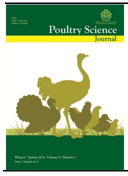

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The Antibiogram Profile of Commensal *Escherichia coli* of the Gastrointestinal Tract of Apparently Healthy Ostriches and Diseased Chickens with Colibacillosis

Salari S[1] & Hoseini A[2]

[1] Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran
[2] Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran

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Corresponding author
Saeed Salari
saeedsalari@uoz.ac.ir

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Abstract

The present study aimed to assess the antibiotic resistance of commensal *Escherichia coli* (*E. coli*) of the healthy ostriches (E_{ho}) and the diseased chickens with colibacillosis (E_{pc}) and to determine if the odds that E_{ho} (test group) shows antimicrobial resistance different from the E_{pc} (reference group). In this descriptive cross-sectional study, we calculated the odd ratio (OR) after determination of the resistance and multidrug resistance (MDR) rates, MDR pattern, and the antibiotype of E_{ho} (n= 49) and E_{pc} (n= 39) against ampicillin, amoxicillin, gentamicin, amikacin, oxytetracycline, sultrim, lincospectin, and chloramphenicol. All of the E_{ho} (100%) were resistant to ampicillin, gentamicin, and amoxicillin ($P < 0.05$) and 100% of E_{pc} were resistant to ampicillin ($P < 0.05$). Thirty point two percent of E_{ho} and 87.2% of E_{pc} isolates were MDR. MDR E_{ho} ($P < 0.05$) and MDR E_{pc} ($P < 0.05$) showed two (P1 and P3) and four (P1-4) MDR patterns, respectively. E_{ho} and E_{pc} showed seven ($P < 0.05$) and 21 ($P > 0.05$) antibiotypes, respectively. The odds of E_{ho} being resistant to ampicillin, amoxicillin, and gentamicin ($P > 0.05$) and P1 MDR pattern ($P < 0.05$) and three ($P > 0.05$) and one ($P < 0.05$) antibiotypes were higher in E_{ho} compared to those in E_{pc}. Our findings emphasized the development of antibiotic resistance in commensal *E. coli* and indicated that not only one antibiotic may not treat the disease in chickens, but antibiotic susceptibility testing is also of great necessity for veterinary health. The possible contamination of meat, carcasses, and eggs of apparently healthy ostriches by their fecal MDR *E. coli* threatens human health.

Introduction

Antibiotic resistance has significantly increased over the recent years among different families of bacteria and has become a universal problem threatening public health. Scientific committees underline the necessity of evaluation of antibiotic sensitivity of indicator bacteria from different origins to set the criteria for the determination of the evolution cycle of the mechanism of antibiotics resistance. This goal requires investigating the prevalence of the antibiogram profile isolated from different origins/animals, particularly country by country (Nhung *et al*., 2017).

Escherichia coli (E. coli) has been isolated from the normal intestinal flora of both healthy and diseased birds (Hasani *et al*., 2017; Amani *et al*., 2020). Diseases caused by *E. coli* have been considered important economic losses for humans and animals (Nhung *et al*., 2017). Only some specific strains that possess certain virulence factors, such as Avian Pathogenic *E. coli*, can cause poultry colibacillosis (Scerbova and Laukova, 2016; Amani *et al*., 2020). Nowadays, antimicrobial agents have been used as control and therapeutic strategies for colibacillosis (Zakeri and Kashefi, 2012; Ranjbar-Malidareh *et al*., 2013; Scerbova and Laukova, 2016; Amani *et al*., 2020). Inordinate use of antibiotics has led to an increase in antimicrobial resistance rate in poultry, with a considerable effect on public health (Ranjbar-Malidareh *et al*., 2013; Scerbova and Laukova, 2016; Amani *et al*., 2020).

World Health Organization has listed the critically important antimicrobials for human medicine (Scott *et al*., 2019). The beta-lactams, tetracyclines, phenicols, and aminoglycosides classes of antimicrobial are referred to as "medically

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important antimicrobials" (Scott *et al.*, 2019). lincospectin is widely used in poultry farms (Faghihi *et al.*, 2017) since its prophylactic application during the first 3-5 days after hatching decreases the mortality rate in growing chicken (Tavakkoli *et al.*, 2014). Among poultry producers, sultrim administration during the first week of broiler management to prevent and control *E. coli* infections is a common and routine program (Ranjbar-Malidareh *et al.*, 2013).

