

Antibiotic Resistance Profile in Relation to Phylogenetic Background in *Escherichia coli* Isolated From Fecal Samples of Healthy Ostrich

Elham Mohamadi¹; Hesam Alizade^{2,*}; Nasrin Askari¹; Mahmood Salehi¹; Mitra Porjafarian¹; Reza Ghanbarpour^{1,3}

¹Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University, Kerman, IR Iran

²Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, IR Iran

³Zoonosis Research Committee, Kerman University of Medical Sciences, Kerman, IR Iran

*Corresponding author: Hesam Alizade, Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, IR Iran. Tel: +98-9132456562, Fax: +98-3412112794, E-mail: alizade.h2000@yahoo.com

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Background: *E. coli* is regarded as a reservoir for antibiotic resistance in foods of animal origin. *E. coli* can be categorized into four main phylogenetic groups (A, B1, B2 and D). The commensal *E. coli* strains mostly are assigned to the phylo-groups A and B1.

Objectives: The purposes of this study were to determine the phylogenetic group/subgroups and antibiotic resistance patterns of ostrich *E. coli* isolates in Iran.

Materials and Methods: A total of 126 *E. coli* isolates were obtained from cloacae swabs of the healthy ostrich in Kerman, Iran. The *E. coli* isolates were confirmed using biochemical API 20E identification system. The confirmed isolates were studied to determine phylogenetic background by PCR. The isolates were tested for antibiotic resistance against 12 different antibiotic disks by disk diffusion method.

Results: Phylotyping of *E. coli* isolates indicated that 74 isolates belonged to A, 27 isolates to B1, 7 isolates to B2, and 18 isolates to D groups. Also the isolates fell into six phylogenetic subgroups, including 34 isolates in A₀, 40 isolates in A₁, one isolate in B2₂, 6 isolates in B2₃, 11 isolates in D₁ and 7 isolates in subgroup D₂. In the examined *E. coli* isolates, the maximum rate of resistance was against tetracycline, and the minimum rate of resistance was against amoxicillin. Twenty three antibiotic resistance patterns were detected among the isolates. The cefoxitin and tetracycline resistance pattern was the most prevalent in the isolates that belonged to phylo-group A.

Conclusions: In conclusion, the result of the present study revealed a low frequency of antibiotic resistance in ostrich *E. coli* isolates. The antibiotic resistance patterns were in relation to A and D phylogenetic groups. Further studies are needed to better understand the distribution of phylogenetic groups in poultry isolates.

Keywords: *Escherichia coli*; Antibiotic Resistance; Ostrich

1. Background

Since 1990s Iran started to import ostriches. In recent years, the ostrich farming industry has expanded significantly and even is facing a competitive market for its products now (1). *Escherichia coli* (*E. coli*) is a major component of commensals of the gastrointestinal tract of animals and humans (2). Commensal bacteria are able to inhabit in the gut without being omitted, playing a major role in human health and nutrition, by elevating nutrient supply, preventing pathogen colonization and preserving the homeostasis of the intestinal immune system (3). 10 to 15% of *E. coli* isolates are pathogenic; nevertheless most isolates are nonpathogenic which considered being indicators of fecal contamination of food (4). Previous study showed that mostly of the used antibiotics are administered to livestock for the goal of growth promotion or infection treatment (5). *E. coli* is regarded as a serving reservoir for antibiotic resistance and plays a predominant role in many existing surveillance systems for antibiotic resistance in animals and foodstuffs (4). *E.*

coli can be assigned to four main phylogenetic groups (A, B1, B2 and D) and six phylogenetic sub-groups (A₀, A₁, B2₂, B2₃, D1 and D2). The commensal strains mostly are categorized into the phylo-groups A and B1. On the other hand, intestinal pathogenic strains are usually classified into groups A, B1 and D and extra-intestinal *E. coli* infections mainly belong to group B2 and, to a lesser extent, group D (6, 7).

