



## Original article

Antimicrobial resistance profiles of *Escherichia coli* isolated from companion birds

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## ABSTRACT

The increase of antimicrobial resistance in companion animals has already been reported worldwide; however, there is a deficiency of data and studies focusing on the resistance profiles of *Escherichia coli* isolates from Passeriformes and Psittaciformes which regarded to be among the most common and popular pet/companion bird species. For this reason, the current research was aimed to evaluate the presence of *E. coli* isolates from apparently healthy companion birds and their antimicrobial resistance profiles.

Two hundred sixty-five cloacal swab samples collected from apparently healthy companion birds (116 parakeets, 59 canaries, 56 parrots, 30 Indian nightingales, 3 finches, and 1 Golden finch) were examined by conventional bacteriological procedures for the identification of *E. coli*. Susceptibilities against 16 antimicrobials from 8 different classes and extended-spectrum beta-lactamase (ESBL) production was also determined. Moreover, all isolates were analysed by PCR assays for ESBL, Metallo- $\beta$ -lactamases, serin-carbapenemase, AmpC beta-lactamase, plasmid-mediated quinolone resistance (PMQR), and aminoglycoside resistance genes.

*E. coli* were isolated from 37.7% of the samples. Majority of the isolates were found resistant to tetracycline (84%) followed by sulfamethoxazole/trimethoprim (46%), streptomycin (34%), and kanamycin (25%). Eleven parakeet and 2 parrot isolates were found resistant to all quinolone class antimicrobial agents, and three parakeet isolates showed resistance to all aminoglycosides. Additionally, 67% of the isolates exhibited multi-resistance, defined herein as 3 or more antimicrobial classes.

PMQR determinants (qnrB and qnrS) were determined in 3 *E. coli* isolates from a parrot and two parakeets. Furthermore, seven aminoglycoside resistance genes [aac(3)-IIa(aacC2), strA, strB, aadA (aadA1 or aadA2), aphA1, aphA2, and ant(2'')-Ia(aadB)] were found in 3 parakeet isolates. Also, the most promising result of this study is that ESBL production was not determined phenotypically and genotypically.

Here, we present the first report that various aminoglycosides and quinolone resistance genes of *E. coli* isolates from parakeets, and a parrot was present in Turkey. In summary, the consequences of the current research emphasise that the companion birds may act as substantial reservoirs carrying antimicrobial resistance and estimate the potential risk for humans, it is critical to define their role as reservoirs.

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## 1. Introduction

The population of companion animals has been increased unquestionably in the whole world (Pomba et al., 2017). Pet birds which are the third most common animal as a companion after dogs and cats, are regarded as close friends of humans and play an important role in human life (Cong et al., 2014). The majority of caged birds are from two orders: Passeriformes including canaries, finches, and Psittaciformes including parrots, parakeets, and lovebirds (Boseret et al., 2013).

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The emergence of antimicrobial resistance (AMR) is not a novel phenomenon, nor a surprising one. AMR has been regarded about One Health issue which associated not only human health but also animal health and the environment (Argudín et al., 2017). All over the world, the emergence and spread of AMR continue incessantly and unfortunately leaving devastating health and economic consequences. Household pets are recognised as significant risks for zoonotic diseases and considered as a source of multidrug-resistant (MDR) bacteria. Many authors emphasise that the drug-resistant bacteria or AMR genes may be transmitted from animals to man and vice versa, through contaminated food, the environment or via contact (Damborg et al., 2016, Argudín et al., 2017, Pompa, 2017). The close contact between household pets and humans creates many opportunities for transmission. Besides, the treatment of the domestic birds is carried out empirically, potentially promoting the AMR (Giacopello et al., 2015). *E. coli*, a member of the Enterobacteriaceae family, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and animals. As a commensal, it lives in a mutually beneficial association with hosts and rarely causes disease. It is, however, also one of the most common human and animal pathogens as it is responsible for a broad spectrum of diseases. Also, it can be part of the commensal resident of microbiota and opportunistically colonise birds are considered reservoirs of drug-resistant bacteria and related genes (Machado et al., 2018). It has estimated that the flexibility and adaptability of *E. coli* to continuously changing environs provides a large number of resistance mechanisms and commensal *E. coli* can be considered as a source of AMR (Szmolka and Nagy, 2013).

