Tetracycline resistant genes in *Escherichia coli* isolated from enteric disease in companion birds

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Article Info	Abstract
	Anti-microbial resistant genes could be passed to human via the food chain or by direct contact with infected birds. To evaluate tetracycline resistance genes in the feces of companion birds suspected to enteritis, 100 fecal samples were collected from diarrheic companion birds in Isfahan province, Iran. The presence of *Escherichia coli* was examined by bacteriological, biochemical, and polymerase chain reaction (PCR) tests. The presence of genes associated with resistance to tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetK*, *tetL*, *tetM*, *tetO* and *tetS* genes) was examined using a multiplex PCR. The results showed that in enteric birds, 43.00% of fecal samples contained *E. coli*. In 26 resistant *E. coli,* 11, 12 and 3 strains contained *tetA* (42.30%), *tetB* (46.15) and *tetA* plus *tetB* (11.53%) resistant genes, respectively. In conclusion, *E. coli* isolates from the enteric problem of companion birds contained tetracycline resistant genes that may transfer to human and pose a risk for antibiotic effectiveness in the treatment of infectious diseases in human. © 2022 Urmia University. All rights reserved.

Introduction

Companion birds have a close contact with humans; so that, an emotional relationship may appear between companion birds and owners. Companion birds are primarily *Passeriformes* (e.g., canary, finch and sparrow) and *Psittaciformes* (e.g., Parrot, parakeet, budgerigar and lovebird).[1] These birds are potential carriers and/or transmitters of zoonotic diseases. One group of the most threatening zoonotic diseases that could be transmitted by birds to humans is bacterial infections that can cause illness in birds and impact human health. Following incorrect use or over-consumption of antibiotics in the treatment of bacterial diseases, these birds can involve in the transmission of resistance genes via feces to the environment and humans.[2,3]

Escherichia coli is usually a commensal bacterium of humans and animals. Although, many of the strains of *E. coli* are non-pathogenic, some serotypes are pathogenic. Pathogenic strains cause intestinal and extra-intestinal infectious diseases, such as urinary tract infection, gastroenteritis, peritonitis, meningitis and septicemia.[4] Some of the *E. coli* strains cause bloody diarrhea, anemia and kidney failure which can lead to death. Most of *E. coli* strains can produce Shiga toxin that is harmful to the epithelium of the small intestine. This bacterium can cause many forms of infections including colisepticemia, yolk sac infection, coligranuloma, cellulitis and swollen head syndrome being commonly described as colibacillosis in birds. The treatment of colibacillosis often requires anti-microbial therapy.[5] Tetracycline is one of the members of the family of broad-spectrum antibiotics. Its low cost, high efficacy and trivial side effects make it one of the most popular options in avian medicine. Widespread and incorrect use of tetracycline can potentially lead to the emergence of antibiotic resistance in the bacteria.[3,4] Resistance to the antibiotic is conferred by one or more of the 38 currently described *tet* genes encoding one of the three mechanisms of resistance including efflux pump, ribosomal protection system or direct enzymatic

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inactivation of the antibiotic. Of these mechanisms in Gram-negative bacteria, an efflux pump system is encoded by 32 genes including *tet*A, *tet*B, *tet*C, *tet*D, tetE tetG tetK and *tet*L; while, *tet*M, *tet*O and *tet*S encode ribosomal protection system.[6] Generally, the rapid spread of tetracycline resistance in bacteria is due to the localization of *tet* genes on plasmids, transposons and integrons.[4]

The objective of this study was to evaluate the presence of tetracycline resistance genes in *E. coli* isolates from fecal samples collected from diarrheic companion birds and the prevalence of *tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO* and *tetS* genes in the local population of *E. coli.* To the best of our knowledge, this is the first report regarding the molecular detection of tetracycline resistance genes in *E. coli* isolated from fecal samples of companion birds in Isfahan province, Iran.

Materials and Methods

Sample collection. A total of 100 fecal samples were collected from companion birds suspected to enteritis including parrot, parakeet, budgerigar, lovebird, canary and finch from private pet clinics, breeding aviaries and pet shops in Isfahan province, Iran. The frequency of samples in each companion bird species is listed in Table 1.

