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# RESEARCH ARTICLE

## Antimicrobial resistances of *Escherichia coli* isolated from Buteo rufinus

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## Kızıl şahinlerden izole edilen *Escherichia coli* suşlarının antimikrobiyal direnç profilleri

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**Öz**

**Amaç:** Bu çalışmada, kızıl şahinlerden (*Buteo rufinus*) toplanan kloakal sıvap örneklerinin bakteriyolojik muayenesi ve izole edilen *Escherichia coli (E. coli)* suşlarının antibakteriyel dirençlerinin belirlenmesi amaçlandı.

**Gereç ve Yöntem:** Çeşitli şikâyetlerle Afyon Kocatepe Üniversitesi Veteriner Fakültesi kliniklerine getirilen kızıl şahinlerden 24 kloakal sıvap örneği toplandı. Örnekler besi yerlerine ekilerek 37 °C de aerobik ve mikroaerofilik olarak inkübe edildi. *Salmonella* spp. varlığı ISO 6579 protokolüne göre yapıldı. Daha sonra izole edilen bakterilerin antibiyotik duyarlılıkları belirlendi.

**Bulgular:** Örneklerden toplam 24 *E. coli* suşu izole edildi. Ayrıca, bir *Enterococcus* faecalis izolatı da izole edildi. *E. coli* izolatlarının tamamı florfenikol ve siprofoksasine karşı duyarlı olarak bulunurken diğer antibiyotiklere karşı değişen oranlarda dirençlilik tespit edildi.

**Öneri:** Bu çalışma ile ülkemizdeki kızıl şahinlerden elde edilen kloakal sıvapların bakteriyolojik muayenesi ilk kez yapıldı. Ayrıca, antibiyotik kullanılmayan kızıl şahinlerden izole edilen *E. coli* izolatlarının farklı antibiyotiklere değişen oranlarda dirençli olması da anlamlı bulundu.

**Anahtar kelimeler:** Kızıl şahin, kloakal sıvap, *Escherichia coli*, antimikrobiyal direnç

**Abstract**

**Aim:** In this study, it was aimed to determine microbiological examination of cloacal swab specimens taken from Long-legged Buzzards (*Buteo rufinus*) and antibiotic resistance of isolated *Escherichia coli (E. coli)* strains.

**Materials and Methods:** The cloacal swab specimens were obtained from 24 Long-legged Buzzards which were admitted to Afyon Kocatepe University Veterinary Faculty clinics. The samples were cultured on different media for various bacteria (*Salmonella* species, *E. coli, Mycoplasma*, Gram-positive cocci, etc.). The media were incubated at 37°C in aerobic and microaerophilic conditions. ISO 6579 protocol was applied for Salmonella. In addition, antibiotic susceptibilities of the isolated bacteria were determined.

**Results:** Twenty-four *E. coli* were isolated from all cloacal swab samples. In addition, *Enterococcus faecalis* was isolated from one sample. While all *E. coli* isolates were susceptible to florfenicol and ciprofloxacin, other antibiotics were found to be susceptible at different rates.

**Conclusion:** This study is important because it is the first microbiological examination of cloacal swaps of Long-legged Buzzards in this Country. In addition, it was significantly evaluated that *E. coli* strains were resistant to various antibiotics with different ratios while no antibiotics are used using antibiotics in Long-legged Buzzards.

**Keywords:** Long-legged Buzzards, *Escherichia coli*, cloacal swap, antimicrobial resistance

## Introduction

Four Buzzard species are observed in Turkey, 3 of which are naturally bred in this country. These 4 species are the Common Buzzard (*Buteo buteo*), the Long-legged Buzzard (*Buteo rufinus*), the Rough-legged Buzzard (*Buteo lagopus*), and the European Honey Buzzard (*Pernis apivorus*). The Common Buzzard is mostly observed between the İstanbul-Borçka region. In Turkey, the breeding site of the European Honey Buzzard covers the Black Sea region and Thrace. This species is a summer migrant and spends the winter in Central and West Africa. The Rough-legged Buzzard, which breeds in the tundra of Northern Europe, is observed in few numbers only during the winter in Turkey. On the other hand, the Long-legged Buzzard is the most typical bird of prey in Anatolia, and is endemic to this region. The Long-legged Buzzard is observed throughout Turkey and inhabits open fields, in particular steppes and agricultural land (Kiziroğlu 2013).