The increase of AMR in companion animals has already been reported, but there is a lack of studies focusing on the effect of antimicrobials on the pathogens isolated from Passeriformes and Psittaciformes. The purpose of the current research was to detect the presence of *E. coli* in good condition Psittacines and Passeriformes, which become a common companion in the home and to evaluate their antimicrobial resistance profiles.

## 2. Material and methods

In total 265 cloacal swab samples from apparently healthy companion birds (116 parakeets, 59 canaries, 56 parrots, 30 Indian nightingales, 3 finches, and 1 Golden finch) were collected in a random manner, from Faculty of Veterinary Medicine clinics, breeding aviaries, and from different pet shops, all located in Istanbul. The protocols were approved by the Animal Care Committee of Istanbul University (Approval no: 2015/105).

All the swabs were cultured in buffered peptone water and incubated at 37 °C for 24 h. After incubation, a loopful of each culture was streaked onto MacConkey agar plates and incubated at 37 °C for 24 h. Strains initially were identified by conventional methods as *E. coli* (Krieg and Holt, 2005) and confirmed with API 20E strips.

Isolates were tested for antibiotic susceptibility by standard disk diffusion procedures to 16 different antimicrobials from 8 antimicrobial classes: amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin-sulbactam (20 µg), aztreonam (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), levofloxacin (5 µg), meropenem (10 µg), nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (10 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg) and tetracycline (30 µg). Extended-spectrum beta-lactamase (ESBL) production was analysed by the double disk diffusion test, and the results were based on CLSI breakpoints (CLSI, 2018). As quality controls, *Escherichia coli* ATCC 25,922 were tested in each run. Multiresistance was considered as resistance at least three different antimicrobial classes.

Multiplex PCR assays were used to screen all *E. coli* isolates for ESBL (*OXA*, *CTX-M*, *TEM*, *SHV*, *GES*, *VEB*, *PER*, *KPC*, *VIM*, and *IMP*), metallo-β-lactamases (*GIM*, *NDM*, *SIM*, and *SPM*), serin-carbapenemase (*IMI*, *SME* and *NMC-A*), AmpC beta-lactamase (*ACC*, *ACT*, *DHA*, *CMY*, *FOX*, *LAT*, *MIR*, and *MOX*) and PMQR genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, and *aac(6′)-Ib-cr*) (Dallenne et al., 2010, Voets et al., 2011, Ciesielczuk et al., 2013). Moreover, PCR assays were performed to determine the aminoglycoside resistance genes (*strA*, *strB*, *aph(3)-Ia*, *aac(3)-IIa*, *aac(3)-III*, *aac(3)-IV*, *aac(6′)-Ib*, *ant(3′′)-Ia*, *aadA*, *aphA1*, *aphA2*, *ant(2′′)-Ia*, *armA*, and *armB*) (Maynard et al., 2004, Saenz et al., 2004, Doi et al., 2007, Costa et al., 2008, Karczmarczyk et al., 2011, Zhang et al., 2014).

## 3. Results

Entirely 100 (37.7%) of the 265 cloacal swabs were positive for *E. coli*. 82/116 (70.6%) of the parakeets, 5/59 (8.4%) of the canaries, 8/56 (14.2%) of the parrots, 4/30 (13.3%) of the Indian nightingales, and 1/3 (33.3%) of the finches isolates were identified as *E. coli* (Table 1).

Table 1. Number of *E. coli* isolates/Number of samples among species and sources

It was determined that 11 of the isolates were susceptible to all tested antimicrobials. Other isolates presented different resistance rates to the tested antimicrobials. Majority of the isolates were found resistant to tetracycline (84%) followed by sulfamethoxazole/trimethoprim (46%), streptomycin (34%), and kanamycin (25%). The results are presented in Fig. 1.

Thirteen isolates (11 parakeets and 2 parrots' samples collected from the same breeder) were resistant to all quinolone class antimicrobial agents phenotypically. Also, 3 of the strains isolated from parakeets showed resistance to all aminoglycoside class of antimicrobial agents. In particular, MDR occurred in 67% of the isolates. MDR was observed in 57/116 (49.1%) of the parakeets, 3/59 (5%) of the canaries, 6/56 (10.7%) of the parrots, and 1/3 (33.3%) of the finches. Canaries' MDR isolates were identified from a same pet shop.

Also, ESBL production was not determined phenotypically. None of the isolates was resistant to meropenem.