It is plausible that antibiotic-resistant *E. coli* expands among animals (e. g., chickens and ostriches). Ostrich farms play an important role in the agriculture, economy, and meat production of Iran (Hosseini *et al.*, 2019). Carcasses of ostrich may be contaminated with their droppings, which can be considered as a potential source for transmission of resistant bacteria to other animals, particularly in the common slaughterhouse of poultry industries (Carrique-Mas *et al.*, 2008). Subsequently, these bacteria may be transmitted to humans via (i) consumption of contaminated chicken meat and (ii) direct contact with feces and/or the carcasses of the ostrich in the polluted poultry industry. Thus, it leads to an increase in the prevalence of resistant bacteria and unfortunately, reduces the effectiveness of antibiotic therapy in humans and/or poultry (Nhung *et al.*, 2017). For this reason, the determination of antimicrobial resistance in different meat-production animals necessitates finding out the role of animals, especially domestic animals, in the epidemiology of drug resistance and colonization and proliferation of resistant bacteria in humans (Namkung *et al.*, 2004).

There is little information about the antibiogram profile of *E. coli* as bacterial flora of the digestive system of ostriches in Iran (Rezaei Far *et al.*, 2013; Hemmatinezhad *et al.*, 2015; Mohamadi *et al.*, 2015; Amani *et al.*, 2020). Furthermore, scanty findings are associated with the antibiogram of *E. coli* isolated from diseased chickens in the southeast of Iran (Zakeri and Kashefi, 2012; Rahimi, 2013; Kazemnia *et al.*, 2014; Talebiyan *et al.*, 2014; Jahantigh and Esmailzade Dizaji, 2015; Mohammadi *et al.*, 2018).

The present study aimed (i) to determine the antimicrobial resistance of commensal *E. coli* isolated from healthy ostriches (E_{ho}) and infected chickens with colibacillosis (E_{pc}) and (ii) find the relative odds of the occurrence of antimicrobial resistance in E_{ho} compared to the E_{pc} in Birjand, southeast of Iran.

Materials and Methods

Herein, we investigated the antimicrobial resistance rate of 82 *E. coli* isolates, recovered from the droppings of 54 chickens with colibacillosis (E_{pc}, n=39), and 59 healthy ostriches (E_{ho}, n=43). These strains were previously isolated, then confirmed by our earlier published work (Hosseini *et al.*, 2019), and subsequently stored at -80˚C in the archive of the Laboratory of Microbiology, Faculty of Veterinary Medicine, University of Zabol, Sistan and Baluchistan, Iran. These confirmed strains were previously isolated from the fecal samples, which were directly collected from droppings of diseased chickens with colibacillosis and healthy ostriches. The diseased chickens with colibacillosis and healthy ostriches were randomly selected from various farms(Hosseini *et al.*, 2019). Ethical approval and/or consent form was not obtained since the present research did not involve animals/human participants.

All antimicrobial agents were purchased from Padtan Teb Co., Tehran, Iran. The antimicrobial resistance rate of the E_{pc} and E_{ho} were evaluated against eight customary antibacterial agents, used in veterinary health fields, via disc diffusion method on Muller-Hinton agar (Table 1; Shahbazi *et al.*, 2018).

Table 1. The concentration and the class of the antibacterial agents used for profiling the resistant E_{pc} and E_{ho}

antibacterial agents (abbreviation)	Concentration (μg)	Class
Ampicillin (AMP)	10	Beta-lactams
Amoxicillin (AMO)	30	
Oxytetracycline (OXY)	10	Tetracyclines
Chloramphenicol (C)	30	Phenicols
Amikacin (AN)	30	Aminoglycosides
Gentamicin (GM)	10	
Lincospectin (LS)	15/200	Combined$_a$ (Lincomycin+Spectinomycin)
Sultrim (SLT)	1.25/23.75	Combined$_b$ (Sulfamethoxazole+Trimethoprim)

The antimicrobial resistance of E_{pc} and E_{ho} was interpreted as sensitive, intermediate, and resistant via the guidance of the Clinical and Laboratory Standards Institute. The isolate resistant to ≥ 3 classes of antibiotics was considered as multidrug resistance (MDR) isolate. The MDR pattern of the MDR isolate was characterized based on the number of antibiotic classes among the six classes of the tested antibiotics against resistant isolate (CLSI, 2018; Quinn *et al.*, 2002; Salari, 2020a). Furthermore, the antibiotics were listed that the isolate was resistant against to assign an organism to a particular antibiotype (Salari, 2020b).

Descriptive statistics were used to tabulate the rate of antibiotic resistance of the isolates and the MDR isolate. The frequency of the MDR patterns and antibiotypes were also tabulated. The Chi-square test was used to statistically test the association between the antibiotic and the resistance pattern of the organism.

