



## Antimicrobial Resistance Pattern of *Escherichia coli* Isolated from Chickens with Colibacillosis in Yazd, Iran

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### HIGHLIGHTS

- Out of 200 avian specimens, 100 (50%) were contaminated with *Escherichia coli*.
- The highest antimicrobial resistances were seen against nalidixic acid, enrofloxacin, ciprofloxacin, and erythromycin, respectively.
- The highest antimicrobial susceptibilities were seen against colistin, meropenem, and gentamicin, respectively.
- Multidrug resistance in *E. coli* isolates indicated heavy usage of drugs in poultry flocks from Yazd.

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PCR=Polymerase Chain Reaction

### ABSTRACT

**Background:** The antibiotic resistance is considered as one of the biggest public health concerns in most countries. The aim of this study was to determine antibiotic resistance pattern of *Escherichia coli* isolated from chickens with colibacillosis in Yazd, Iran.

**Methods:** A total of 200 carcasses of Ross chickens with colibacillosis were collected from farms located around Yazd, central Iran. After autopsy, specimens were collected from air bags of carcasses using sterile swaps and transferred immediately to the laboratory. After microbiological culture, the isolates were confirmed by the conventional Polymerase Chain Reaction (PCR) assay. Antimicrobial susceptibility of *E. coli* isolates were determined by disk diffusion method.

**Results:** Out of 200 specimens, 100 (50%) *E. coli* isolates were identified and confirmed by PCR method. The results of antimicrobial susceptibility tests showed the highest resistance against nalidixic acid (100%), enrofloxacin (87%), ciprofloxacin (86%), and erythromycin (82%), respectively. Also, the highest susceptibility was seen for colistin (100%), meropenem (94%), and gentamicin (93%), respectively.

**Conclusion:** In this study, multidrug resistance was observed in avian pathogenic *E. coli* isolates that represents the heavy usage of these drugs in poultry flocks in Yazd, central Iran. Improper or overuse of antibiotics usage for treatment of the poultry diseases could play an important role in spreading of the antimicrobial resistance genes among pathogenic bacteria from human and animals especially through food chain.

### Introduction

*Escherichia coli*, as a facultative anaerobic Gram-negative bacterium, is a member of the intestinal microbiota of human, animals, and birds that is often responsible for various diseases, including gastrointesti-

nal and non-gastrointestinal disorders (Guerra et al., 2003; Holko et al., 2006). Virulence factors of *E. coli* in poultry often include fimbriae, flagella, aerobactin production, bacteriocin, hemolysin, and also drug resistance (Emery et al., 1992; Foley et al., 2000). Colibacillosis is one of the infectious diseases of young poultry (4 to 9 weeks olds) that is caused by *E. coli* (Fadly and Nair,

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2008; Shane, 2001). This disease could cause pericarditis, accumulation of gelatin on the liver and peritoneum, inflatable air sacs, reducing the quality of the carcass, and increasing the cost of treatment due to resistance of bacteria to commonly used antibiotics (Salehi and Bonab, 2006; Sharada and Ruban, 2010).

The results of some studies have shown that avian pathogenic *E. coli* strains causing colibacillosis may show resistance to antibiotics. They could transfer the resistance genes to the other bacteria of human gut (Emery et al., 1992; Guerra et al., 2003). Due to inappropriate use of antimicrobial agents in the production of poultry feed and the use of the same antibiotics for treatment of human infections and poultry diseases, antibiotic resistant *E. coli* strains may be transmitted to human through the use of contaminated poultry products (Hammerum and Heuer, 2009; Johnson et al., 2005).

Given that antibiotic resistance is considered as one of the biggest public health concerns in most countries, the aim of this study was to determine antibiotic resistance pattern of *E. coli* isolated from chickens with colibacillosis in Yazd, Iran.

## Materials and methods

### Sampling

In this 4-month descriptive study, from March 2016 to July 2017, a total of 200 carcasses of Ross chickens with colibacillosis were collected from farms located around Yazd, central Iran. Studied chickens were aged between 3-5 weeks and died because of colibacillosis disease that diagnosed clinically by a trained poultry veterinarian. After the autopsy, specimens were collected from air bags of the carcasses, using sterile swaps and transferred immediately to the Laboratory of Microbiology.

### Microbial analysis

Specimens were cultured on eosin methylene blue agar medium and incubated at 37 °C for 18 h. Lactose-positive colonies were identified using biochemical tests, including fermentation of glucose and lactose with gas production in the triple sugar iron agar, production of indole and motility in SH<sub>2</sub>-indole-motility agar, the reaction of methyl red in Methyl Red-Voges Proskauer (MR-VP) broth, and growth in simmon citrate agar (Moulin-SchoUleur et al., 2006). All media were manufactured in Merck-Darmstadt Company, Germany.

