

Evaluation of Antibiotic Resistance Pattern of *Escherichia coli* Isolated From Broiler Chickens With Colibacillosis in Ardabil Province, Iran

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Abstract

Introduction: Colibacillosis is one of the most important bacterial diseases of birds that is caused by *Escherichia coli*. This disease causes considerable economic damage to the poultry industry every year. Various antimicrobial agents are used to reduce the damage caused by this infection. But in recent decades, the increased use of antibiotics has led to the development of resistant genes and, consequently increasing antibiotic resistance of bacteria, leading to a decrease in the efficacy of antibiotics. The purpose of this study was to determine the susceptibility and drug resistance of 178 isolates of 40 chicken flocks in Ardabil province northwest of Iran.

Methods: Five carcasses were randomly selected from each flocks with colibacillosis and sampled from liver and heart using sterile swabs. After culture and isolation, colonies were identified by biochemical and serological methods. Antibiotic resistance of all isolates to 19 antibiotics was determined using disk diffusion method based on CLSI guidelines.

Results: Of 200 samples, 178 (89%) were isolated, and 22 (11%) did not grow. In this study the highest antibiotic resistance was observed against flumequine (98.31%), nalidixic acid (97.25%), tylosin (97.20%), oxytetracycline (97.20%), chlortetracycline (95.50%), difloxacin (89.32%), doxycycline (81.47%), enrofloxacin (77.53%), sulfamethoxazole + trimethoprim (71.91%), and the lowest antibiotic resistance was recorded for Linco-Spectin (36.52%), chloramphenicol (22.47%), gentamycin (7.30%), fuzbac (5.05%) and ceftriaxone (3.93%). All isolates were highly sensitive to ceftazidime.

Conclusion: The results of this study showed a high level of resistance to antibiotics commonly used in poultry industry, which is probably due to improper use of antibiotics in poultry.

Keywords: Colibacillosis, Antibiotic resistance, Broiler chicken, Isolates



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Introduction

Escherichia coli is a natural flora of the digestive tract of humans, mammals and birds, which is generally regarded as an opportunistic pathogen and accounts for about 10-15% of its population as pathogenic strains for birds (APEC; Avian pathogenic *Escherichia coli*).^{1,2} Often following the damage to the immune system or broken barrier of domestic birds, APEC strains cause systemic or local infections.^{1,3} Colibacillosis is one of the most important bacterial diseases of poultry and turkeys caused by infection with the APEC strains of *E. coli*.

It causes septicemia, respiratory tract infections, cellulite, pericarditis,

peritonitis, salpingitis, synovitis, colon granulomas, and airbag infections.^{4,5} Non-infectious agents such as stress, exposure to the dust and ammonia inside breeding farms increase the likelihood of damage to the lining cells of the mucosal layer of the respiratory system and cause the invasion of the respiratory system by *E. coli*.^{5,6} It can also occurs secondary to primary infectious diseases such as infectious bronchitis, Newcastle (including vaccine strains), mycoplasmosis, pasteurellosis, etc., and lead to respiratory complex disease.⁷ However, infection with *E. coli* is of particular importance and imposes significant economic losses to the poultry industry annually worldwide due to

increase in mortality and increased carcass removal in the slaughter inspection process.⁸

There are many antimicrobial agents used in poultry industry to control the morbidity and mortality of colibacillosis. In fact, antibiotic therapy is one of the important tools to reduce the incidence and overall damage of bacilli.^{2,6} But in recent years, the overuse of drugs as preventive and therapeutic agents for infections or growth promoters of poultry has led to the emergence and spread of resistant genes and consequently increasing in antibiotic resistance, leading to reduced efficiency has finally made it difficult to treat the disease.⁹ On the other hand, the phenomenon of antibiotic resistance due to the spread of resistant bacteria in human population is also of great importance.^{8,10,11} The aim of this study was to evaluate the frequency of drug resistance of *E. coli* isolates obtained from poultry flocks in Ardabil province against different antibiotics.

Materials and Methods

Sample Collection

In this study, 40 broiler flocks (2-6 weeks old) from different parts of Ardebil province with clinical symptoms and suspected autopsy injuries were studied in Mehr Veterinary Laboratory within a year. Five birds were randomly selected from each flock and after sampling and initial diagnosis, the liver and heart were sampled after heating.