The odds ratios (OR) were calculated to find the relative odds of the occurrence of "resistance against each antibiotic", "various patterns of MDR" and "various antibiotypes" in E_{ho} (test group) compared to those in E_{pc} (reference group) through the methodology explained by Szumilas (2010) in which the odds ratio was estimated via

OR= (a × d) / (b × c)

For OR of resistance against each antibiotic, the resistant status of the isolates against each antibiotic was defined as resistant and non-resistant. For each antibiotic, a 2 × 2 table was prepared where (a) is the number of *E. coli* that were resistant in the test group, (b) is the number of *E. coli* that were not resistant in the test group, (c) is the number of *E. coli* which were resistant in the reference group, and (d) is the number of *E. coli* which were not resistant in the reference group. For OR of various patterns of MDR, a 2 × 2 table was prepared for each pattern where (a) is the number of *E. coli* which shows specific pattern of MDR (e. g., P1; see Figure 1 and Table 2) in the test group, (b) is the number of *E. coli* which does not show the specific pattern of MDR (i. e., P1; see Figure 1 and Table 2) in the test group, (c) is the number of *E. coli* which show the specific pattern of MDR in the reference group, and (d) is the number of *E. coli*, which does not show the specific pattern of MDR in the reference group. For OR of various antibiotypes, a 2 × 2 table was prepared for each antibiotypes where (a) is the number of *E. coli* which shows specific antibiotypes (for example, AN-GM-AMP-C; see Figure 1 and Table 2) in the test group, (b) is the number of *E. coli* which does not show the specific antibiotypes (for example, AN-GM-AMP-C; see Figure 1 and Table 2) in the test group, (c) is the number of *E. coli* which show the specific pattern of MDR in the reference group, and (d) is the number of *E. coli*, which does not show the specific pattern of MDR in the reference group. Once zeros caused problems with computation of the odds ratio or its standard error, 0.5 was added to all the cells of the 2 × 2 table (a, b, c, d).

The statistical tests were performed via SPSS software. *P* values of < 0.05 were considered for statistical significance in the Chi-square test. For OR results, if the 95% confidence interval spans 1.0, the OR did not reach the statistical significance.

Results

Determination of the antimicrobial resistance rate of E_{ho} and E_{pc}

As can be seen in Figure. 1A, E_{ho} showed the highest resistance against ampicillin, amoxicillin, and gentamicin (100%) followed by amikacin (83.7%), oxytetracycline (27.9%), and chloramphenicol (4.7%), and the least resistance was observed against both sultrim and lincospectin (2.3%). There is a statistically significant association between the antibiotic and resistance pattern of E_{ho} ($\chi(14) = 310.1$, $P = .000$).

As can be seen in Figure. 1A, E_{pc} showed the highest resistance against ampicillin (100%) followed by amoxicillin (94.9%), gentamicin (94.9%), amikacin (84.6%) oxytetracycline (69.2%), chloramphenicol (64.1%), and sultrim (61.5%), and the least resistance was observed against lincospectin (35.9%). There was a statistically significant association between antibiotic and resistance patterns of E_{pc} ($\chi(14) = 90.8$, $P = .000$).

Determination of the MDR rate, MDR pattern, and antibiotypes of E_{ho} and E_{pc}

As represented in Figure 1B, 30.2% (13/43) of E_{ho} were MDR. Among MDR isolates, two different MDR patterns (P1 (60%) and P3 (10%)) were detected (Figure 1B). The majority of MDR E_{ho} with statistically significant difference showed P1 MDR pattern (resistance to three antibiotics; $\chi(1) = 9.3$, $P = .000$). Seven antibiotypes belonged to E_{ho} (Figure 1C). The prevalent antibiotype of E_{ho} was AMO-AN-GM-AMP (62.8%; $\chi(3) = 33.5$, $p = .000$; Figure 1C).

As can be seen in Figure 1B, 87.2% (34/39) of E_{pc} isolates were MDR. Among MDR isolates, four different MDR patterns (P1 (40%); P2 (100%); P3 (90%); P4 (100%)) were detected (Figure 1B). The majority of MDR E_{pc} with no statistically significant differences showed P4 (resistance to six antibiotics; $\chi(3) = 1.5$, $P = 0.675$) MDR pattern. Twenty-one antibiotypes belonged to E_{pc} (Figure 1C). The prevalent antibiotype of E_{pc} was AMO-AN-LS-GM-AMP-C-OXY-SLT (20.51%; $\chi(3) = 4.6$, $p = .204$; Figure 1C).