PCR analysis revealed that none of the susceptible isolates harboured the tested genes. However, 6 of the 67 (8.9%) MDR isolates harboured at least one of these genes (Table 1). PMQR determinants were found in 3 isolates: *qnrB* (1%) and *qnrS* (2%). Furthermore, seven aminoglycoside resistance genes were found in 3 resistant isolates: *aac(3)-IIa(aacC2)* (1%), *strA* (2%), *strB* (3%), *aadA* (*aadA1* or *aadA2*) (1%), *aphA1* (2%), *aphA2* (1%), and *ant(2′′)-Ia(aadB)* (1%). ESBL production was not determined genotypically. The distribution of the genes among MDR *E. coli* isolates is presented in Table 2.

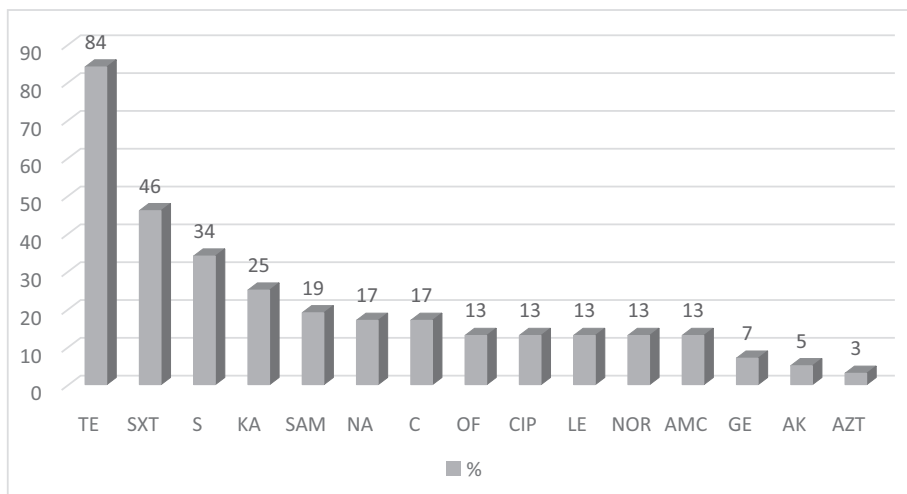
## 4. Discussion

The complex hazard of AMR transmission from companion animals to man has not been fully established. Consequently, studies on AMR, performed in many countries of the world, unceasingly continue to provide more information. Unfortunately, data regarding AMR in companion birds are scarce. The current study is, to our knowledge, one of the scarce and comprehensive reviews on AMR that focuses explicitly on Passeriformes and Psittaciformes as companion birds.

In the current study, the obtained results demonstrated a 37.7% *E. coli* isolation rate in the Passeriformes and Psittaciformes. The isolation rates were 70.6% of the parakeets, 33.3% of the finches, 14.2% of the parrots, 13.3% of the Indian nightingales, and 8.4% of the canaries. In many studies focused on various bird species,

**Table 1**  
Number of *E. coli* isolates/Number of samples among species and sources.

Species	Sources																No. of isolates/samples (%)	
	Breeder no				Clinic no					Pet shop no								
	1	2	3	4	1	2	3	4	5	1	2	3	4	5	6	7		8
<b>Parakeets</b>	9/13	13/15	11/17	-	3/4	-	6/9	11/12	3/6	6/9	-	9/10	9/14	0/5	-	2/2	-	82/116 (70.6%)
<b>Canaries</b>	-	-	0/10	0/5	2/4	0/1	-	-	0/4	0/5	3/5	0/9	0/10	-	0/6	-	-	5/59 (8.4%)
<b>Parrots</b>	-	2/2	0/10	0/5	-	0/1	0/1	0/2	-	2/11	-	3/10	1/7	0/5	-	-	0/2	8/56 (14.2%)
<b>Indian Nightingales</b>	-	1/5	1/6	0/3	-	-	-	1/3	-	0/8	-	-	-	-	-	1/5	-	4/30 (13.3%)
<b>Finches</b>	-	-	-	-	1/2	-	-	-	-	-	-	-	-	-	-	-	0/1	1/3 (33.3%)
<b>Golden Finch</b>	-	-	-	-	0/1	-	-	-	-	-	-	-	-	-	-	-	-	0/1
<b>Total</b>	9/13	16/22	12/43	0/13	6/11	0/2	6/10	12/17	3/10	8/33	3/5	12/29	10/31	0/10	0/6	3/7	0/3	100/265 (37.7%)



**Fig. 1.** The rates of resistance decreasing in the order. TE: Tetracycline, SXT: Sulfamethoxazole/Trimethoprim, S: Streptomycin, KA: Kanamycin, SAM: Ampicillin-Sulbactam, C: Chloramphenicol, NA: Nalidixic Acid, OF: Ofloxacin, CIP: Ciprofloxacin, LE: Levofloxacin, NOR: Norfloxacin, AMC: Amoxicillin-Clavulanic Acid, GE: Gentamycin, AK: Amikacin, AZT: Aztreonam.

