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Evaluation of Antibiotic Resistance in *Escherichia coli* Isolates Obtained from Broiler Chicken Flocks at Young Ages in Kermanshah Province

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ABSTRACT

Introduction: The widespread use of antibiotics for growth promotion or therapeutic purposes in poultry farming has led to increased antibiotic resistance. *Escherichia coli* is one of the gastrointestinal bacteria capable of transferring resistance genes and causing antibiotic resistance in humans and poultry. Evaluating antibiotic resistance in poultry flocks can provide researchers with a clear picture of the health status of poultry flocks and the human community.

Methods: This study was conducted on 60 broiler chicken flocks aged 1 to 28 days. These flocks had no history of antibiotic use. In the laboratory, after necropsy, sampling was carried out from five chicken pieces in each flock. After confirming the diagnosis and purification of *E. coli* using biochemical methods, antibiotic sensitivity testing against 19 antibiotics was performed using the disc diffusion method, following the CLSI guidelines.

Results: Out of 300 samples collected, 270 (90%) isolates of *E. coli* were obtained. In this study, the sensitivity of antibiotics was as follows: fosfomycin (100%), lincomycin (94.81%), neomycin (48.52%), amoxicillin (48.15%), norfloxacin (41.48%), thiamphenicol (38.52%), enrofloxacin (38.52%), sulfamethoxazole (36.66%), florfenicol (31.85%), tilmycosin (31.85%), danofloxacin (30%), flumequine (25.19%), difloxacin (21.85%), chlortetracycline (16.66%), trimethoprim (16.66%), doxycycline (11.85%), erythromycin (10%), tylosin (1.48%), and colistin (0%). Additionally, resistance was observed only against tylosin (91.85%). No multiple resistance was observed among the isolated strains, and at least sensitivity to two antibiotics was detected in all samples.

Conclusion: The findings of this research indicate that the level of antibiotic resistance in broiler chicken flocks at young ages in the Kermanshah region is low. However, the sensitivity rate to 17 antibiotics is less than 50%, demonstrating a relatively high level of sensitivity in poultry at these ages.

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Introduction

In the past three decades, the poultry farming industry has witnessed remarkable growth due to the significant increase in the human population (1,2,3). The substantial rise in the number of farms alongside increased farming density per unit area has led to an upsurge in disease occurrences during the rearing period. Factors such as improved performance, disease prevention, and treatment have resulted in increased consumption of antimicrobial compounds in the broiler chicken industry, turning it into a major hub for antibiotic compound usage (4,5). Accordingly, the estimated consumption of antimicrobial compounds is projected to grow by 67%, from 63,151 tons in 2010 to 105,596 tons in 2030 (1,2).

Antibiotics serve various roles in therapeutic treatment, disease prevention, and performance enhancement in poultry. However, their usage contributes to the emergence of resistance against these compounds (4,6). The initial prescription of antibiotics in poultry dates back to 1946, while the first instance of antibiotic resistance was recorded in 1956 (7). Antibiotic resistance occurs through four primary pathways: alteration in the target, antibiotic

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inactivation, decreased permeability, and increased efflux (the pump of antibiotics out of the cell). Genes play a role in antibiotic resistance, transferring among bacteria via both vertical and horizontal methods. Approximately 30-90% of administered antibiotics in poultry are excreted via urine and feces, entering the environment, exerting selective pressure, and increasing antibiotic resistance (7,8,9).

The rise in antibiotic resistance in bacteria poses a potential threat to human health as the ultimate consumers in the production chain. *Escherichia coli* is an ubiquitous bacterium present in the intestines of humans and animals (3,5). This bacterium has the capability of transferring resistance genes to other bacteria, including pathogenic ones, and is considered an indicator bacterium in studies of antibiotic resistance in Gram-negative bacteria. Recent years have witnessed extensive studies conducted in various geographical regions of Iran on antibiotic resistance. This study aims to further update the status of the level and pattern of antibiotic resistance in 60 broiler chicken flocks in Kermanshah province, continuing these investigations.