*Escherichia coli* (*E. coli*), which is found in the intestinal flora of birds and chickens, is also frequently isolated from environmental samples. This bacterium is classified under several pathogenic and non-pathogenic serogroups, on the basis of its antigenic properties (Wasteson 2001). *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, motile bacterium, which belongs to the Enterobacteriaceae family and measures 1.1-1.5x2.0-6.0 um in size (İzgür 2006).

In avian species, *E. coli* causes air sacculitis, enteritis, yolk infection, coli-septicaemia (colibacillosis), pericarditis, coli-granuloma, peritonitis, arthritis, perihepatitis, omphalitis and cellulitis (Barnes et al 2003). Research has shown that poultry meat and faeces are among the main sources of *E. coli* contamination (Schoeni and Doyle 1994, Naylor et al 2005). Generally, *E. coli* is detected by the conventional culture technique, which is based on isolation and identification. Furthermore, molecular techniques, serological tests, immunomagnetic separation and biosensors are also used for this purpose (Gülhan et al 2009).

Antibiotic-resistant bacteria are generally of human and animal origin, and their environmental contamination causes public health risks (Martine 2009). Recently, antibiotic-resistant bacteria have also been detected in mountainous areas frequently visited by humans (Cole et al 2005). Although known not to be directly exposed to antibiotics, wild birds can be exposed to antibiotic-resistant *E. coli* strains by consuming contaminated water and feed (Cole et al 2005). Thereby, wild animals and wild birds become reservoirs and carriers of antibiotic-resistant bacteria (Dolejska et al 2007).

While there are a limited number of studies on the antibacterial resistance profile of *E. coli* strains carried by wild birds (Cole et al 2005, Dolejska et al 2007, Guenther et al 2009), to the authors' knowledge, the antibiotic resistance of *E. coli* strains carried by long-legged buzzards has not been investigated before. This study was aimed at determining the antibacterial resistance profile of *E. coli* strains isolated from cloacal swab samples taken from Long-legged Buzzards inhabiting Central Anatolia, and at investigating whether Long-legged Buzzards serve as a reservoir of antibiotic-resistant *E. coli* strains.

## Materials and methods

### *Samples*

In this study, A total of 24 cloacal swab samples from Long-legged buzzards were taken brought to Afyon Kocatepe University Veterinary Faculty clinics due to various diseases. Swab samples were brought to the Department of Microbiology at Aksaray University Veterinary Faculty under cold chain conditions.

### *Microbiological examination*

Swab samples were cultured on blood agar (Oxoid) with 5% sheep blood, Mac Conkey agar (Oxoid) and XLD agar (Oxoid) and plates were incubated at 37°C for 18-24 hours. Gram negative colonies growing in the plates were subjected to various biochemical tests for *Salmonella* spp and *E. coli* (Winn ve ark 2006). In addition, swab samples were cultured to to investigate mycoplasma presence and evaluated according to the method reported by Frey et al (1968).

### *Antibiotic susceptibility test*

Antibiotic susceptibilities of *E. coli* strains were determined by standard disk diffusion method (CLSI 2012). Briefly, growing the bacteria into tryptic soy broth (Oxoid) for 18-24 hours at 37°C were planted on Mueller-Hinton agar (Oxoid). Antibacterial susceptibility test discs were placed at 3 cm intervals, and the plates were incubated at 37°C for 24 hours. Antibiotic discs (Oxoid) were used: amoxicillin (25 µg), penicillin (10 µg), trimethoprim-sulfamethoxazole (1.5 µg-23.5 µg), gentamicin (10 µg), tetracycline (30 µg), streptomycin (10 µg), erythromycin 30 µg), florfenicol (30 µg), ciprofloxacin (5 µg) and cefquinome (30 µg).

## Results

Twenty-four *E. coli* was isolated in all (100%) of cloacal swab samples. Also, Enterococcus spp was isolated from one sample. However, *Salmonella* spp and mycoplasma species were not isolated in any of the samples.