**Table 2**  
Resistance genes among the MDR isolates.

Source	Species	Aminoglycoside Resistance Genes							PMQR genes	
		<i>aac(3)-IIa (aacC2)</i>	<i>strA</i>	<i>strB</i>	<i>aadA (aadA1 or aadA2)</i>	<i>aphA1</i>	<i>aphA2</i>	<i>ant(2'')-Ia (aadB)</i>	<i>qnrB</i>	<i>qnrS</i>
Breeder No 2	Parakeet 1		+	+						
	Parakeet 2	+	+	+	+					
	Parakeet 3									+
	Parakeet 4									+
Pet shop No 8	Parrot 1								+	
	Parakeet 5			+				+		

*E. coli* isolation rates have been reported as 48% from the necropsy samples and cloacal swabs of psittacines living in captivity (Corrêa et al., 2013), 59.5% from the necropsy samples and cloacal swabs of canaries from domiciliary breeding locations (Horn et al., 2015), 46.5% from the cloacal swabs of psittacine birds maintained in the Wildlife Rehabilitation Center (de Souza Lopes et al., 2015), 10% from the cloacal swabs of captive cockatiels (De Pontes et al., 2018) and 36.1% from the cloacal swabs of free-living grey-breasted parakeets (Machado et al., 2018). The differences between rates could be based on multiple criteria, including geographical differences, sampling techniques, and detection procedures.

In the list of “Critically Important Antimicrobials for Human Medicine” of World Health Organization (WHO), chloramphenicol and tetracycline were stated as “highly important antimicrobials”. Erythromycin, streptomycin, ciprofloxacin, gentamycin and ampicillin were considered as “critically important antimicrobials” (World Health Organization, 2017). According to the “List of Antimicrobials of Veterinary Importance” of the World Organisation for Animal Health (OIE), tetracycline and ampicillin were considered as “very important” agents and erythromycin and streptomycin were regarded as extremely important (OIE, 2015). In this study, among the 100 *E. coli* isolates, 89 (89%) were resistant

to at least one of the antimicrobials and the most frequently displayed resistances were determined to tetracycline (84%). Several authors have also observed a resistance rate to tetracycline; 41% from the cloacal swabs of captive cockatiels (De Pontes et al. (2018)), and 28.6% from the cloacal swabs of free-living grey-breasted parakeets (Machado et al. (2018)). Regarding previous reports in Turkey (65–100%), our results are consistent (Yilmaz and Dolar, 2017, Diren Sigirci et al., 2019). However, tetracycline resistance rate in Turkey is higher than the other countries. In our country, the wide uncontrolled use of antimicrobial compounds in human and veterinary practices, animal production, agriculture and industrial technology, and an increase in population mobility and the circulation of food might be the reason for this crucial problem. Factors responsible for the emergence and dissemination of resistant and MDR strains establish risk for human and animal health due to an increase in morbidity, mortality and the cost associated with the treatment of infections (Clemente et al., 2015).

Trimethoprim-sulfamethoxazole exhibits broad-spectrum activity through oral efficacy, and it is particularly proper used for therapy of birds. Diren Sigirci et al. (2019) reported that 38% of the isolates from cloacal swabs of the synanthropic birds were resistant to SXT. Relatively, the high resistance rate to sulphamethoxazole/trimethoprim (46%) was observed in this study.

Otherwise, 13% of the isolates obtained from the same breeder (11 parakeets and two parrots) were resistant to all analysed quinolone class antimicrobial agents. De Pontes et al. (2018) presented 41% resistance to quinolones from healthy captive cockatiels, and it is interpreted that this high percentage of resistance may be the result of empirical treatments without veterinary supervision. Also, the risk of contamination and spread should be taken into account in the places where the breeder is sold.