### Molecular diagnosis

Boiling method was used for DNA extraction of the isolates (Saei et al., 2012). The conventional Polymerase

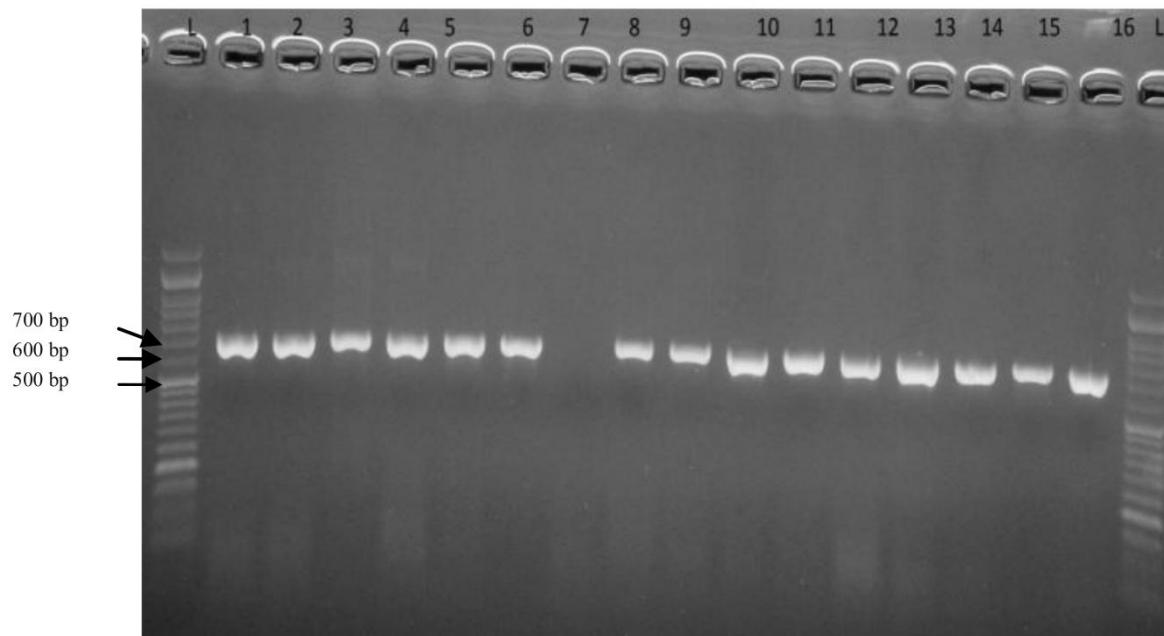
Chain Reaction (PCR) assay was performed in a 25 µl reaction mixture of PCR 2X master mix (50 mM MgCl<sub>2</sub>, 2.5 mM dNTP mixture, 0.2 units/µl Ampliqon Taq DNA polymerase; Ampliqon; Denmark), specific primer pair of Eco 2083 (5'- GCT TGA CAC TGA ACA TTG AG-3') and Eco 2745 (5'-GCA CTT ATC TCT TCC GCA TT-3') with the concentration of 10 pmol (Saei et al., 2012), as well as 5 µl DNA template (20 ng/µl). Temperature conditions of PCR were as follows: initial denaturation at 94 °C for 240 s, 35 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for 60 s, extension at 72 °C for 120 s; and final extraction at 72 °C for 600 s. After that, PCR products were analyzed using 1.5% agarose gel electrophoresis alongside with 100 bp DNA ladder.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* isolates were determined by disk diffusion method (Kirby-Bauer) according to CLSI (2016). The tested antibiotics disks (MAST, UK) were as follows: ampicillin (10 µg), chloramphenicol (28 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), cefixime (30 µg), meropenem (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), erythromycin (10 µg), nitrofurantoin (32 µg), furazolidone (34 µg), imipenem (10 µg), nalidixic acid (10 µg), gentamicin (10 µg), doxycycline (24 µg), phosphomycin (32 µg), colistin (10 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). Briefly, a bacterial suspension with a dilution equivalent to opacity of 0.5 McFarland tube was prepared (1.5×10<sup>8</sup> colony forming unit/ml). Then, the suspension was inoculated on the plates containing mueller-hinton agar medium (Merck-Darmstadt, Germany) and then antibiotics disks were put on the medium. The inoculated plates were incubated at 37 °C for 18 h. After incubation period, diameter of inhibition zones of bacterial growth around the disks was measured (in mm) and compared to the table provided in the standard protocol. Results were reported as sensitive (S), intermediate (I), and resistant (R). Standard strain of *E. coli* ATCC 25922 was used as the control.

## Results

Out of 200 specimens, 100 (50%) *E. coli* isolates were identified and confirmed using PCR method (Figure 1). The results of antimicrobial susceptibility tests presented in Table 1, showed the highest resistance against nalidixic acid (100%), enrofloxacin (87%), ciprofloxacin (86%), and erythromycin (82%), respectively. Also, the highest susceptibility was seen for colistin (100%), meropenem (94%), and gentamicin (93%), respectively.