Identification of *E. coli*

The samples were first cultured in MacConkey agar and incubated at 37°C for 24 hours. The lactose-positive colonies were then separated and re-cultured on MacConkey agar until pure culture.⁹ To confirm the bacterial identity, a colony of pure culture was cultured on EMB medium (Eosin methylene blue) and if produced by metallic green glaze, finally was identified and confirmed with biochemical subtraction tests including indole production, methyl red reaction and *Voges-Proskauer* reaction, urea production, citrate intake, fermentation of glucose, and decarboxylation of ornithine and lysine based on standard bacteriological methods of final diagnosis and confirmation.^{1,2} The *E. coli* isolates confirmed by biochemical tests were stored in LB medium with 30% glycerol at -70°C until the test.

Antibiotic Susceptibility Test

To determine antibiotic resistance pattern of 178 isolates by 19 drugs commonly used in Iranian Poultry Industry was used disc qualitative diffusion method by Kirby Bauer procedure² in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.¹² For this study, antibiotic discs (in micrograms concentration) sulfamethoxazole + trimethoprim (23.75 / 1.25), amoxicillin (10), ciprofloxacin (5), ceftriaxone (30), tylosin (30), enrofloxacin (5), gentamicin (10), fuzbac

(200), ceftazidime (30), chloramphenicol (30), kanamycin (30), lincospectin (200/15), furazolidone (100), chlortetracycline (30), doxycycline (30), oxytetracycline (30), difloxacin (10), colistin, and nalidixic acid (30) were provided from Padtan Teb Company of Iran.

For disk diffusion experiments, each bacterial isolate was removed from 70°C freezer and cultured on MacConkey agar for 24 hours at 37°C. Then 2 to 3 colonies were transferred from the MacConkey agar medium to the test tube containing TSB (Tryptic Soy Broth) and incubated for 3 hours at 37°C to prepare 0.5 McFarland turbidity. Subsequently, sterile swabs from the half-McFarland bacterial suspension were cultured on Müller-Hinton agar medium. After about 10 minutes, antibiotic discs were placed on the inoculated Müller-Hinton medium and plates were incubated at 35°C for 18 hours.^{1,7} Then the diameter of the inhibition growth zone of each disk was measured and finally the resistance and sensitivity of each isolate were compared with CLSI standard.

Results

The results of the present study are shown in Tables 1 to 2. Out of 200 samples of broilers infected with colibacillosis in Ardebil province, *E. coli* was isolated in 178 cases (89%) and was not grown in 22 cases (11%). The results of the disk diffusion test of *E. coli* isolates from cases of broiler poultry colibacillosis in Ardabil province have been presented in Table 1. According to Table 1, 178 *E. coli* isolates from broiler chickens with colibacillosis were highly susceptible to ceftazidime and the resistance of the isolates to 18 other antibiotics ranged from 3.93% to 98.31%, which varied in the three resistance groups. In the first group there were 5 antibiotics with low resistance (between 0%-40%), chloramphenicol, gentamicin, lincospectin, fosbac and ceftriaxone. The second group of 5 antibiotics colistin, amoxicillin, ciprofloxacin, furazolidone and kanamycin had moderate resistance (between 40% and 70%).

In the third group of 8 antibiotics tylosin, oxytetracycline, chlortetracycline, doxycycline, sulfamethoxazole + trimethoprim, flumequine, difloxacin and nalidixic acid showed high resistance rate (between 70 and 100%). The isolates were highly resistant (over 95%) to 5 antibiotics flumequine, tylosin, oxytetracycline, chlortetracycline and nalidixic acid.

Among the resistant isolates, multiple resistance was observed, with 100% resistant isolates to at least 3 compounds and 0.5% resistant to at least 18 compounds of the total 19 antibiotics tested (Table 2).

Discussion

Colibacillosis is one of the most economically important bacterial diseases of poultry industry, especially broilers worldwide. Today, the fight against this disease relies heavily on the use of antimicrobial compounds, which have been widely used and prolonged use of antibiotics,

Table 1. Frequency of Resistance of 178 *E. coli* Isolates From Broiler Chickens With Colibacillosis to 19 Antibiotics Tested