Isolation and identification of *E. coli.* All samples were prepared and cultured in peptone water and then on MacConkey's agar and incubated at 37.00 ℃ for 24 hr. A single colony from each plate was inoculated on MacConkey's agar plates containing tetracycline (30.00 µg mL⁻¹). The tetracycline resistant growth of suspected *E. coli* colonies was subjected to Gram staining. The presence of *E. coli* was confirmed by growing the isolates on the eosin methylene blue agar medium. Gram-negative colonies grown on this medium were subjected to biochemical tests (indole, methyl red (MR), Voges Proskauer (VP) and citrate utilization (IMViC) tests) to confirm the colonies as *E. coli*. The isolates with typical IMViC patterns (indole and MR positive and VP and citrate utilization negative) were considered as *E. coli.*[7] For extraction of DNA, colonies of overnight growth bacteria were used. The colonies were put in a test tube containing one mL of distilled water and boiled for 10 min and then, centrifuged for five min at 1000 rpm. Five microliters of the supernatant were used

for the polymerase chain reaction (PCR).[5] After DNA extraction, molecular confirmation of colonies was achieved according to the 16S rRNA gene of *E. Coli* described by Sabat *et al.*[8] The primer set of ECP79F (forward; targeting bases 79 to 96: 59-GAAGCTTGCTT CTTTGCT-39) and ECR620R (reverse; targeting bases 602 to 620: 59-GAGCCCGGGGATTT CACAT-39) were used for identification of *E. coli.*[8] The *E. Coli* ATCC 25922 was used as reference and quality control organisms in PCR method for *E. coli* band confirmation.

Tetracycline resistance gene detection. The presence of genes associated with resistance to tetra-cycline (*tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO* and *tetS* genes) was examined using a multiplex PCR. The primers were used according to Ng *et al.*[9] The PCR was achieved in three separate categories. In category I, the PCR was performed for *tetB, tetC* and *tetD* tetracycline resistance genes. In category II, the *tetA, tetE* and *tetG* genes were amplified simultaneously. In category III, the PCR was performed for *tetK, tetL, tetM, tetO* and *tetS* tetracycline resistance genes. The PCR reactions were performed in a total volume of 25.00 µL including 3.00 mM MgCl₂ (Sigma-Aldrich, St. Louis, USA), 500 mM KCl (Sigma-Aldrich), 100 mM Tris-HCl (Sigma-Aldrich), 0.10% Triton X-100 (Sigma-Aldrich), 200 µm of each dNTP (Fermentas, St. Leon-Rot, Germany), 1.00 µm primers, 2.50 IU of Taq DNA polymerase (Fermentas) and 5.00 µL (200 ng µL⁻¹) of DNA. The amplification reactions were carried out using a DNA thermocycler (Eppendorf, Hamburg, Germany) for 5 min of initial denaturation at 94.00 ℃, followed by 35 cycles of 94.00 ℃ for one min, 55.00 ℃ for one min and 72.00 ℃ for 90 sec. The PCR products were analyzed by gel electrophoresis in 1.50% (wt/vol) agarose gels and stained with ethidium bromide.[10] A 100 bp DNA Marker (Fermentas) was also used.

Results

A 544 bp fragment of 16S rRNA gene of *E. coli* was amplified in the samples identified as *E. coli* in bacteriological methods. In diarrheic companion birds, *E. coli* was isolated from 43 out of 100 fecal swab samples (43.00%). In diarrheic companion birds, 6/20 (30.00%) of the canaries, 5/20 (25.00%) of the finches, 6/10 (60.00%) of the parrots, 7/10 (70.00%) of the parakeets, 10/20

Table 1. The frequency of tetracycline resistance genes in *Escherichia coli* isolates from enteric disease in some species of companion birds.

Bird Species	Total samples	Total *E. coli* isolates (%)	Resistance *E. coli* (%)	*TetA*	*TetB*	*TetA + TetB*
Canary	20	6 (30.00)	3 (50.00)	1	2	0
Finch	20	5 (25.00)	2 (40.00)	1	1	0
Parrot	10	6 (60.00)	4 (66.60)	2	1	1
Parakeet	10	7 (70.00)	5 (71.40)	2	2	1
Budgerigar	20	10 (50.00)	7 (70.00)	3	3	1
Lovebird	20	9 (45.00)	5 (55.50)	2	3	0
Total	100	43	26	11 (42.30)	15 (57.69)	3 (11.53)

(50.00%) of the budgerigars and 5/9 (45.00%) of the lovebird isolates were identified as *E. coli* (Table 1). In 26 resistance *E. coli* detected from diarrheic birds, 11, 12 and 3 strains contained *tet*A (42.30%), *tet*B (46.15) and *tet*A plus *tet*B (11.53%) genes, respectively.

None of the isolates contained the *tet*C, *tet*D, *tet*E, *tet*G, *tet*K, *tet*L, *tet*M, *tet*O and *tet*S tetracycline resistant genes. In the PCR, only the *tet*A (210 bp) and *tet*B (659 bp) genes were amplified (Fig. 1).
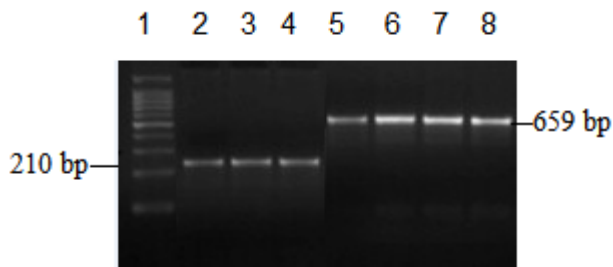

Fig. 1. Polymerase chain reaction product electrophoresis. C1: 100 bp marker; C2 to C4: 210 bp fragment of *tet*A gene; C5 to C8: 659 bp fragment of *tet*B gene.