**Figure 1:** Evaluation of *Escherichia coli* Eco gene using PCR and assessed by agarose gel electrophoresis. L: 50 bp DNA ladder; lane 1: *E. coli* ATCC 25922 (positive control); lanes 2-6 and 8-16: PCR product from isolates with 662 bp in length; lane 7: negative control

**Table 1:** Antimicrobial resistance pattern of *Escherichia coli* isolated from chickens with colibacillosis in Yazd, Iran

Antibiotics	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Ampicillin	10 (10)	13 (13)	77 (77)
Chloramphenicol	70 (70)	0 (0)	30 (30)
Ciprofloxacin	86 (86)	4 (4)	10 (10)
Enrofloxacin	87 (87)	0 (0)	13 (13)
Cefixime	12 (12)	0 (0)	88 (88)
Meropenem	3 (3)	3 (3)	94 (94)
Fosfomycin	18 (18)	0 (0)	82 (82)
Ceftriaxone	10 (10)	6 (6)	84 (84)
Cefotaxime	6 (6)	30 (30)	64 (64)
Trimethoprim/sulfamethoxazole	70 (70)	4 (4)	26 (26)
Nitrofurantoin	6 (6)	8 (8)	84 (84)
Imipenem	10 (10)	12 (12)	78 (78)
Nalidixic acid	100 (100)	0 (0)	0 (0)
Gentamicin	7 (7)	0 (0)	93 (93)
Doxycycline	58 (58)	16 (16)	26 (26)
Colistin	0 (0)	0 (0)	100 (100)
Erythromycin	82 (82)	10 (10)	8 (8)

## Discussion

*E. coli* is known as the most important member of the Enterobacteriaceae family as humans', birds', and mammals' intestinal microbiota. This bacterium is one of the most important pathogens in poultry and main cause of the overall bacillus, which is started with respiratory tract infection and continued with infection in the internal organs (Antao et al., 2008; Kwon et al., 2008; Moulin-SchoUleur et al., 2006). Poultry colibacillosis is the most economically important disease in the poultry industry in the world. The aminoglycoside family, fluoroquinolones, third and fourth generation cephalosporins are used for diseases treatment as well as the increasing of the growth rate of poultry. There is a concern in the world and Iran that the use of these antibiotics leads to antibiotic resistance transmission from poultry to humans. Heavy or inappropriate usage of antibiotics in the diet of poultry flocks transfers resistance genes through the food chain and resulted in spreading of antibiotic resistance to humans (Hammerum and Heuer, 2009).

In present study, 100 *E. coli* isolates showed the most resistance against nalidixic acid, enrofloxacin, ciprofloxacin, and erythromycin indicating high prevalence of multidrug resistance. Such high rate of multidrug resistance was similarly reported in Iran by Nasirian and Rafiei (2004) who showed that among 50 *E. coli* isolates from chicken flocks, all had multidrug resistance. They showed that the highest resistances were reported for tetracycline (94%), rifampin (90%), oxytetracycline (80%), Tiamulin (76%), chloramphenicol (58%), sulfadiazine (56%), trimethoprim/sulfamethoxazole (50%), streptomycin (48%), neomycin (48%), and ampicillin (28%). In another study conducted in Iran by Haghghi Khoshkho and Peyghambari (2004) on drug-resistant of *E. coli* isolated from chickens with colibacillosis, all of the 150 isolates were reported sensitive to gentamicin and ceftiofur. They also found that 96% isolates were resistant to colistin, nalidixic acid, erythromycin, and ampicillin. According to their study, the resistance to tetracycline, nitrofurantoin, furazolidone, enrofloxacin, norfloxacin, neomycin, chloramphenicol, ciprofloxacin, trimethoprim/sulfamethoxazole, and streptomycin were ranged between 26- 94%, indicating some variations with our findings. These differences could be due to the limited usage of some antibiotics for treatment of the broilers in Yazd province. Also, Kim et al. (2007) revealed that more than 60% *E. coli* isolated from chickens with minor injuries in South Korea were resistant to enrofloxacin, tetracycline, streptomycin, and ampicillin. In Brazil, Cunha et al. (2014) reported multidrug resistance in 92% of turkeys with airsacculitis. They showed the highest levels of resistance to sulfametazine (94%), tetracycline (83%), and erythromycin (82.6%).

The differences between the results of our study and that of Cunha et al. (2014), especially in cases of quinolones and fluoroquinolones, may be due to differences in the type of specimens, sample size, and the different policies of antibiotics usage for treatment of infections. In another study by Obeng et al. (2012) in Australia, among 251 *E. coli* strains isolated from poultry, the resistance to antibiotics are as follows: 40.6% for tetracycline, 26.7% for ampicillin, 12.4% for trimethoprim/sulfamethoxazole, 10.8% for streptomycin, 9.6% for spectinomycin, 6% for neomycin, and 2% for phenolphenicol; however, no resistances find against ceftiofur, ciprofloxacin, or gentamicin which represent appropriate usage of these antibiotic in Australia.

## Conclusion

In this study, multidrug resistance was observed in avian pathogenic *E. coli* isolates that represents the heavy usage of these drugs in poultry flocks in Yazd, central Iran. Improper or overuse of antibiotics usage for treatment of the poultry diseases could play an important role in spreading of the antimicrobial resistance genes among pathogenic bacteria of human and animals especially through food chain.

## Conflicts of interest

The authors have no conflict of interest in this research.

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