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Comparing the difference of antimicrobial resistance of *Escherichia coli* between broiler breeder and broiler farms with *Colibacillosis* in East Azerbaijan province

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ABSTRACT

Antimicrobial therapy is one of the important tools in reducing the economic losses in the poultry industry caused by Escherichia coli infections (colibacillosis), but the abusive use of antibiotics is the cause of the high percentages of resistance to antibiotics. At this moment there is no information available about the difference of antibiotic resistance in broiler breeder and broiler farms. To asses susceptibility of Escherichia coli to antimicrobial drugs in broiler breeder and broiler farms in East Azerbaijan, In vitro antibiotic activities of seven antibiotic substances against the Escherichia coli were determined by the standard disk diffusion method in Mueller-Hinton agar. Antibiograms in broiler breeder farms revealed that Fosbac (94.12%) has the highest sensitivity and it found to be most effective followed by Florfenicole (76.47%), Enrofloxacin (35.29%), Trimethoprim-Sulfamethoxazole (29.41%), Danofloxacin (23.54%), Difloxacin (17.65%) and Doxycyclin (10.34). On the other hand in broiler farms again Fosbac (92.38%) was the most effective antibiotic followed by Florfenicole (45.71%), Enrofloxacin (16.19%), Danofloxacin (9.52%), Trimethoprim-Sulfamethoxazole (5.72%), Doxycyclin (5.71) and Difloxacin (4.76%). These findings confirm significant increase in the incidence of antimicrobial resistance in the E. coli isolates and the abusive use of antibiotics can be the cause of the high percentages of resistance detected in East Azerbaijan province. Also these findings indicate that antibiotic resistance in broiler farms is higher than broiler breeder farms and it might be due to the fact that broiler breeder farms pay more attention to their farms and most of the time they use antibiograms before using antibiotics.

Keywords: Escherichia coli, antimicrobial Resistance, broiler breeder, broiler, East Azerbaijan province

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INTRODUCTION

E. coli is a major pathogen of commercially produced poultry which is responsible for large economical problems in poultry industry and it is prevalent throughout the world. It is known as one of the microbial flora of gastrointestinal tract of poultry but may become pathogenic (9, 10) and usually only certain pathogenic serotypes that show virulent factors cause conditions of disease (7).

Colibasillosis was first discovered in chickens in 1894 and since then there have been numerous reports of colibasillosis in poultry industry throughout the world. Escherichia coli is one of the main causes of morbidity in poultry and its mortality is about 5- 50 in poultry flocks (11). E. coli can be either as primary pathogen or as

secondary pathogen and Colibacillosis begins, in general, with an infection of the upper respiratory tract, followed by septicemia. In poultry it causes airsacculitis, pericarditis, peritonitis, yolk sac infection, swollen head syndrome, colisepticemia, colibacillosis, coligranuloma and death, it also affects layers resulting failure of productivity and fertility of eggs (4). It usually occurs after mycoplasmal or viral infections. Because there is not any efficient vaccine for colibacillosis, antibiotics are using widely in poultry flocks for its treatment and reducing its morbidity and mortality. Extensive use of antimicrobial drugs to prevent infections causes an increased resistance to commonly used antibiotics in the public health and veterinary sectors and it also limits the therapeutic possibilities in treatment of bacterial disease. Unfortunately this resistance to exiting antimicrobials is widespread and this antimicrobial resistance can be transmitted from animals to humans through consumption of contaminated food which causes a public health risk (13) and this can cause an extensive trouble in the management of infections caused by *E. coli*.

Because of the importance of *E. coli* in poultry disease and because most of the bacterial infections treated without first establishing of antibiogramm this study was designed to determine the antimicrobial resistance of Escherichia coli in poultry farms and its difference between broiler breeders flocks and broiler farms as a industry which fulfill world to animal protein through fast growing chickens.

MATERIALS AND METHODS

Samples collection The study was done in east-Azerbaijan (Iran). From 29 broiler breeder farms, aged between twenty six to seventy three week-old, A total of 145 samples and total of 145 fifteen to forty five day old birds from 29 broiler farms which showed clinical signs of colibacillosis (weight loss, acute septicemias, and death) and showing characteristic lesions at necropsy (enteritis, pericarditis, airsacculitis and perihepatitis) were collected during 2009 and 2010 and transported to laboratory. Tissues were collected based on clinical and pathognomic lesions observed during postmortem examination, in addition in all cases bacteria were obtained in profuse culture from both heart and liver tissues.

Bacteriological analysis Visceral organs such as liver and spleen were cultured on Mac Conkey agar (Merck) and incubated at 37ºC for 24 hours. The lactose fermenting colonies were subsequently cultured on Eosin Methylene Blue agar (Merck) and colonies that have a dark green, black metallic sheen transferred to nutrient agar slants and incubated at 37ºC for 24 hours and stored at 4ºC for further identification.

Identification was done according to Whitman *et al.*, (2012) following a series of biochemical tests included gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, catalase, urea hydrolysis, Gelatin hydrolysis, nitrate reduction, TSI test (16). The biochemical tests used for identification of Escherichia coli are shown in table 1.