2. Objectives

The ostrich is an important animal in the commercial farming section. The expanded of antibiotic resistance among bacterial pathogens in foods of animal origins is a major public health concern in human healths. Currently, little information is available about the phylogenetic group/subgroups and antibiotic resistance patterns of ostrich *E. coli* isolates. This is the first study in Iran on the status of phylogenetic background of ostrich.

3. Materials and Methods

3.1. *E. coli* Isolates

In this study 126 *E. coli* isolates were obtained from cloacae swabs of the healthy Ostrich in Kerman, Iran. The samples were directly streaked on Mac-Conkey and EMB agar (Biolife Laboratories, Milan, Italy) for isolation of *E. coli*. The *E. coli* isolates were confirmed using biochemical API 20E identification system (BioMe[®] rieux, Marcy l'Etoile, France). All isolates were stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30% sterile glycerol at -70°C.

3.2. Antibiotic Susceptibility

The antibiotic susceptibility of the studied strains to gentamicin, streptomycin, ciprofloxacin, sultrim, tetracycline, cephalixin, bacitracin, cefazolin, erythromycin, amoxicillin, chloramphenicol and ceftiofur were determined by using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI 2013) (8) using commercial antimicrobial disks (Pad Tan Teb. Co., Iran).

3.3. Phylogenetic Group/Subgroups

The DNA of the *E. coli* isolates were extracted by boiling. The triplex PCR assay was used to assign the *E. coli* isolates. Strains were categorized to phylogenetic group/subgroups on the basis of presence or absence of the *chuA*, *yjaA* genes and an anonymous DNA fragment, TspE4.C2, which each was subdivided as follows: *chuA* -, *yjaA* -, TspE4.C2 -, group A subgroup A₀; *chuA* -, *yjaA* +, TspE4.C2 -, group A subgroup A₁; *chuA* -, *yjaA* -, TspE4.C2 +, group B1; *chuA* +, *yjaA* +, TspE4.C2 -, group B2 subgroup B₂; *chuA* +, *yjaA* +, TspE4.C2 +, group B2 subgroup B₂; *chuA* +, *yjaA* -, TspE4.C2 -, group D subgroup D₁; *chuA* +, *yjaA* -, TspE4.C2 +, group D subgroup D₂ (6, 7). One strain from the ECOR collection was used as positive control for phylogenetic grouping: ECOR62 and *E. coli* strain MG1655 as a negative control for phylogenetic background. The primers used in this study are listed in Table 1 according to the Clermont et al. (6) method. All the reference strains were from the bacterial culture collection of Microbiology Department of Ecole Nationale Veterinaire Toulouse, France.

4. Results

Phylogenetic results showed that 126 *E. coli* isolates segregated into four phylo-groups including 74 isolates (58.73%) in A, 27 isolates (21.42%) in B1, 7 isolates (5.55%) in B2, and 18 isolates (14.28%) in D groups. Phylotyping of studied isolates showed that the isolates fell into six subgroups, including 34 isolates (26.98%) in A₀, 40 isolates (31.74%) in A₁, one isolate (0.79%) in B₂, 6 isolates (4.76%) in B₂, 11 isolates (8.73%) in D₁ and 7 isolates (5.55%) in subgroup D₂ (Figure 1).

According to the disk diffusion results, the maximum rate of resistance in *E. coli* isolates were against tetracycline (21.42%), ceftiofur (15.87%) and streptomycin (11.90%)

(Table 2). Twenty three antibiotic resistance patterns in the present study were described among the ostrich *E. coli* isolates. The ceftiofur and tetracycline resistance pattern was the most prevalent pattern in the isolates which fell into phylogenetic group A. The antibiotic resistance patterns were distributed among the isolates in the four phylogenetic groups (Table 3).