Antimicrobial Agent	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Macrolide groups			
Tylosin	173 (97.20)	5 (2.80)	0
Tetracycline groups			
Oxytetracycline	173 (97.20)	5 (2.80)	0
Chlortetracycline	170 (95.50)	8 (4.50)	0
Doxycycline	145 (81.47)	30 (16.85)	3 (1.68)
Fenicol groups			
Chloramphenicol	40 (22.47)	62 (34.83)	73 (42.70)
Quinolone and Fluoroquinolone groups			
Flumequine	175 (98.31)	3 (1.69)	0
Nalidixic acid	174 (97.75)	4 (2.25)	0
Difloxacin	159 (89.32)	11 (6.18)	8 (4.50)
Ciprofloxacin	105 (58.98)	34 (19.11)	39 (21.91)
Aminoglycoside groups			
Kanamycin	105 (58.98)	28 (15.73)	45 (25.27)
Gentamicin	13 (7.30)	61 (34.27)	104 (58.43)
Lincosamide groups			
Lincospectin	65 (36.52)	40 (22.47)	73 (41.01)
Polymyxin groups			
Colistin	122 (68.54)	43 (34.16)	13 (7.30)
Penicillin groups			
Amoxicillin	124 (69.67)	48 (26.96)	4 (2.25)
Nitrofurazone groups			
Furazolidone	91 (51.13)	67 (37.64)	20 (11.23)
Sulfonamide groups			
Sulfamethoxazole+ trimethoprim	128 (71.91)	21 (11.80)	29 (16.29)
Fosbac	9 (5.05)	49 (27.53)	120 (67.42)
Cephalosporin groups			
Ceftriaxone	7 (3.93)	10 (5.62)	161 (90.54)
Ceftazidime	0	0	100 (100.0)

leading to resistance to some bacterial strains, led to drug inefficiencies and treatment failure.^{6,9} Bacterial resistance to antibiotics is usually based on mechanisms such as the production of drug-degrading enzymes, changes in bacterial permeability to drugs, changes in drug receptors at the bacterial level, changes in bacterial cell wall structure, and access to sub-metabolic pathways which compensate for the drug-inhibited reaction, which is transmitted either from one bacterium to another through spontaneous mutation in genes that control bacterial susceptibility or through plasmid transferring^{1,10} In the present study, resistance to 10 antibiotics with common use in poultry industry (tylosin, amoxicillin, oxytetracycline, chlortetracycline, doxycycline, sulfamethoxazole + trimethoprim, flumequine,

difloxacin, colistin and nalidixic acid) is high that 53% of the antimicrobial compounds constituted. More than 95% of *E. coli* isolates were resistant to the five antibiotics (27%) including flumequine, tylosin, oxytetracycline, chlortetracycline and nalidixic acid that the main reasons being non-normatively and prolonged use of these compounds, particularly refers to usage of tetracyclines and macrolides group in poultry farms as treatment and growth-promoting therapies. Other factors, such as the presence of resistance genes to antimicrobial drugs, as well as the ability to exchange resistance genes and their activity in different hosts, may play an important role in drug resistance. In this study, the resistance of the tested isolates to fluoroquinolones other than ciprofloxacin was almost similar to tetracyclines and macrolides. Percentage of low resistance to lincospectin, chloramphenicol, gentamicin, fuzbac and cephalosporins group compared to the drugs investigated in this study is due to limited use (due to the combination of structure and high cost) of these medicinal compounds in the prevention and treatment of commercial poultry flocks.

In recent decades, increasing resistance of *E. coli* poultry pathogens strains to antimicrobial compounds used in poultry industry has been reported, and the pattern of antibiotic resistance in various geophysical regions has been changing.¹³⁻¹⁵ Mellata et al⁵ examined the sensitivity of 101 *E. coli* isolates from poultry flocks in Algeria, although they showed the highest resistance to tetracycline, but resistance to sulfamethoxazole + trimethoprim, ampicillin and neomycin was at the next

Table 2. Multiple Resistance of 178 *E. coli* Isolates From Broiler Chickens With Colibacillosis Against 19 Tested Antibiotics

Antibiotics No.	Resistant No. (%)
1	178 (100.0)
2	178 (100.0)
3	178 (100.0)
4	172 (96.6)
5	167 (93.8)
6	160 (89.8)
7	151 (84.8)
8	145 (81.4)
9	126 (70.7)
10	113 (63.4)
11	105 (58.9)
12	84 (47.2)
13	38 (21.3)
14	25 (14.0)
15	20 (11.2)
16	11 (6.1)
17	5 (2.8)
18	1 (0.5)

site.