Materials and Methods

Sample Collection

From June 2021 to December 2022, 60 broiler flocks were randomly selected from farms responsible for the major part of broiler production in Kermanshah province. The age range of these flocks was between 1 to 28 days, and they had no history of antibiotic consumption. Five birds were chosen for sampling from each referred flock, and sampling was conducted from the heart and liver.

Isolation and Identification of Escherichia coli Bacteria

Following the sample collection, the samples were cultured on MacConkey agar medium using sterile swabs and were then incubated in a 37-degree Celsius incubator for 24 hours. Subsequently, lactose-positive colonies were transferred to Eosin Methylene Blue (EMB) medium and incubated until pure culture was obtained. For the identification of *Escherichia coli* bacteria, bacteria with a metallic green sheen on EMB medium were confirmed using Gram staining and biochemical tests. (10)

Determination of Antibiotic Resistance Patterns of Isolates

To determine the resistance and sensitivity patterns of 270 isolates against 19 antibiotics commonly used in the poultry industry, the disc diffusion method based on the Kirby Bauer standard of the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines was employed (11). Nineteen antibiotics were evaluated for antimicrobial testing, including their potential concentrations in micrograms: tylosin (30), danofloxacin (10), amoxicillin (25), difloxacin (5), fosfomycin (200), chlortetracycline (30), neomycin (30), doxycycline (30), colistin (10), norfloxacin (10), thiamphenicol (30), erythromycin (15), tilmycosin (30), trimethoprim (5), florfenicol (30), lincomycin (15/200), trimethoprim-sulfamethoxazole (sulfadiazine) (1.25/23.75), enrofloxacin (5), and flumequine (30) obtained from Padtan Teb Iran Company.

After biochemical diagnosis confirmation, each isolate was cultured on Tryptone Soya Agar (TSA) medium and incubated at 37 degrees Celsius for 2 to 8 hours to achieve a turbidity matching the 0.5 McFarland standard. Subsequently, it was cultured on Mueller-Hinton medium, and after 5 minutes, antibiotic discs were placed on the culture medium. The plates were then incubated at 37 degrees Celsius for 18 hours, followed by measuring the diameter of growth inhibition zones. (12)

Results

In this study, sampling was conducted from 300 carcasses of broiler chickens, from which 270 (90%) *Escherichia coli* isolates were separated following confirmation through biochemical tests. As evident in Table-1, among the isolates, 248 (91.85%) showed resistance to the antibiotic tylosin, while no resistance to other antibiotics was observed within the isolate population. Concerning the percentage of sensitivity to the investigated antibiotics in the isolated strains, the rates were as follows: fosfomycin 100%, lincomycin 94.81%, neomycin 48.52%, amoxicillin 48.15%, norfloxacin 41.48%, thiamphenicol 38.52%, enrofloxacin 38.52%, sulfamethoxazole 36.66%, florfenicol 31.85%, tilmycosin 31.85%, amoxicillin 30%, flumequine 25.19%, difloxacin 21.85%, chlortetracycline 16.66%, trimethoprim 16.66%, doxycycline

11.85%, erythromycin 10%, tylosin 1.48%, and colistin (0%).

Except for the antibiotic fosfomycin, degrees of resistance (semi-sensitivity) to other antibiotics within the population of examined isolates were observed as follows: colistin 100%, erythromycin 90%, neomycin 88.15%, chlortetracycline 83.33%, trimethoprim 83.33%, difloxacin 78.15%, flumequine 74.81%, danofloxacin 70%, florfenicol 68.15%, tilmycosin 68.15%, sulfamethoxazole 63.33%, enrofloxacin 61.48%, thiamphenicol 61.48%, norfloxacin 58.52%, amoxicillin 51.85%, tylosin 6.66%, lincomycin 5.19%, and fosfomycin 0%.

The results related to the resistance pattern are visible in Table-2. No multiple resistance patterns among the antibiotics were observed within the separated isolates. Minimum sensitivity to at least two antibiotics was observed in 100% of the isolates, with 80% showing sensitivity to five antibiotics and 65.18% displaying sensitivity to six antibiotics.