Table 1. Primers Used in This Study

| Primer Sequence (5'-3') | Annealing Temp, °C | Product Size, bp |
|-------------------------|--------------------|------------------|
| TspE4.C2 | 55 | 152 |
| CTGGCGAAAGACTGTATCAT | | |
| CGCGCCAACAAAGTATTACG | | |
| yjaA | 55 | 211 |
| TGAAGTGTCTCAGGAGACGCTG | | |
| ATGGAGAATGCGTTCCTCAAC | | |
| chuA | 55 | 279 |
| GACGAACCAACGGTCAGGAT | | |
| TGCCGCCAGTACCAAAGACA | | |

Table 2. Antibiotic Resistance of *E. coli* Strains Isolated From Fecal Samples of Healthy Ostrich^a

| Antibiotic | Healthy Ostrich |
|-----------------|-----------------|
| Gentamicin | 10 (7.93) |
| Streptomycin | 15 (11.90) |
| Ciprofloxacin | 8 (6.34) |
| Sultrim | 7 (5.55) |
| Tetracycline | 27 (21.42) |
| Cephalixin | 6 (4.76) |
| Cefazolin | 11 (8.73) |
| Bacitracin | 8 (6.34) |
| Erythromycin | 3 (2.38) |
| Amoxicillin | 2 (1.58) |
| Chloramphenicol | 6 (4.76) |
| Ceftiofur | 20 (15.87) |

^a Data are presented as No. (%).

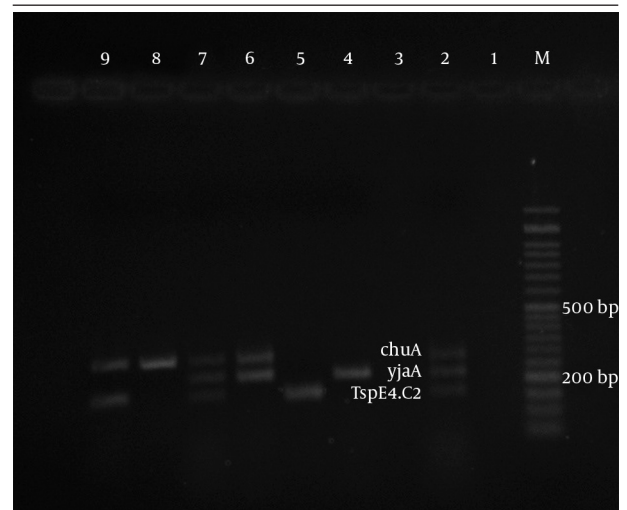


Figure 1. Positive Multiplex PCR Results for the Detection of *E. coli* Phylogenetic Group/Subgroups

Table 3. Antibiotic Resistance Patterns in Relation to Phylogenetic Group^a

| Antibiotic Resistance Patterns | Phylogenetic Group | | | | Total |
|--------------------------------|--------------------|----------|----------|----------|-----------|
| | A | B1 | B2 | D | |
| S, SXT | - | - | 1 | - | 1 |
| BAC, CX | - | - | - | 1 | 1 |
| CX, GM | - | - | 1 | - | 1 |
| BAC, CZ | 1 | - | - | - | 1 |
| CX, T | 5 | 1 | 1 | - | 7 |
| S, T | 1 | - | - | 1 | 2 |
| BAC, CP | - | - | 1 | - | 1 |
| AM, SXT | 1 | - | - | - | 1 |
| CX, E | 1 | - | - | - | 1 |
| CP, S | 1 | - | - | - | 1 |
| C, T | 1 | - | - | - | 1 |
| GM, S | 1 | - | - | - | 1 |
| SXT, T | - | 1 | - | - | 1 |
| CP, T | 1 | - | - | - | 1 |
| BAC, T | 1 | - | - | 1 | 2 |
| AM, T | - | 1 | - | - | 1 |
| CZ, T | 1 | - | - | - | 1 |
| GM, T | 2 | 1 | - | - | 3 |
| E, CX, CZ | 1 | - | - | - | 1 |
| CX, CZ, T | 1 | - | - | - | 1 |
| C, CX, T | - | - | - | 1 | 1 |
| CL, CP, CX, S | 1 | - | - | - | 1 |
| C, CL, CX, T | - | 1 | - | - | 1 |
| Total | 20 | 5 | 4 | 4 | 33 |

^a Abbreviations: AM, amoxicillin; BAC, bacitracin; C, chloramphenicol; CL, cephalixin; CP, ciprofloxacin; CX, cefoxitin; CZ, cefazolin; E, erythromycin; GM, gentamicin; S, streptomycin; SXT, sultrim; T, tetracycline.