Determination of the relative odds of antibacterial resistance and MDR patterns and antibiotypes of E_{ho} compared to E_{pc}

As depicted in Table 2, the odds of resistance against ampicillin (1.1), amoxicillin (5.8), and gentamicin (5.8) in E_{ho} were more than those in E_{pc} while the odds of resistance to amikacin (0.93), oxytetracycline (0.17), chloramphenicol (0.02), sultrim (0.00), and lincospectin (0.04) in E_{ho} were less than those in E_{pc}. Statistically, the results observed for ampicillin, amoxicillin, gentamicin, and amikacin were not significant, indicating no differences between E_{ho} and E_{pc} regarding the odds of resistance to ampicillin, amoxicillin, gentamicin, and amikacin.

As to be seen in Table 2, the odds of the presence of P2 (0.2), P3 (0.2), and P4 (0.1) in the test group (E_{ho}) were insignificantly lower than those in the reference group (E_{pc}), except for the odds of the presence of P1 (39), which was higher in E_{ho} compared to that in E_{pc} with a statistically significant difference. As shown in Table 2, out of 23 discriminated antibiotypes, the odds of the presence of AMO-AN-GM-AMP (11.5) and AMO-AN-LS-GM-AMP-C-OXY-SLT (0.0) were statistically in favor of the test group (E_{ho}) and the reference group (E_{pc}), respectively. Notably, with no statistically significant differences, the odds of the presence of three antibiotypes (AMO-AN-GM-AMP-OXY, AMO-GM-AMP, and AMO-GM-AMP-OXY) and 18 antibiotypes (i. e., the rest of antibiotypes) were respectively higher and lower in E_{ho} compared to that in E_{pc} (Table 2), indicating no differences between E_{ho} and E_{pc} regarding the odds of occurrence of 22 antibiotypes among 23 discriminated antibiotypes.