Table 1. The prevalence of resistance and sensitivity among 270 isolated *Escherichia coli* strains from general broiler bacterial cases compared to 19 antibiotics

	Antibiotic	Abbreviation	Resistant Isolates		Intermediate Isolates		Sensitive Isolates	
			Quantity	Percent	Quantity	Percent	Quantity	Percent
1	Tylosin	TY	248	91.85	18	6.66	4	1.48
2	Danofloxacin	DFX	0	0	189	70	81	30
3	Amoxicillin	AMX	0	0	140	51.58	130	48.15
4	Difloxacin	DF	0	0	211	78.15	59	21.85
5	Fosbac	F	0	0	0	0	270	100
6	Chlortetracycline	CTE	0	0	225	83.33	45	16.66
7	Neomycin	N	0	0	139	51.48	131	48.52
8	Doxycycline	D	0	0	238	88.15	32	11.85
9	Colistin	CL	0	0	270	100	0	0
10	Norfloxacin	NOR	0	0	158	58.52	112	41.48
11	Tiamulin	TM	0	0	166	61.48	104	38.52
12	Erythromycin	E	0	0	243	90	27	10
13	Tilmicosin	TMS	0	0	184	68.15	86	31.85
14	Trimethoprim	TMP	0	0	225	83.33	45	16.66
15	Florfenicol	FF	0	0	184	68.15	86	31.85
16	Linco-Spectin	LS	0	0	14	5.19	256	94.81
17	Sultrim	SLT	0	0	171	63.33	99	36.66
18	Enrofloxacin	NFX	0	0	166	61.48	104	38.52
19	Flumequine	FM	0	0	202	74.81	68	25.19

Table 2. Pattern of multiple resistance in 270 isolated *Escherichia coli* from poultry to 20 antibiotics

Number of Antibiotics	Number of Resistant isolates	Number of Intermediate isolates	Number of Sensitive isolates
1	248 (91.85%)	270 (100%)	270 (100%)
2	0	270 (100%)	270 (100%)
3	0	270 (100%)	257 (95.18%)
4	0	266 (98.52%)	239 (88.52%)
5	0	266 (98.52%)	216 (80%)
6	0	261 (96.66%)	176 (65.18%)
7	0	256 (94.81%)	117 (43.33%)
8	0	252 (93.33%)	86 (31.85%)
9	0	230 (85.18%)	72 (26.66%)
10	0	203 (75.18%)	50 (18.52%)
11	0	189 (70%)	23 (8.52%)
12	0	162 (60%)	18 (6.66%)
13	0	108 (40%)	14 (5.18%)
14	0	59 (21.85%)	9 (3.33%)
15	0	41 (15.18%)	9 (3.33%)
16	0	18 (6.66%)	5 (1.85%)
17	0	0	0
18	0	0	0
19	0	0	0

Discussion

The widespread use of antibiotics in broiler chickens has led to selective pressure and an increase in resistance to these antibiotics in the population of *Escherichia coli* bacteria. This flora bacterium, residing in the lower part of the digestive system, is an opportunistic pathogen and is considered an indicator of Gram-negative bacteria. Both its pathogenicity and its ability to transfer resistance genes to other bacteria, including pathogenic ones, are significant (6,7).

In the current study, 300 samples were obtained from 60 broiler flocks during the year 1401 in Kermanshah province, from which 270 (90%) *Escherichia coli* isolates were separated following biochemical tests. Similar studies conducted by Azizpour in 2020, and Azizpour Saeidi Namin in 2018 in Ardabil province, reported isolating the bacteria from 92.5% and 89% of the samples, respectively. In this study, 91.85% of the isolates showed resistance to the antibiotic tylosin. Comparable studies by Rajaeiyan et al. in 2003 in Shiraz and Azizpour in 2021 in Ardabil reported resistance percentages of 84.1% and 86.48%, respectively, to this antibiotic. Another study conducted by Madadi et al. in 2014 on resistance to tylosin in Urmia between 2006-2011 reported resistance between 95.5-99.6%, validating the results obtained in this research. No resistance to other studied antibiotics was recorded among the isolated strains (13).