All *E. coli* isolates were susceptible to florfenicol and ciprofloxacin. Resistance to penicillin was detected in 7 (20.1%), amoxicillin in 6 (25%), tetracycline in 5 (20.8%), trimethoprim-sulfamethoxazole in 4 (16.6%), streptomycin and eryt-

Table 1. Antibacterial resistance of *E. coli* isolates

| Antibiotic | S (%) | I (%) | R (%) |
|---|---|---|---|
| Amoxicillin | 14 (58.3) | 4 (16.6) | 6 (25.0) |
| Penicillin | 11 (45.8) | 6 (25.0) | 7 (29.1) |
| Trimethoprim-sulfamethoxazole | 18 (75.0) | 2 (8.3) | 4 (16.6) |
| Gentamicin | 19 (79.1) | 2 (8.3) | 3 (12.5) |
| Tetracycline | 10 (41.6) | 9 (37.5) | 5 (20.8) |
| Streptomycin | 16 (66.6) | 4 (16.6) | 4 (16.6) |
| Erythromycin | 15 (62.5) | 5 (20.8) | 4 (16.6) |
| Florfenicol | 23 (95.8) | 2 (4.1) | 0 (0) |
| Ciprofloxacin | 24 (100) | 0 (0) | 0 (0) |
| Cefquinome | 20 (83.3) | 3 (12.5) | 1 (4.1) |

S: sensitive; I: intermediate; R: resistance

hromycin in 4 (16.6%), gentamicin in 3 (12.5%) and sefquinome in 1 (4.1%) strains (Table 1).

## Discussion

The multiple antibiotic resistance in *E. coli* strains isolated from humans and animals is a major threat to public health. Antibiotic-resistant strains have been reported to be isolated from wild birds, which are not normally exposed to antibiotics, is considered to be linked to the occurrence of antibiotic resistance in domestic animals (Dolejska et al 2007). In this study, the presence of *E. coli* strains, displaying multiple antibiotic resistance was investigated for the first time in Long-legged Buzzards inhabiting Anatolia.

In this study, in total 24 *E. coli* strains were isolated from cloacal swab samples taken from Long-legged Buzzards. Antibacterial sensitivity tests demonstrated that 29.1% of the isolated strains were resistant to one or more antibiotic. In a research carried out in Portugal, 115 *E. coli* strains were isolated from various wild bird species and 24.3% of these strains were determined to be resistant to one or more antibiotic (Santos et al 2013). Literak et al (2010) reported that 27% of the *E. coli* strains they isolated from wild ducks in Poland had multiple antibiotic resistance. The investigation of the antimicrobial resistance profile of *E. coli* strains isolated from wild cranes in Japan showed that 18.8% of the strains were resistant to more than one antibiotic (Kitadai et al 2012). The antibiotic resistance rate of 26.3% detected in the present study shows similarity to the multiple antibiotic resistance rates previously reported in studies conducted in wild birds in different parts of the world.

The two most commonly used antibiotics in human and veterinary medicine are penicillin and tetracycline (Dolejska et al 2007). In this study, the 24 *E. coli* isolates displayed resistance rates of 29.1%, 25% and 20.8% to penicillin, amoxicillin and tetracycline, respectively. Upon investigating the antibacterial resistance profile of *E. coli* strains isolated from wild birds distributed throughout Europe, Guenther et al (2010) ascertained that 60% of these strains were resistant to amoxicillin and ampicillin, and 47% were resistant to tetracycline. In a study conducted in Portugal, a total of 115 *E. coli* strains were isolated from wild birds, and of these isolates 4 were reported to be resistant to ampicillin and 5 were reported to be resistant to tetracycline (Santos et al 2013). Dolejska et al (2007) reported that, of the *E. coli* strains they isolated from seagulls in the Czech Republic, 12% were resistant to ampicillin and 19% were resistant to tetracycline.

In this study, all of the isolates were found to be sensitive to florphenicol and ciprofloxacin. Similar sensitivity rates have been reported in studies carried out in the Czech Republic, Portugal and Germany (Dolejska et al 2007, Guenther et al 2010, Santos et al 2013). Furthermore, the present study demonstrated that, of the *E. coli* strains isolated from Long-legged Buzzards, 15.8% were resistant to streptomycin, 10.5% to gentamycin, and 15.8% to trimethoprim-sulfamethoxazole. Previous research carried out on *E. coli* strains isolated from wild birds has revealed varying levels of resistance to these antibiotics (Dolejska et al 2007, Guenther et al 2010, Santos et al 2013). The differences observed between the resistance levels reported in these studies may be related to differences in the levels of the contact of wild birds with humans and farm animals and the exposure of wild birds to antibiotic-resistant bacteria.

## Conclusion

In conclusion, this study demonstrated for the first time that Long-legged Buzzards inhabiting Central Anatolia are carriers of *E. coli* strains with multiple antibiotic resistance. It was also concluded that Long-legged Buzzards could be involved in the expansion of antimicrobial resistance by shedding

antibiotic-resistant bacteria into the environment, and by contaminating environments of humans, farm animals and water resources.

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