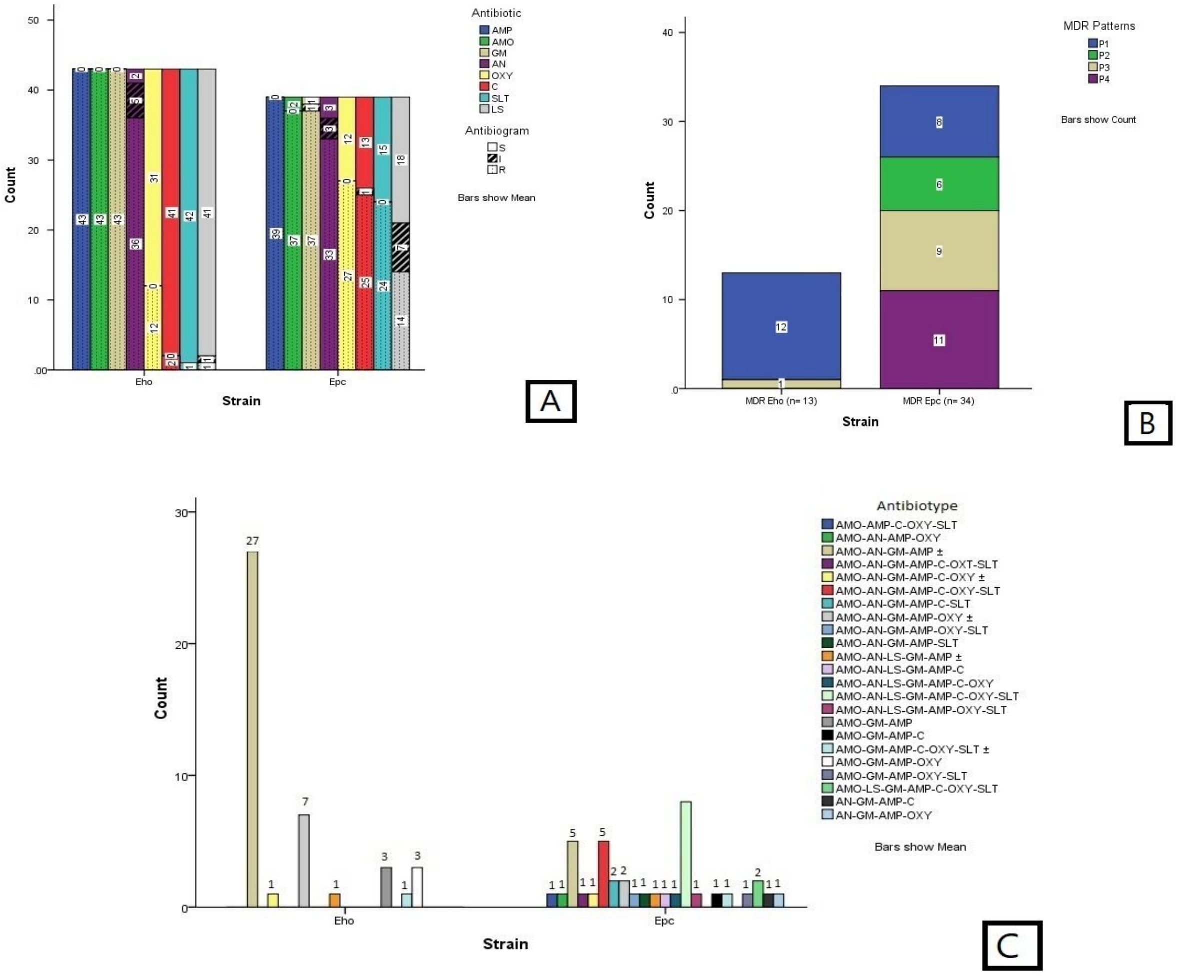

Figure 1. The number of different phenotypes of antibacterial resistance (A), MDR patterns (B), and antibiotypes (C) of the commensal *Escherichia coli* isolated from the healthy ostriches (E_{ho}; n = 43) and infected chickens with colibacillosis (E_{pc}; n = 39); MDR: multidrug resistance; Resistant to 3 (P1), 4 (P2), 5 (P3), and 6 (P4) antibiotic classes out of the six antibiotic classes tested; LS: Lincospectin; SLT: Sultrim; C: Chloramphenicol; OXY: Oxytetracycline; AN: Amikacin; GM: Gentamicin; AMO: Amoxicillin; AMP: Ampicillin; $^{\pm}$ indicates antibiotypes common between E_{pc} and E_{ho}.

Table 2. The relative odds [OR (95% CI)] of the various antibiogram profiling of E_{ho} compared to E_{pc}

Various antibiogram profiling	*Categorization*	*OR*
ANTIBACTERIAL RESISTANCE	Ampicillin	1.10 [0.0 - 56.8]
	Amoxicillin	5.80 [0.3 - 124.7]
	Gentamicin	5.80 [0.3 - 124.7]
	Amikacin	0.93 [0.3 - 3.1]
	Oxytetracycline	0.17 [0.1 - 0.4] #
	Chloramphenicol	0.02 [0.0 - 0.1] #
	Sultrim	0.00 [0.0 - 0.1] #
	Lincospectin	0.04 [0.0 - 0.3] #
PATTERN of MDR	P1	39 [4.4 – 348.0] #
	P2	0.2 [0.0 - 3.1]
	P3	0.2 [0.0 - 2.0]
	P4	0.1 [0.0 - 1.4]
	Total	0.1 [0.0 to 0.2] #
ANTIBIOTYPE	AMO-AMP-C-OXY-SLT	0.3 [0.0 - 7.4]
	AMO-AN-AMP-OXY	0.3 [0.0 - 7.4]
	AMO-AN-GM-AMP ±	11.5 [3.7 - 35.3] #
	AMO-AN-GM-AMP-C-OXT-SLT	0.3 [0.0 - 7.4]
	AMO-AN-GM-AMP-C-OXY ±	0.9 [0.0 – 15.0]
	AMO-AN-GM-AMP-C-OXY-SLT	0.1 [0.0 - 1.3]
	AMO-AN-GM-AMP-C-SLT	0.2 [0.0 - 3.7]
	AMO-AN-GM-AMP-OXY ±	3.6 [0.7 - 18.5]
	AMO-AN-GM-AMP-OXY-SLT	0.3 [0.0 - 7.4]
	AMO-AN-GM-AMP-SLT	0.3 [0.0 - 7.4]
	AMO-AN-LS-GM-AMP ±	0.9 [0.0 – 15.0]
	AMO-AN-LS-GM-AMP-C	0.3 [0.0 - 7.4]
	AMO-AN-LS-GM-AMP-C-OXY	0.3 [0.0 - 7.4]
	AMO-AN-LS-GM-AMP-C-OXY-SLT	0.0 [0.0 - 0.7] #
	AMO-AN-LS-GM-AMP-OXY-SLT	0.3 [0.0 - 7.4]
	AMO-GM-AMP	6.8 [0.3 - 136.5]
	AMO-GM-AMP-C	0.3 [0.0 - 7.4]
	AMO-GM-AMP-C-OXY-SLT ±	0.9 [0.0 – 15.0]
	AMO-GM-AMP-OXY	6.8 [0.3 - 136.5]
	AMO-GM-AMP-OXY-SLT	0.3 [0.0 - 7.5]
	AMO-LS-GM-AMP-C-OXY-SLT	0.2 [0.0 - 3.7]
	AN-GM-AMP-C	0.3 [0.0 - 7.5]
	AN-GM-AMP-OXY	0.3 [0.0 - 7.5]
	Total	1.1 [0.0 - 56.8] #