Regarding the sensitivity level of the isolated strains, only two antibiotics, fosbac (100%) and linco-spectin (94.81%), exhibited more than 50% sensitivity. In similar studies by Azizpour in 2020, Azizpour Saeidi Namin in 2018, Haghighi Khoshkhoo and Ali Nejad in 2010, and Haghighi Khoshkhoo and Peighambari in 2005, the sensitivity percentages for lincomycin were 72.7%, 63.48%, 10.7%, and 35.3%, respectively. Fosfomycin, being a newer antibiotic with a higher price, is less commonly used. The differences observed in lincomycin sensitivity between this study and similar research could be due to regional variations, differences in the age of broiler chickens, or variations in the therapeutic background of these studies compared to the present one (14,15,16).

For neomycin and norfloxacin antibiotics, the sensitivity rates in this study were 48.52% and 41.48%, respectively. In similar studies conducted by Azizpour Saeidi Namin in 2018, Azizpour in 2020, Pourhossein et al. in 2023, and Haghighi Khoshkhoo and Peighambari in 2005, the sensitivity to neomycin was 24.16%, 18.95%, 30.33%, and 48%, respectively. Norfloxacin sensitivity in Haghighi Khoshkhoo and Ali Nejad's 2010 study and Haghighi Khoshkhoo and Peighambari's 2005 study was 22% and 47.3%, respectively. These variations might be anticipated due to extensive use of norfloxacin in the broiler industry, explaining the disparity in results.

The sensitivity to other antibiotics like enrofloxacin and danofloxacin was 22.47% and 30.34%, respectively, in Azizpour Saeidi Namin's 2018 study, whereas Azizpour in 2020 reported rates of 8.28% and 44.15%, respectively. In Haghighi Khoshkhoo and Peighambari's 2005 study, sensitivity to enrofloxacin was 34%, aligning with the results of this research, but in Haghighi Khoshkhoo and Ali Nejad's 2010 study, the values for both antibiotics were 3.3%. The findings from these initial two studies align with the outcomes of this research. However, they contrast with the findings of Khoshkhoo and Alinejad's 2010 study, which was anticipated given the extensive utilization of the antibiotic enrofloxacin in the broiler industry. For the antibiotic Soltrim, the sensitivity rates were 25.33% in Azizpour in 2020 and 19.66% in Azizpour Saeidi Namin in 2018. The sensitivity rate to florfenicol in this study was 31.85%, whereas it was 37.3%, 41.01%, and 54.96% in Haghighi Khoshkhoo and Peighambari in 2005, Azizpour Saeidi Namin in 2018, and Azizpour in 2020, respectively. (14,15,16,17,18)

The sensitivity rate to flumequine in this study was 25.19%. In the studies by Khoshkhoo and Alinejad in 2010, Khushkho and Naghemiri in 2005, and Azizpour in 2020, it was 0%, 6%, and 5.4%, respectively. Our research indicates a higher sensitivity of the isolated strains to this antibiotic compared to similar studies. As for sensitivity to the cholestin antibiotic in this study, it was 0%. While Khoshkho and Propheti found a sensitivity rate of 3.3% in their 2005 study, Azizpour in 2020 and Pourhossein et al. in 2023 reported rates of 45.06% and 95.01%, respectively. These findings contradict the results of our research. (14,15,17,18)

No multiple resistance patterns were observed among the isolates in this study. However, 100% of the isolates exhibited sensitivity to at least two antibiotics, and 65.18% showed sensitivity to six antibiotics. In a similar study by Haghighi Khoshkhoo and Ali Nejad in 2010, 100% of the isolated strains were resistant to at least five antibiotics, and 62.7% were resistant to 13 antibiotics. Another study by Azizpour in 2020 reported that 100% of the isolates were resistant to at least three antibiotics, and 50.45% showed resistance to 10 antibiotics. While the first two studies align with the current research, the latter contradicts it, likely due to factors such as the younger age of the flocks in this study, no history of antibiotic use in these flocks, and geographical differences (14,17).

Conclusion

The results of this study indicate that the level of antibiotic resistance in broiler flocks aged 1-28 days is lower compared to other examined studies in the country. However, the sensitivity level of the isolated strains to 17 antibiotics is less than 50%, suggesting a relatively high level of sensitivity in these broiler flocks.

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