens including drug-resistant bacteria.

The economic and public health burden of these diseases have made this topic time demanding. It is suggested that more effective application of existing therapeutic control methods would greatly reduce the risks to veterinary public health.

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