E_{pc}: *E. coli* isolated from infected chickens with colibacillosis; E_{ho}: *E. coli* isolated from apparently healthy ostriches; OR: odd ratio; CI: confidence interval; # indicates statistical significance; MDR: multi-resistance; Resistance to 3 (P1), 4 (P2), 5 (P3), and 6 (P4) antibiotic classes out of six antibiotic classes tested; LS: Lincospectin; SLT: Sultrim; C: Chloramphenicol; OXY: Oxytetracycline; AN: Amikacin; GM: Gentamicin; AMO: Amoxicillin; AMP: Ampicillin; ± indicates antibiotypes which are common between E_{pc} and E_{ho}.

Discussion

Poultry is one of the most widespread food industries in the world. Chicken is the most commonly farmed species in Iran. Ostrich meat is introduced as a suitable alternative to beef, making ostrich important livestock for several countries, such as Iran (Hosseini *et al*., 2019). The magnitude of the ostrich is expected to be discussed considerably over the coming years on account of the intense ostrich farming practices next to the chicken farming practices in numerous developing countries(Amani *et al*., 2020).

In the present study, we focused on the antibiotic resistance of the known member of the bacterial flora of the gastrointestinal tract (*E. coli*) of apparently healthy ostriches (E_{ho}) and infected chickens (E_{pc}). On the other hand, the OR is one of the most commonly used measures of association in preventive medicine (Persoskie and Ferrer, 2018).

Determination of the antimicrobial resistance rate of E_{ho} and E_{pc}

In the present investigation, E_{ho} indicated higher resistance against six antibiotics, including ampicillin (100%), amoxicillin (100%), gentamicin (100%), amikacin (83.7%), oxytetracycline (27.9%), and chloramphenicol (4.7%) and lower resistance against two antibiotics, including sultrim (2.3%) and lincospectin (2.3%) compared to the other investigation which revealed that the mean frequency of the resistant isolates of E_{ho} against ampicillin, amoxicillin, gentamicin, amikacin, oxytetracycline, chloramphenicol, sultrim, and lincospectin was 77.2%, 10.5%, 22.6%, 28.1%, 19.9%, 3.6%, 9.4%, and 25%, respectively (Rezaei Far *et al*., 2013; Hemmatinezhad *et al*., 2015; Mohamadi *et al*., 2015; Amani *et al*., 2020).

In previous studies, the mean frequency of the resistant isolates of E_{pc} against ampicillin, amoxicillin, gentamicin, amikacin, oxytetracycline, chloramphenicol, sultrim, and lincospectin have been reported to be 70%, 65%, 23%, not reported, 74%, 58%, 51%, and 51%, respectively (Zakeri and Kashefi, 2012; Rahimi, 2013; Kazemnia *et al*., 2014;

Talebiyan *et al*., 2014; Jahantigh and Esmailzade Dizaji, 2015; Seifi *et al*., 2015; Hasani *et al*., 2017; Mohammadi *et al*., 2018). For E_{pc}, the frequency of resistant isolates against five antibiotics, including ampicillin (100%), amoxicillin (94.9%), gentamicin (94.9%), chloramphenicol (64.1%), and sultrim (61.5%) was higher in the study area compared to that in the previous works. On the other hand, the frequency of resistant isolates against two antibiotics, including oxytetracycline (69.2%) and lincospectin (35.9%), was lower in the study area compared to that in the previous works. Notably, according to our literature review, the frequency of resistant E_{pc} against amikacin have not been reported; thus, the present study revealed the resistant isolates of E_{pc} against amikacin (84.6%) evidently for the first time in Iran (Zakeri and Kashefi, 2012; Rahimi, 2013; Kazemnia *et al*., 2014; Talebiyan *et al*., 2014; Jahantigh and Esmailzade Dizaji, 2015; Seifi *et al*., 2015; Hasani *et al*., 2017; Mohammadi *et al*., 2018).

