

Geographical and seasonal variation in antimicrobial susceptibility of *Escherichia coli* isolated from broiler chicken carcasses in Iran

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ABSTRACT

Avian colibacillosis is responsible for large economic losses in poultry industry. Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. Antimicrobial-resistant *Escherichia coli* can be transferred from animals to humans through consumption of contaminated food and it causes a public health risk. The present study was conducted to estimate the Geographical and seasonal variation in antimicrobial susceptibility of *Escherichia coli* isolated from broiler chicken in Iran. A total of 401 samples were collected in two geographical regions in Iran. 184 isolates of *E. coli* from North of Iran and 217 isolates of *E. coli* from Northwest of Iran were collected since 2012 to 2013, and were analyzed for resistance to 7 antimicrobial agents. Statistical analysis was carried out using SPSS 16.0 where applicable. Antibigrams revealed that the highest level of resistance was observed against Sulfamethoxazole+trimethoprim (Sultrim[®]) (69.4%) and Enrofloxacin (53.2%) in North and Sulfamethoxazole+trimethoprim (Sultrim[®]) (53.9%) and Doxycycline (34%) in Northwest of Iran. The most sensitive antibiotics in North were Colistin and Lincomycin+Spectinomycin (Lincospectine[®]) (20.3% and 13.3% respectively); and in Northwest Colistin and Lincomycin+Spectinomycin (Lincospectine[®]) (14.8% and 3.8% respectively). Multi drug resistance was also detected in high percentage of *E. coli* isolates. There were some differences in susceptibility pattern of *E. coli* isolates between seasons of year and two Geographical regions. This study showed that very high level of resistance to some common antibiotics in poultry farms causes treatment failure, because they do not have remedial effects.

Key words: *Escherichia coli*, Antibigram, Broiler chicken, Antimicrobial-susceptibility

INTRODUCTION

The poultry industry plays a very important role in national economy of Iran. The continuing regularly rise in human population in Iran and the background of food requirements have contributed to the development of this important industry. Throughout the past 30 years, a considerable number of private and state firms have been established in Iran [1].

Escherichia coli is the most common pathogen isolated from broiler chickens. *E. coli* are Gram negative bacteria under the family Enterobacteriaceae and widely distributed in nature, the important reservoir of *E. coli* is the intestinal tract of Humans and poultry [2]. *E. coli* cause colibacillosis in chickens, Avian colibacillosis is responsible for large economic losses in poultry industry which is characterized by cellulitis, omphalitis, air sacculitis,

salpingitis, meningitis, endocarditis, urinary tract infection, swollen head syndrome, coligranuloma, and colibacillosis peritonitis, synovitis and coligranuloma [3],[4]. *E. coli* may be sensitive to many antibiotics, but also the *E. coli* isolated from chicken are frequently resistant to one or more antibiotics.

Antibiotics are extensively used in poultry industry for the function of growth promoter and control infectious diseases. There is clear evidence of abuse of antibiotics causes continuously increasing multi-drug resistant of *E. coli* [5]. This condition is due to uncontrolled use of antimicrobial drugs in poultry industry without former testing that might have resulted antibiotic resistance causing a important problem because it limits the therapeutic possibilities in the controlling of bacterial disease [5]. antimicrobial-resistant *E. coli* can be transferred from animals to humans through consumption of contaminated food and it causes a public health risk.

There are Geographical and seasonal variation in antimicrobial resistance profiles in veterinary medicine for a lot of bacteria such as *E. coli* [6], and also these variation are exist in human medicine for big range of bacteria [7],[8],[9]. The present study was designed to estimate the Geographical and seasonal variation in antimicrobial susceptibility of *E. coli* isolated from broiler chicken in two geographical region and seasons of a year in Iran.

MATERIALS AND METHODS

Sample collection

From October 2012 to October 2013, a total of 401 samples were collected in two geographical regions in Iran, 184 isolates of *E. coli* were collected from North of Iran (Behshahr) and 217 isolates of *E. coli* from Northwest of Iran (Urmia) (fig 1). The bacteria were originally recovered from a variety of tissues including the air sac, pericardial sac, heart, liver and trachea.

Isolation and identification of *E. coli*

Standard methods were used for the enrichment, isolation and identification of *E. coli* isolates. All strains were isolated from broiler chickens that had died from colisepticemia. Specimens were cultured on McConkey and EMB agar and the colonies suspected to be *E. coli* were identified by standard methods (nccl) [10].

Antimicrobial agent susceptibility testing

Antimicrobial susceptibility determination was routinely tested by the single-disc diffusion method (NCCLS, 2001) [10]. These antimicrobial agents were chosen on the basis of their importance in treating human or animal *E. coli* infections. The following antibiotic discs on Mueller Hinton agar were applied: Sulfamethoxazole+trimethoprim (SXT/ 25 µg), Enrofloxacin (NFX/5 µg), Florfenicol (FFC/ 30 µg), Colistin (CL/ 10 µg), Doxycycline (D/ 30 µg), Lincospectine (LIN / 200 µg) and Fosbac (200 µg).