Hanson et al¹⁶ in the study of 54 *E. coli* isolates in Thai broiler farms reported the highest resistance to tetracycline (77.8%), fluorophenicol (50%), ampicillin (38.9%) and enrofloxacin (9.3%), respectively. In the study of Shecho et al¹⁷ on a total of 14 antimicrobials included in the study panel, 0%-96.15% *E. coli* isolates were likely to express resistance to erythromycin, clindamycin, spectinomycin and ciprofloxacin. Multidrug resistance to more than two antibiotics was found in 24 (92.30%) isolates. This study showed the high presence of antimicrobial resistant in isolates of *E. coli*. Zhao et al¹⁸, in a study of 95 APEC strains of *E. coli* in Georgia, also found the highest resistance to sulfamethoxazole (93%) followed by tetracycline (87%) and enrofloxacin (52%). A study of 83 APEC strains of *E. coli* in Japan by Ozawa et al¹⁴ also reported the highest resistance to ampicillin (77.1%) followed by oxytetracycline (75.9%) and trimethoprim (25.3%), Enrofloxacin (21.7%) and fluorophenicol (0.6%). According to Gregova and Kmet¹⁹ in Slovakia, the highest percentage of resistance of *E. coli* isolates in slaughter poultry was related to ampicillin (89%), enrofloxacin (43%), tetracycline (33%) and fluorophenicol (18%). In a study by Khan et al,²⁰ 30 commercial poultry *E. coli* isolates in Bangladesh reported erythromycin and tetracycline resistance percentages of 100 and 93.3, respectively. Wakawa et al²⁶ examined 67 *E. coli* isolates isolated from commercial poultry flocks with colibacillosis in Nigeria with the highest resistance to chloramphenicol (11.9%) and tetracycline (10.5%) and subsequently to them. Neomycin (5.9%), sulfamethoxazole (4.5%), enrofloxacin (0.3%), ampicillin (2.9%) and erythromycin (1.5%) were observed. Jahantigh et al.²² In Sistan and Baluchestan province had the highest resistance of *E. coli* isolated from broiler herds with infected with colibacillosis against tetracycline (95.0%) and ciprofloxacin (88.3%), co-trimoxazole (86.7%) , lincospectin (52.5%) and gentamicin (21.7%) and also the prevalence of *tetA*, *tetB*, *tetC* and *tetD* genes of tetracycline resistance among 60 isolates were 96.7%, 38.3%, 7.731% and 8.30%, respectively. Bakhshi et al²³ In Yazd also reported the highest resistance to nalidixic acid (100%) and ciprofloxacin (86%) and the highest sensitivity to colistin (100%) and gentamicin (93%). Joshi et al⁸ reported that in 20 *E. coli* isolates collected from laying flocks involved with colibacillosis in India, the highest antibiotic resistance to cephalixin (73.68%) and the lowest was related to chloramphenicol (0%). Also, the percentages of resistance to enrofloxacin, neomycin, sulfamethoxazole, furazolidone and amikacin were 31.58, 31.58, 15.79, 15.79 and 5.26, respectively. Sjölund et al²⁴ examined 51 isolates obtained from poultry in Italy and found the highest resistance to ampicillin and nalidixic acid and the lowest in the cephalosporin group. Of the 51 isolates identified, 16 were APEC strains that possessed genes *iucD*, *iss*, *cvi/cva*, *irp2* and *papc*. Ibrahim et al²⁵ studied 269 APEC strains isolated from broiler

herds in northern Jordan with the highest resistance related to sulfamethoxazole + trimethoprim (95.5%), amoxicillin (93.3%) and doxycycline (92.2%). The lowest were ceftriaxone (3.93%) and ceftazidime (0%) and the frequencies of *SitA*, *iss*, *iucD*, *iucC*, *astA*, *tsh*, *cvi* and *irp2* genes were 97.4%, 93.3%, 75%, 74.00%, 71.00%, 46.5%, 39.00% and 34.00%, respectively.

In Iran, there have been several studies on the antibiotic resistance of *E. coli* in poultry, indicating that the drug resistance is varied and high in different parts of the country. Khoshkhoo and Peighambari²¹ examined 150 *E. coli* isolates from 30 broiler flocks in Tehran province showed resistance to difloxacin, sulfamethoxazole + trimethoprim, enrofloxacin, Linco-Spectin and neomycin were 84%, 72.6%, 66%, 76.4% and 52% respectively.