Discussion

Anti-microbial resistant bacteria could be passed to humans via direct contact with an infected host.[11] Close contact with companion birds or their feces may result in colonization of resistant bacteria in the gastrointestinal tract that may transfer resistance genes to human endogenous flora.[3] In the present study, (43/100) 43.00% of the fecal samples collected from birds suspected to enteric infection contained *E. coli* and 60.46% (26/43) of the *E. coli* isolates were resistant to tetracycline. All of the *E. coli* strains resistant to tetracycline contained *tet*A, *tet*B or *tet*A plus *tet*B resistant genes. Similar to the present study, other studies have discussed the isolation of *E. coli* from fecal samples of birds.[11-13] Beleza *et al.*, have stated that *E. coli* is isolated from 4.50% (4/88) of all isolated *Enterobacteriaceae*.[12] In contrast, some studies have demonstrated higher frequencies of *E. coli* in companion birds. Hidasi *et al.*, have reported that *E. coli* is detected from 300 fecal samples of psittacine birds with a frequency of 33.87%.[14] In this regard, there are some reports about *Passeriformes* birds as well. Giacopello *et al.*, have found 62.00% positivity for *E. coli* in 50 fecal samples from canaries with signs of illness in Italy.[10] Therefore, it can be assumed that environmental conditions such as sanitary status may influence the isolation rate of *E. coli*. Comparison of the results of different researches has demonstrated that intestinal infectious diseases increase the probability of *E. coli* detection from fecal samples and healthy companion birds only have minimal numbers of *E. coli* in their gastrointestinal tract.

Several researchers have also observed different resistance rates to tetracycline in companion birds;

41.00% of the cloacal swabs of cockatiels,[15] 28.60% of the cloacal swabs of free-living parakeets,[16] 39.30% of canaries in Brazil [13] and 69.00% of fecal samples of *Psittacines* were found to be resistant to tetracycline.[14] The tetracycline resistance rate in diarrheic birds in our research was the same as other reports. It seems that wide uncontrolled and empirical use of antibiotics in companion birds might be the reason for the resistance rate in these birds. The factors responsible for the emergence and dissemination of resistant and multi-drug resistant strains cause health risk for human and animal health through developing resistance to antibiotics in infectious diseases treatment.[4]

There were no significant differences between the frequency of *tet*A and *tet*B in *E. coli* isolates. Although, the frequency of *tet*B and *tet*A in animal isolates of *E. coli* was varying. Some studies have shown an increase in the prevalence of *tet*A in animal isolates of *E. coli* and others have reported an increase in the prevalence of *tet*B [17,18] Moreover, Koo and Woo have reported that *tet*A and *tet*B are the most frequent genes in tetracycline resistant *E. coli* strains in Korea (52.40 and 41.30%, respectively).[19] However, the distribution and incidence of tetracycline resistance genes being mediated by efflux mechanism depend on the geographical location, species and origin of the isolate.[17]

Different *tet* resistant genes are responsible for tetracycline resistance in Gram-negative bacteria like *E. coli*.[6] However, the most common *tet* resistance mechanism in *E. coli* is tetracycline efflux pump exporting the drug out of the cell.[19] The form genes studied in the present investigation being related to efflux pump were comprised of *tet*A, *tet*B, *tet*C, *tet*D, *tet*G, *tet*K and *tet*L. Failure to detect other resistant genes such as the genes associated with ribosomal protection could be due to the efficacy of reflux genes in tetracycline resistance in *E. coli*.[18] The tetracycline resistance genes except for reflux pump related genes have not been detected in similar studies.[17,19] Studies have reported that over 30.00% of *E. coli* isolated from turkeys, pigs and horses contain two resistance genes and 4.50% of the pig isolates contain three *tet* genes. Bryan *et al.*, have reported that the most frequent *tet* genes in human, bird, and animal are *tet* B and *tet* A, followed by *tetC, tetD* and *tet*M genes being more frequent than others.[17] Bryan *et al.*, have shown that failure to detect other genes may be due to their low importance or no effect.[17]

In conclusion, it was demonstrated that *E. coli* isolates from enteric problem of companion birds contained tetracycline resistant genes that may be a risk factor for antibiotic effectiveness in the treatment of infectious diseases in human.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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