The diameters of the zones of inhibition were measured by using precision callipers and were interpreted by referring to the table which represents the NCCLS subcommittee's recommendation (NCCLS, 2001) [10].

Statistical analysis

Statistical analysis was carried out by using SPSS 16.0, for investigation of seasonal pattern χ^2 -test were used as statistical methods. The geometric mean, median and mode of MIC values were determined using the Microsoft Office Excel 2013 software package.



Figure 1. Two geographical regions in Iran that samples were collected from their poultry farms. Urmia city (a) that placed in West Azarbaijan province and the other one is Behshahr city (b) that placed in Mazandran province

RESULTS

Geographical regions:

In North of Iran (Behshahr City) the highest rate of resistance were against Sulfamethoxazole+trimethoprim (Sultrim®) (69.4%), Enrofloxacin (53.2%), Felorfenicol (51.8%), Doxycycline (31%), Fosbac (27.8%), and lowest levels of resistance were against Lincomycin+Spectinomycin (Lincospectine®) (20.3%), Colistin (13.3%) (Table 1). In Northwest of Iran (Urmia City) the highest rate of resistance were against Sulfamethoxazole+trimethoprim (Sultrim®) (53.9%), Doxycycline (34%), Felorfenicol (25.7%), Fosbac (20.5%), Enrofloxacin (15.1%) and lowest levels of resistance were against Lincomycin+Spectinomycin (Lincospectine®) (14.8%), Colistin (3.8%) (Table 2). Multiple resistances were observed in all of the isolates.

Seasonal variation:

The meaningful seasonal variation in North of Iran (Behshahr City) were observed against Enrofloxacin and Doxycycline, and in Northwest (Urmia City) were observed against Colistin, Doxycycline, Lincomycin+Spectinomycin (Lincospectine) and Fosbac ($P < 0.05$). Percentage of antimicrobial sensitivity in different seasons for each geographical regions were showed completely in figure 2 and figure 3.

Table 1. Antimicrobial susceptibility of *Escherichia coli* isolated from broiler chicken carcasses in North of Iran (Behshahr)

DISC	Disc content	Total	Sensitive		Intermediate		Resistance	
			Percentage	N	Percentage	N	Percentage	N
Sultrim	25 µg	184	28.4%	(52)	2.2%	(4)	69.4%	(128)
Enrofloxacin	5 µg	184	42.4%	(78)	4.4%	(8)	53.2%	(98)
Florfenicol	30 µg	184	48.2%	(89)	-	-	51.8%	(95)
Colistin	10 µg	184	84%	(154)	2.7%	(5)	13.3%	(25)
Doxycycline	30 µg	184	58.3%	(107)	10.7%	(19)	31%	(58)
Lincospectine	200 µg	184	77.1%	(141)	2.7%	(5)	20.3%	(36)
Fosbac	200 µg	184	70%	(129)	2.2 %	(4)	27.8%	(51)

Table 2. Antimicrobial susceptibility of *Escherichia coli* isolated from broiler chicken carcasses in Northwest of Iran (Urmia)

DISC	Disc content	Total	Sensitive		Intermediate		Resistance	
			Percentage	N	Percentage	N	Percentage	N
Sultrim	25 µg	217	34.7%	(75)	11.4%	(25)	53.9%	(117)
Enrofloxacin	5 µg	217	75.7%	(164)	9.2%	(20)	15.1%	(33)
Florfenicol	30 µg	217	66%	(143)	8.3%	(18)	25.7%	(56)
Colistin	10 µg	217	87.2%	(189)	9%	(19)	3.8%	(9)
Doxycycline	30 µg	217	45.8%	(99)	20.2%	(44)	34%	(74)
Lincospectine	200 µg	217	79%	(171)	6.2%	(14)	14.8%	(32)
Fosbac	200 µg	217	71.3%	(155)	8.2%	(18)	20.5%	(44)

DISCUSSION

E. coli is one of the major pathogen responsible for various types of disease conditions in poultry leading to economic losses to poultry industry. The antibiotic sensitivity pattern showed that important emphasis need to be given for careful selection of antibiotics after antibiotic sensitivity testing and judicious use of such antibiotics [11]. The results showed that in North of Iran the most resistance was related to Sulfamethoxazole+trimethoprim (Sultrim®) (69.4%) and Enrofloxacin (53.2%), and the least resistance was related to Lincomycin+Spectinomycin (Lincospectine®) (20.3%), Colistin (13.3%). In the Northwest of Iran the most resistance was related to Sulfamethoxazole+trimethoprim (Sultrim®) (53.9%) and Doxycycline (34%), and the least resistance was related to Lincomycin+Spectinomycin (Lincospectine®) (14.8%), Colistin (3.8%). The highest sensitive drug was Colistin in both regions (sensitivity in Behshahr 84% and in Urmia 87.2%) and the lowest sensitive drug in both regions was Sultrim (sensitivity in Behshahr was 28.4% and in Urmia was 34.7%).

very high resistance to some common antibiotics may be related to bacterial properties, Blind antimicrobial therapy, irregular usage of antibiotic drugs, the lack of organization and restrictions on their use [12]. the higher incidence of antibiotic resistance observed in this study is consistent with results obtained in Algeria and Senegal [13].