The massive use of antibiotics as a growth promoter, the time and place of the study, properties of the host, and the rate and usage of the antibiotics, in particular for diseased chickens, may explain our findings regarding E_{pc}, albeit, more investigation is required. Moreover, a low rate of antimicrobial resistance against lincospectin and sultrim, which was observed in both E_{ho} and E_{pc}, may indicate low and limited use in the poultry treatment industry of the study area (Nhung *et al*., 2017; Boireau *et al*., 2018). For note, *E. coli* isolate of apparently "healthy" ostriches were resistant against the combination of antibiotics, including sultrim and lincospectin in the present study, however, further investigations are required to reveal the reason behind the presence of sultrim and lincospectin-resistant *E. coli* isolate in apparently healthy ostriches.

Determination of the MDR rate, MDR pattern, and antibiotypes of E_{ho} and E_{pc}

Regarding the MDR pattern and antibiotype of E_{ho}, our results were fairly different from those of other investigations (Rezaei-Far *et al*., 2013; Mohamadi *et al*., 2015; Amani *et al*., 2020). E_{ho} showed the P1 MDR pattern as the prevalent MDR pattern among two MDR patterns (P1 and P3; $P < 0.05$) in the present study while Amani *et al*. (2020) reported that P3 (3%) was the prevalent MDR pattern followed by P2 (2%) and both P1 (0%) and P4 (0%); on the other hand, Mohamadi *et al*. (2015) illustrated that resistance to two antibiotics was the prevalent MDR pattern (28/33) followed by resistance to three antibiotics (3/33) and resistance to four antibiotics (2/33) in commensal isolates of *E. coli* recovered from ostriches. E_{ho} showed seven antibiotypes among which AMO-AN-GM-AMP was the most prevalent one in the present study while 23 antibiotypes were described among the ostrich *E. coli* isolates in the study of Mohamadi *et al*. (2015), in which the cefoxitin-tetracycline resistance pattern was the most prevalent one. Rezaei-Far *et al*. (2013) showed that the MDR pattern was different and ranged from 2 to 12 drugs. Rezaei-Far *et al*. (2013) suggested 30 antibiotypes in which both carbenicillin-erythromycin-colistin-ampicillin-amoxicillin/clavulanate and carbenicillin-erythromycin-colistin-cephalothin-ampicillin-amoxicillin/clavulanate resistant patterns were the most prevalent ones.

Albeit, several investigations from different regions of Iran, including Kermanshah (Rahimi, 2013), Tabriz (Zakeri and Kashefi, 2012; Hasani *et al*., 2017), Shahrekord (Talebiyan *et al*., 2014), West Azerbaijan (northwestern Iran; Mohammadi *et al*., 2018), and Zabol (southeast of Iran; Jahantigh and Esmailzade Dizaji, 2015) revealed the rate of resistance of E_{pc} against ampicillin, amoxicillin, gentamicin, oxytetracycline, chloramphenicol, sultrim, and lincospectin; none of them reported the MDR pattern and antibiotype in their studies. However, just one study from Mazandaran province, Iran (Seifi *et al*., 2015) reported that both P2 and P3 (12/53) were the prevalent MDR patterns followed by P4 (11/53) and P1 (6/53), which is inconsistent with our results, indicating the equal distribution of four (P1-4) MDR patterns in MDR E_{pc}. Moreover, 14 antibiotypes in the study of Seifi *et al*. (2015) were described among the diseased chicken-origin *E. coli* isolates, in which the ampicillin-tetracycline-oxytetracycline-erythromycin-flumequine resistance pattern (12/53) was the most prevalent (Seifi *et al*., 2015); this is not in line with our findings indicating the presence of 21 antibiotypes in E_{pc}.

The presence of MDR pattern and antibiotype in E_{ho} threatens public health since the healthy ostriches might play a role in the distribution of the resistant *E. coli*, in particular against sultrim and lincospectin, and the MDR *E. coli* by their fecal dropping. The feces of the healthy ostriches contained resistant/MDR *E. coli* strains. As a result, due to the possible contamination of meat, carcasses, and eggs, the feces of apparently healthy ostriches may play a role in transferring or distributing these strains into the environment and consequently, to human or other animal populations. On the other hand, the presence of MDR pattern and antibiotype in E_{pc} sheds light on the fact that not only one antibiotic may not treat the disease in chickens, but antibiotic susceptibility testing is also required to select the best strategy for antibiotic therapy (Nhung *et al*., 2017; Boireau *et al*., 2018). Significant differences in the management of different farms, inappropriate and/or massive use of antibiotics in the form of therapies, metaphylaxis, and prophylaxis are the underlying causes of the difference in antibiotic resistance patterns. Moreover, the presence of resistant isolates at the level of farms would cause problems for the human community in addition to the high cost of treatment of colibacillosis, which requires further investigation (Nhung *et al*., 2017; Boireau *et al*., 2018).