5. Discussion

The use of antibiotics are particularly generated strong debate as growth promoters in livestock production. Concerns about antibiotic resistance in bacteria isolated from foods of animal origin have been developing for recent years. Antibiotic resistant of nonpathogenic bacteria may provide a reservoir of resistance genes which can be transferred to pathogenic bacteria in human clinical medicine (4). Studies on *E. coli* isolates from poultry origin have shown high rates of antimicrobial resistance in recent years in Iran (1, 9, 10). In the current study, minority of *E. coli* isolates were resistant to two or more antibiotics. The high prevalence of resistance could be related with the widespread use of antibiotics in poultry production in countries throughout the world. Unlike the high resistance rate among poultry *E. coli* isolates than commonly used antimicrobials such as erythromycin, ampicillin, sulfamethoxazol & trimethoprim, enrofloxacin, chloramphenicol, ciprofloxacin and streptomycine, present study indicated that *E. coli* isolates from ostrich have been high rate of susceptibility to these antibiotics (11, 12). These differences may be described by the use of

antibiotics as prophylactic and therapeutic in poultry industry. Carneiro et al. (13) found that 16.66% of the studied ostrich fecal *E. coli* isolates resistance to ampicillin, 12.96% to tetracycline, 5.55% to streptomycin and 1.85% to amoxicillin-clavulanic acid, cefoxitin and gentamicin. Another study in Italy on ostrich eggs *E. coli* isolates founded high frequency of resistance to oxytetracycline, ampicillin and amoxicillin-clavulanic acid (14). In this study, 21.42% and 15.87% of the isolates were resistant to tetracycline and cefoxitin, respectively. It was not surprising that ostrich *E. coli* isolates were resistant to these agents. Resistance to these antibiotics probably indicates the long-term use of them in the ostrich industry. The high prevalence of resistance to streptomycin and gentamycin in ostrich *E. coli* isolates are important, because aminoglycosides are critically significant for treating *E. coli* infections in humans (4). Phylogenetic analysis of *E. coli* isolates from ostrich indicated that 50.73% of isolates fall into A group and also isolates possessed antibiotic resistance mostly distributed in A group. Few data are available about phylogenetic groups of ostrich *E. coli* isolates. A study on phylogenetic groups of ostrich isolates indicated that these isolates belonged to B1 group (13). The phylogenetic group B1 is usually in relation to commensal bacteria (7). Cortes et al. (15) reported that phylo-group B1 was predominant group among isolates from poultry farms. Farm poultry *E. coli* isolates were compared with wild birds, by a higher ratio of A and B1 strains and a lower ratio of B2 and D strains. It has been proved that B2 phylo-group strains are less prone to antibiotics resistance than non-B2 strains. This link between phylogenetic background and antibiotic resistance could explain that farm animals subjected to a higher antibiotic pressure more than wild animals. Therefore, A and B1 strains are selected and B2 strains counter selected (16). The *E. coli* data suggest that isolates of the A and B1 phylo-groups are significant in the commensal flora of animal origin. In contrast, the human isolates fell into B2 phylogenetic group which the most common in human samples and, these are frequently responsible for extra-intestinal infections in humans (3, 17). In conclusion, this study revealed a low frequency of ostrich *E. coli* isolates resistant to antibiotics, although these isolates can act as a reservoir of resistance genes to human. Further studies are needed to better understand the distribution of phylogenetic groups in poultry isolates.

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Authors' Contributions

All the authors participated in the manuscript preparation and experiment procedures equally.

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