Variety in Antibigram results in different studies may be related to the properties of pathogenic bacteria in any region and to the difference in usage of antibiotics. Ozawa et al. studied 83 *E. coli* strains from colibacillosis in Japan and reported that the most resistance was against ampicillin and oxytetracycline while the least resistance was against enrofloxacin and florfenicol [14].

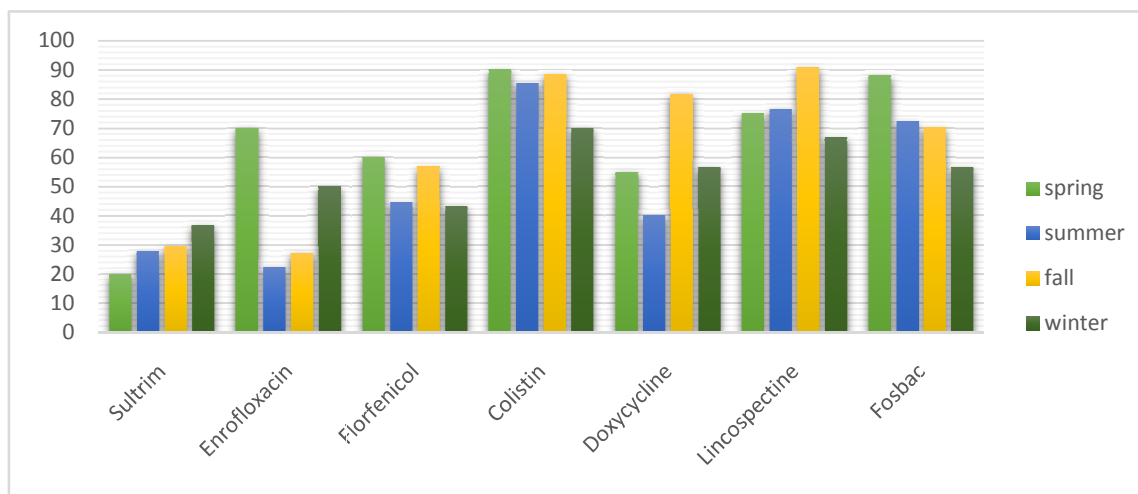


Figure 2. Seasonal variation in antimicrobial sensitivity percentage of *Escherichia coli* isolated from broiler chicken carcasses in North of Iran (Behshahr)

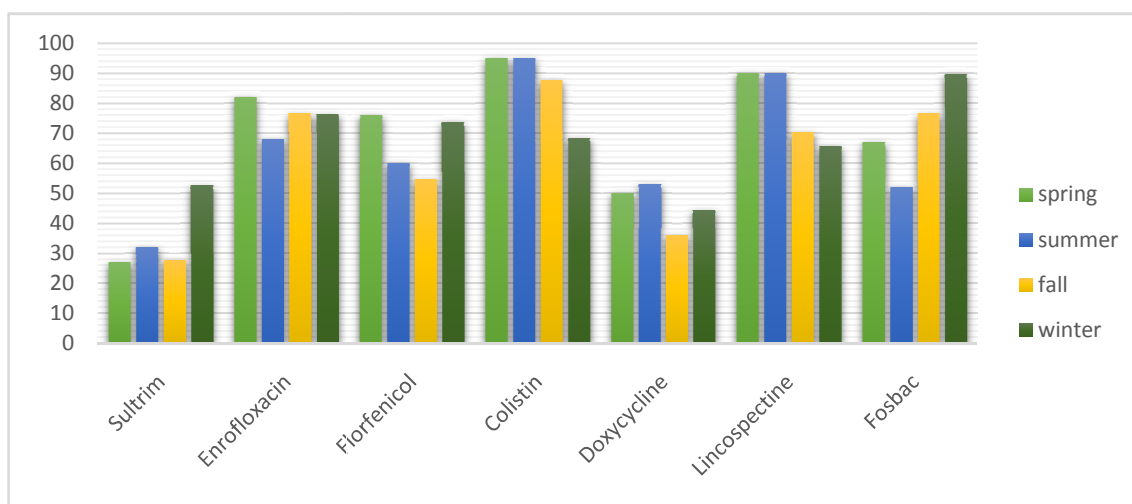


Figure 3. Seasonal variation in antimicrobial sensitivity percentage of *Escherichia coli* isolated from broiler chicken carcasses in Northwest of Iran (Urmia)

CONCLUSION

The results of this study show that very high resistance to some common antibiotics in poultry farms may cause problems of usage of these antibiotics, because they do not have positive effects, increase economic costs and cause side effects of antibiotics abuse and difficulties when antibiotic drugs remain in the flesh. The results of this study emphasize that the need for using multi-antibiotic remedial protocols for effective therapy of colibacillosis in poultry farm.

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