In the study of Zahraei Salehi and Farashi Bonab⁴ on 50 APEC strains of *E. coli* isolated from broilers in Tabriz, the highest resistance was to two antibiotics erythromycin (97%) and tetracycline (94%). Firouzi et al³² study in 58 *E. coli* isolates around Shiraz showed that all isolates were resistant to tylosin and the resistance to doxycycline, oxytetracycline, enrofloxacin and chloramphenicol were 86.2%, 84.5%, 39% and 27.2%, respectively. In the study of Saberfar et al¹⁵ in commercial farms throughout Iran showed the highest resistance to erythromycin (99%) followed by tetracycline (96%), neomycin (87%), Linco-Spectin (79%), difloxacin (78%), enrofloxacin (76%), ampicillin (49%) and fluorophenicol (39%).

Azizpour and Saeidi Namin³⁰ in Ardabil province showed the highest resistance of *E. coli* isolated from broilers with colibacillosis to tetracycline (99.43%) and erythromycin (97.75%) and the lowest to fluorophenicol (58.99%) and lincospectin (36.52%). Ghaniei and Peighambari¹⁰ observed 943 *E. coli* isolates from 8 provinces (including Tehran, Mazandaran, Isfahan, Semnan, Golestan, Kurdistan, Kermanshah and Qazvin) that these isolates showed resistance 65.5% to sulfamethoxazole + trimethoprim and 61.5% to doxycycline, 55.5% to colistin, 41.5% to enrofloxacin, and 34.5% to fluorophenicol. Seifi et al⁷ in Mazandaran broilers reported the highest percentage of resistance to tetracycline (71.25%), erythromycin (65%), ampicillin (62.5%) and enrofloxacin (7.5%), respectively.

The findings of the present study are somewhat consistent with those reported in other geographical regions in terms of drug resistance^{15,20} but with the findings of some researchers are different.¹⁹ This discrepancy may be due to differences in the type, amount and persistence of antimicrobial compounds use, and temporal and spatial differences and differences in the isolates studied. Multiple drug resistance to antibiotic compounds is a major problem and a common phenomenon seen in the majority of *E. coli* strains of the poultry pathogen in which bacterial isolates are resistant to at least two types of antibiotics.^{26,27} Significant increases in the incidence of multiple resistance of *E. coli* isolates to antibiotic

compounds over the last decade have been reported in many countries including Iran^{7,15} Germany,²⁷ China,²⁸ Bangladesh,²⁹ India,²⁰ Algeria,⁵ Nigeria,²¹ and Zimbabwe.¹⁶

In this study, all 178 isolates were resistant to three types of antibiotics and 145 isolates (81.14%) to 8 types of antibiotics out of 19 tested antimicrobial compounds, mainly due to their continued use without careful evaluation of the drug. The level of bacterial susceptibility in poultry flocks is alarm a threat to the transmission of resistance to humans.³¹ This is very serious in Iran and other developing countries, which do not abide the use of antibiotics and avoid consumption of meat and other animal products treated with antibiotics. The present study findings (that the isolates were resistant to more than three type of antibiotic) is consistent with the study Jahantigh et al²² of Saidi et al¹⁶ In the present study, the frequency of drug resistance of isolates belonging to a single poultry unit was varied.

Conclusion

Due to the variability of drug resistance pattern of isolates in farms, antimicrobial susceptibility test should be performed independently for each region or even each poultry unit prior to prescribing antibiotics to select the appropriate and effective drug for the treatment of poultry colibacillosis. The results of this study showed that the spectrum and resistance level of *E. coli* isolates were broad and high compared to the majority of antibiotics commonly used in Iranian poultry industry, which necessitated the implementation of a national monitoring plan for essential antimicrobial resistance seems necessary that prevents the spread of resistant bacteria and consequently increasing antibiotic resistance of the bacteria through the regular use of medicines in poultry farms, along with the promotion of hygiene and biosecurity. Based on the above study and similar results by other researchers, it can be concluded that the indiscriminate use of antibiotics causes the resistance of bacteria to antibiotics and resistance genes are transmitted more rapidly between pathogenic bacteria. Therefore, it is suggested that by studying the prevalence of resistance and transmission of pathogenic genes in bacteria, it is possible to prevent increasing in resistance of these antibiotics.

Ethical Approval

Not applicable.

Conflict of Interest Disclosure

The authors declare no conflict of interest.

Authors' contributions

AA: Supervision, concept, design, data gathering, analysis and interpretation of data, drafting of the manuscript, searching of databases, scientific revision of manuscript; CG: critical revision of the manuscript for important intellectual content.

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