Table 1: Biochemical tests used for identification of *Escherichia coli*

Biochemical Test Properties	*Escherichia coli* Reaction
Gram Staining	G ¯, Small Rod, Pink
EMB	black centered colony with metallic sheen
Citrate Test	-
Oxidase Test	-
Indole Test	+
Methyle Red Test	+
Voges-Proskauer Test	-
Gelatin Hydrolysis Test	+
TSI	(A/A/g+/H_2S-)
Catalase Test	+
Nitrate Reduction Test	-
Urea Hydrolysis test	+

Antimicrobial Sensitivity The antimicrobial resistance to different antibacterial agents was determined by the standard disk diffusion method in Mueller-Hinton agar according to the guidelines of the National Committee for Clinical Laboratory Standards (17). The antibiotics used for this study were Florfenicole (30), Trimethoprim-sulfamethoxazole (125/2375), Enrofloxacin (5), Danofloxacin (5), Doxycyclin (30) (Padtan Teb, Iran), Difloxacin

(10) (BBL, USA) and Fosbac (Bedson, Argentina) which are commonly using in poultry flocks in Iran. The plates were incubated for 24 h at 37°C and inhibition zones measured.

RESULTS AND DISCUSSION

Post-Mortem Examination The pathological lesions of avian collibacillosis are varied and wide but basically the observed lesions at necropsy were enteritis, pericarditis, airsacculitis and perihepatitis.

Antibiogram The sensitivity and resistance pattern for various antibiotics in broiler breeder farms and boiler farms are shown in table 2 and table 3. From the table 2 Fosbac (94.12%) showed the highest sensitivity and it found to be most effective followed by Florfenicole (76.47%), Enrofloxacin (35.29%), Trimethoprim-Sulfamethoxazole (29.41%), Danofloxacin (23.54%), Difloxacin (17.65%) and Doxycyclin (10.34).

Table 2: Antibiogram of *E. coli* in broiler breeder farms

S.NO	Antibiotic	Concentration per disc	Sensitive (%)	Moderately sensitive (%)	Resistant (%)
1.	Fosbac		94.12	-	5.88
2.	Florfenicole	30mcg	76.47	-	23.53
3.	Trimethoprim-sulfamethoxazole	125/2375	29.41	11.77	58.82
4.	Enrofloxacin	5mcg	35.29	11.77	52.94
5.	Difloxacin	10mcg	17.65	17.65	64.70
6.	Danofloxacin	5mcg	23.54	23.52	52.94
7.	Doxycyclin	30mcg	10.34	13.79	75.87

On the other hand from table 3 in broiler farms again Fosbac (92.38%) was the most effective antibiotic followed by Florfenicole (45.71%), Enrofloxacin (16.19%), Danofloxacin (9.52%), Trimethoprim-Sulfamethoxazole (5.72%), Doxycyclin (5.71) and Difloxacin (4.76%). Fosbac remained at a low level of resistance in both broiler and broiler breeder farms, because of infrequent use of this antibiotic in poultry rearing in Iran due to its high cost.

Table 3: Antibiogram of *E. coli* in broiler farms

S.NO	Antibiotic	Concentration per disc	Sensitive (%)	Moderately sensitive (%)	Resistant (%)
1.	Fosbac		92.38	1.91	5.71
2.	florfenicol	30mcg	45.71	2.87	51.42
3.	Trimethoprim-sulfamethoxazole	125/2375	5.72	0	94.28
4.	Enrofloxacin	5mcg	16.19	10.48	73.33
5.	Difloxacin	10mcg	4.76	9.53	85.71
6.	Danofloxacin	5mcg	9.52	11.44	79.04
7.	Doxycyclin	30mcg	5.71	.96	93.33

Considering the numerous types of antibiotics available in Iran and wide use of antibiotics, the higher incidence of antibiotic resistance observed in this study is predicable.

In vitro antibiotic sensitivity results obtained in our study agreed with several previous reports such as Allan (1993), Amara (1995) and Zahraei and Farashi, (2006) which have indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens with colibacillosis (2, 3, 12).

Resistance to trimethoprim-sulfamethoxazole in broiler breeder farms is 58.82% which is similar to reported by Blanco, *et al*. (1997) and Aggad et al (2010) which was 63- 76% and 70% resistance shown by them(1, 6), but higher than reports of Hammoudi and Aggad (2008) which was shown 42% resistance for trimethoprim-sulfamethoxazole (8). The broiler farms showed very high resistance to trimethoprim-sulfamethoxazole 94.28% which were in partial agreement with Zahraei and Farashi, (2006) who showed 80% resistance against trimethoprim-sulfamethoxazole (12).

In the case of Enrofloxacin in broiler breeder farms our data were in agreement with Aggad et al. (2010) (1)which were reported 45% resistance to Enrofloxacin but far higher than Sharada et al, (2010) (14) and Hammoudi et al, (2008) (8) studies which was shown 15.38% and 6% resistance by them respectively. In broiler farms our data was higher (73.33%) and were in close agreement with Zahraei and Farashi, (2006) (12) that showed 76% resistance to Enrofloxacin, because Enrofloxacin is one of the antibiotics which is using widely for treatment of mycoplasma

galysepticum and other bacterial disease and this broad use of Enrofloxacin can cause high resistance pattern for this antibiotic.