Determination of the relative odds of antibacterial resistance and MDR patterns and antibiotypes of E_{ho} compared to E_{pc}

To the best of our knowledge, this is the first report for the calculation of the odds ratio of the antibacterial resistance, MDR patterns, or antibiotypes of E_{ho} compared to those of E_{pc}; therefore, discussions about the odd ratio and comparison with the literature are limited (Zakeri and Kashefi, 2012; Rahimi, 2013; Rezaei Far *et al*., 2013; Kazemnia *et al*., 2014; Talebiyan *et al*., 2014; Hemmatinezhad *et al*., 2015; Jahantigh and Esmailzade Dizaji, 2015; Mohamadi *et al*., 2015; Seifi *et al*., 2015; Hasani *et al*., 2017; Mohammadi *et al*., 2018; Amani *et al*., 2020).

Given the odds of the presence of the antibacterial resistance, MDR patterns, or antibiotypes, our findings provided evidence concerning the threat for human health and veterinary health because of the following reasons: (I) the gastrointestinal tract of the healthy ostriches could harbor *E. coli* strains resistant against four customary antibacterial agents used in veterinary medicine fields (ampicillin, amoxicillin, gentamicin, and amikacin), more likely than diseased chickens with colibacillosis; (II) the *E. coli* of apparently healthy ostriches, like the *E. coli* of diseased chickens with colibacillosis, can host various MDR patterns/antibiotypes and consequently, contaminate the carcasses, meat, and egg of ostriches in the study area (Mohammadi *et al*., 2018; Nhung *et al*., 2017; Scerbova and Laukova, 2016; Szumilas, 2010).

Biological features of isolates, point mutations in the chromosome of resistance phenotypes of *E. coli,* and particularly, horizontal gene transfer, as a key source to receive and disseminate resistance genes, may explain our findings, which should be carefully studied (Nhung *et al*., 2017; Sadeghi Bonjar *et al*., 2017; Boireau *et al*., 2018). The conjugation in bacteria is considered a common way to transfer resistant genes (Græsbøll *et al*., 2014). Furthermore, the gene transfer rate differs in various media and environments (Quinn *et al*., 2002). A selective antibiotic treatment-associated pressure, even inside the body of birds, might be the reason behind the fluctuation of antibiotic resistance, which consequently might produce different patterns of MDR and/or antibiotype (Nhung *et al*., 2017; Boireau *et al*., 2018).

It is noteworthy that a drawback of our study was the study subjects which were the archived strains. We could thus suggest a larger sample size of fresh *E. coli* isolated from two hosts (apparently healthy ostriches and diseased chickens with colibacillosis) associated with wider geographical distribution. However, it can be inferred from our analysis that it is likely to accelerate the development of antibiotic resistance in *E. coli* organisms in ostriches and chickens. In addition, it is possible to expand antibiotic-resistant *E. coli* between two hosts (ostriches and chickens) if they are reared, slaughtered, and processed close to each other. In addition to the concerns due to the emergence of antibiotic resistance in bacteria from poultry production, there are human health concerns about the presence of antimicrobial residues in meat, carcasses, and eggs of ostriches (Nhung *et al*., 2017).

In conclusion, our results implied the importance of further scrutiny and monitoring measures for preventive veterinary medicine, regarding *E. coli* in various hosts. This study will improve the treatment and control of colibacillosis in chicken cases in the study area. Our results revealed that one type of antibiotic may not support the colibacillosis in chickens because of the presence of various MDR patterns/antibiotypes in E_{pc}, which emphasize the valuability of the antibiotic susceptibility testing for these strains. Furthermore, the higher odds of the presence of some MDR pattern/antibiotype in E_{ho} compared to those in E_{pc}, besides the presence of various MDR pattern/antibiotype in E_{ho}, revealed that the gastrointestinal tract of the healthy ostriches can harbor *E. coli* strains resistant against certain MDR pattern/antibiotype more likely than that of diseased chickens with colibacillosis; this could be regarded as a threat for human and veterinary health. We could suggest a larger sample size of fresh *E. coli* associated with wider geographical/host distributions in future investigations.

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ORCID

Saeed Salari
https://orcid.org/0000-0003-2460-9518

Afsaneh Hoseini
https://orcid.org/0000-0001-8783-0266

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