In the current study, three of the parakeet isolates sampled from the same breeder showed resistance to all aminoglycoside class of antimicrobial agents. Herein, if we consider rates severally, various resistances for other antimicrobials were recorded; streptomycin (34%), kanamycin (25%), gentamycin (7%), and amikacin (5%). In contrast to the present study, the higher resistance rates to streptomycin were also reported. Horn et al. (2015) determined that *E. coli* strains isolated from necropsy samples and cloacal swabs of canaries were resistant 4% to gentamycin and 40% to streptomycin. Therewithal, De Pontes et al. (2018) notified the high resistance rate to aminoglycosides (74%), and streptomycin (67%) from cockatiels kept in captivity.

A few studies were focused on the AMR of Gram-negative bacteria isolated from cage pet birds. Lopes et al. (2018) stated that ESBL-producing *E. coli* from faecal samples of psittacines had not been published yet. In this research, ESBL were not detected, which is the most promising result of this study. Contrary, the authors reported a 2.7% isolation rate for ESBL from cage birds, in Turkey (Yilmaz and Dolar, 2017). This promising result has to be confirmed by future studies and needs to be underlined.

MDR presence in companion animals may present a severe risk. Probably the most worrying result in the current study was the detection of 67% multiple drug resistance rates. MDR rate detected are quite higher than those described by 33.8% Hidasi et al. (2013), 55.7% Horn et al. (2015), and 59% de Pontes et al. (2018).

The majority of the studies focus mostly on phenotypic resistance profiles. Due to this deficiency, in the current study, PCR analysis was conducted for screening some of the AMR genes. As a result of the current data, it was revealed that the AMR genes were disseminated unequally among the isolates, besides some isolates harboured multiple genes.

PMQR is a significant phenomenon, and the most common PMQR genes found among Enterobacteriaceae isolates have been

*qnrS*, *qnrB* and *aac(6′)-Ib-cr* (Araújo et al., 2017). In the current research, PMQR genes were found in 3 MDR isolates: *qnrB* (1%) and *qnrS* (2%). Whereas some researchers are already working on it, currently there is still little work focused on the cage birds. Alcalá et al. (2016) pointed out that it is also noteworthy the finding of PMQR genes in an only single isolate. Even though, a report concluded that PMQR were not detected from parrot (Clemente et al., 2015). This is the first paper of the *qnrB* gene presence from a parrot and *qnrS* from two parakeets, in Turkey.

Aminoglycosides are clinically important therapeutic agents commonly applied to treat infections particularly severe infections by Gram-negative bacteria, but they are less preferred at birds because of the toxicity disadvantage (Flammer, 2006). Unfortunately, a considerable resistance to aminoglycosides has been noticed, it is worth noting the detection of seven aminoglycoside resistance genes (*aac(3)-IIa(aacC2)*, *strA*, *strB*, *aadA(aadA1* or *aadA2)*, *aphA1*, *aphA2*, and *ant(2′′)-Ia(aadB)*) in 3 MDR isolates from parakeets. Here, we demonstrated, for the first time in Turkey, the presence of aminoglycosides resistance in *E. coli* isolates from parakeets. It should be appreciated that direct contact with the owners of the cage birds may probably facilitate the possible transmission. It should be noted that resistant bacteria could also have been horizontally transmitted by the owners (Machado et al., 2018).

## 5. Conclusion

There is a deficiency of data concerning antimicrobial resistance in companion birds, more studies should be conducted, and more data should be collected in order to monitor future trends to build further knowledge. Nevertheless, infected/carrying/untreated birds could become a potential reservoir for humans, and they have consequences on public health. In an ideal situation, surveillance and early diagnosis should be performed in every imported bird bunch including animals captured from the wild. In both humans and animals, the use of antimicrobials caused an increase in the incidence of resistance in both pathogenic and endogenous bacteria, highlighting a serious health problem to human medicine. Information obtained from systematic surveillance studies is essential for monitoring changes in the antimicrobial resistance among pathogens, and appropriate antibiotic treatments.

In conclusion, the current results showed that resistance to various antimicrobials, unfortunately, and extensively, are available in companion birds, and this study present novel data that will support to fill knowledge. This outcome highlights the requirement to avoid the usage of unnecessary and inadequate antimicrobials, and to update continuous data by providing appropriate scientific support and to carry out multidisciplinary studies in the scope of “One health”.

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