Zahraei and Farashi, (2006) and Siddiqui et al, (2008) reported a high resistance to Doxycyclin which was 88% and 71.43% and it was in close agreement with our results both in broiler breeder and broiler farms, at the same time the present findings differed with the report of Islam et al, (2004), who showed 25% resistance to Doxycyclin (12, 15).

Zahraei and Farashi, (2006) showed resistance to florfenicol (27%) which is in close agreement with our results in broiler breeder farms (23%). Also our results in broiler breeder farms are in partial agreement with Shaohua et al, (2001) which showed 10% resistance to florfenicol among *E. coli* non-O157 isolates. At the same time, the present findings in broiler farms that showed 51.42% resistance were contradictory with them (12). Zahraei and Farashi, (2006) showed resistance to Difloxacin (83%) which is similar to our results in broiler farms (85.71%) and in partial agreement with our data in broiler breeder farms (64.70%) (12).

The current study also revealed resistance to Danofloxacin in broiler breeder farms and broiler farms (52.94%) and (79.04%) respectively, these findings could not be compared due to unavailability of relevant literature.

CONCLUSION

Based on the present study, it can be concluded that that fosbac is the best antibiotic for treatment of colibasillosis and it shows the lowest resistant which is 5.88% and 5.71% in broiler breeder and broiler farms respectively, these findings could not be compared due to unavailability of relevant literature.

The current study also showed intermediate reaction to all of antibiotics which tested except for fosbac and florfenicole in broiler breeder farms. Our field observations indicate that resistance to different antimicrobial agents in broiler farms is higher than broiler breeder farms and it prove that antibiotics are using widely and abusively in broiler farms. The abusive use of antibiotics especially in broiler farms is the cause of the high percentages of resistance detected in Iran avian *E. coli* infections and this resistance to antibiotics is continuously increasing day-by-day. Such practices without prior antibiotic sensitivity testing of bacterial isolates are common especially in broiler farms. It may lead to the development of a pool of antibiotic-resistant genes and *Escherichia coli* of avian origin could act as a possible source for the transfer of antibiotic restriction to other bacterial species including human pathogens (5). Further studies are necessary in order to determine bacterium mechanisms of resistance.

REFERENCES

[1] H. Aggad, A. Ammar, A. Hammoudi, M. Kihal, *Global Vet*, **2010**, 4(3)**:** 303-306.
[2] B. J. Allan, J. V. Van Den Hurk, A. A. Potter, *Canadian Journal of Veterinary Research*, **1993**, 57(3)**:** 146.
[3] A. Amara, Z. Ziani, K. Bouzoubaa, *Veterinary Microbiology*, **1995**, 43(4)**:** 325-330.
[4] H. J. Barnes, L. K. Nolan, J. P. Vaillancourt, *In:* Y. M. SAIF (ed.) *Disease of Poultry*.(Wiley-Blackwell Publishing,Iowa, IA, **2008**) 691-737.
[5] L. Bebora, J. Oundo, H. Yamamoto, *East African Medical Journal*, **1994**, 71(10)**:** 624-627.
[6] J. E. Blanco, M. Blanco, A. Mora, J. Blanco, *J. Clin. Microbiol* **1997**, 352184-2185.
[7] E. Gonzalez, J. Blanco, S. Baloda, G. Fröman, M. Dho, J. Lafont, T. Wadström, *Microbios*, **1989**, 62(251)**:** 113-127.
[8] A. Hammoudi, H. Aggad, *Turkish Journal of Veterinary and Animal Sciences*, **2008**, 32(2)**:** 123.
[9] E. Jawetz, J. L. Melnick, E. A. Adelberg: Review of medical microbiology, Springer, **1980**.
[10] M. M. Levine, *Journal of Infectious Diseases*, **1987**, 155(3)**:** 377-389.
[11] P. Roy, P. G. Edwin, V. Purushothaman, *Indian Veterinary Journal*, **2006**, 8775-82.
[12] T. Z. Salehi, S. F. Bonab, *International Journal of Poultry Science*, **2006**, 5(7)**:** 677-684.
[13] C. M. Schroeder, C. Zhao, C. Debroy, J. Torcolini, S. Zhao, D. G. White, D. D. Wagner, P. F. Mcdermott, R. D. Walker, J. Meng, *Appl Environ Microbiol.*, **2002**, 68(2)**:** 576-81.
[14] R. Sharada, S. Wilfred Ruban, M. Thiyageeswaran, *American-Eurasian Journal of Scientific Research*, **2010**, 5(1)**:** 18-22.
[15] M. Siddiqui, L. Khan, U. Suradkar, M. Mendhe, S. Rindhe, P. Sirsat, *Veterinary World*, **2008**, 1(2)**:** 52-53.
[16] W. B. Whitman, M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, W. Ludwig, K.-I. Suzuki, A. Parte: Bergey's manual® of systematic bacteriology, Springer, **2012**.

[17] M. A. Wikler: Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement, Clinical and Laboratory Standards Institute, **2006**.