

The Potential Risk in Epizootiology of Bacterial Zoonosis: Pigeon (*Columba livia domestica*) Feces

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Summary

The purposes of this study were to determine the profile of some Gram-negative bacteria in domestic pigeon intestinal microflora and isolated *Enterococcus* and *E. coli* strains to some antibiotics resistance were investigated by disc diffusion methods. For this purpose, cloacal swab samples were collected from a total of 215 pigeons from 8 different pigeon-houses, in Kırıkkale, Turkey. A total of 180 isolates were obtained from 215 samples and these isolates were identified as 46% *E. coli*, 10% *Enterobacter* spp., 7% *Shigella* spp., 4.5% *C. diversus*, 3% *K. ozoenae*, 1.5% *B. dispar*, 1% *P. alcalifaciens*, 1% *S. rubidae* and 25% *Enterococcus* spp. It was determined that the isolates of *E. coli* were not resistant to enrofloxacin and gentamicin, while, they were found resistant to ampicillin-sulbactam, nalidixic acid and oxytetracycline at the rates of 70%, 49% and 64% respectively. Moreover, 42.3% of the *E. coli* strains were identified as positive for β -lactamase activities.

Keywords: Antibigram, *Escherichia coli*, *Enterococcus*, Pigeons

Bakteriyel Zoonozların Epizootiyolojisinde Potansiyel Risk: Güvercin (*Columba livia domestica*) Dışkı

Özet

Bu çalışmada, hobi amacıyla yetiştirilen evcil güvercinlerin bağırsak florasındaki bazı gram negatif bakteri profilini belirlemek ve izole edilen *E. coli* ile enterokok suşlarının bazı antibiyotiklere direncini disk difüzyon metodu ile araştırmak amaçlandı. Bu amaçla Kırıkkale ve çevresindeki 8 güvercin kümesinden toplam 215 adet güvercinde kloakal svap örneği alındı. 215 numunede 180 izolat elde edildi ve bu izolatların %46'sı *E. coli*, %10'u *Enterobacter* spp., %7'i *Shigella* spp., %4.5'i *C. diversus*, %3'ü *K. ozoenae*, %1.5'ü *B. dispar*, %1'i *P. alcalifaciens*, %1'ü *S. rubidae* ve %25'i *Enterococcus* spp. olarak tanımlandı. *E. coli* izolatlarının enrofloksasin ve gentamisin karşı direnç taşımadığı, ampicilin-sulbaktam %70, nalidiksik asit %49, oksitetrasikline %64 dirençli olduğu tespit edildi. Ayrıca *E. coli* suşlarının %42.3'ünün, β -laktamaz aktivitesi yönünden pozitif olduğu belirlendi.

Anahtar sözcükler: Antibiyogram, *Escherichia coli*, Enterokok, Güvercin

INTRODUCTION

The breeding of domestic pigeons (*Columba livia domestica*) are quite common in Turkey. Domestic pigeons pose the risk of contamination due to their close environment and contact with the other avian species, most importantly for humans because of carrying many bacteria.

Lately, the increase in the number of pigeons leading to anxiety in terms of public health concerns¹. Pigeons are potential reservoir for humans that host many of patho-

genic microorganisms with zoonotic features including *Erysipelothrix insidiosa*, *Listeria monocytogenes*, *Pasteurella multocida*, *Yersinia* spp., *Campylobacter* spp., *Rickettsia rickettsii*, *Chlamydia*, *Salmonella* spp., *Enterobacter* spp., *Escherichia coli*, *Mycobacterium* spp.²⁻⁶. Pigeon breeding for domestic purposes have become quite popular and human contact with pigeon feces in public parks play an important role in the spread of infectious agents^{7,8}.

Ishiguro et al.⁹ were obtained 36 of *E. coli* isolates from



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22 of pigeon cloacal swab samples taken from domestic pigeons, pigs, cattle and horses in their study. Tanaka et al.¹⁰ conducted the study concerning the isolation and identification of *Salmonella* spp., *Mycobacterium* spp., *E. coli* O157 and *Chlamydia* spp. and they detected the presence of these bacteria in the samples of pigeon feces that were taken from 38 different public parks, located in 7 regions.

Recently, the misuse of antibiotics have become widespread among the pigeon breeders, therefore, this situation may result in the emergence of antibiotic-resistant strains of microorganisms. Results reported by Kimpe et al.¹¹, revealed that the *E. coli* strains, isolated from pigeons show resistance, to the antibiotics of ampicillin, gentamycin, kanamycin, trimethoprim/sulfamethoxazole, oxytetracycline, enrofloxacin, erythromycin.

Silva et al.⁸, obtained 184 of *E. coli* isolates from 100 fresh urban pigeon feces sampled in their study. This researchers indicated resistance profiles of 184 *E. coli* strains to Amikacin, Ampicillin, Ampicillin-sulbactam, Cefotaxime, Ceftriaxone, Gentamicin, Levofloxacin, Trimethoprim-sulfamethoxazole.

Gülhan et al.¹² isolated 150 enterotoxigenic *E. coli* (ETEC) strains from various animal feces samples. They reported, 7 enterotoxigenic *E. coli* isolates were detected in pigeon feces.

The purposes of this study were to determine the profile of some Gram-negative bacteria in domestic pigeons intestinal microflora and isolated *Enterococcus* and *E. coli* strains to some antibiotics resistance were investigated by disc diffusion methods.

MATERIAL and METHODS

A total of 215 cloacal swab samples collected (197 tumbler, 14 ornamental, 4 carrier pigeons) from 8 pigeon-houses in Kirikkale and the surrounding region between September 2005 - April 2006. The swab samples were inoculated directly onto agar Slanetz-Bartley (SB) agar and Eosine Methylene Blue (EMB) agar. The inoculated media were incubated at 37°C for 24-48 h. The colonies that grew on EMB agar and resemble *Enterobacteriaceae* colony morphology were passaged into Tryptic Soy (TS) agar and incubated for 24 h. Then samples were identified by conventional test methods¹³. The colonies that grew in SB agar and resemble *Enterococcus* colony morphology, were subjected to catalase test and Gram staining. Catalase negative, PYR (L-pyrrolidonyl-beta-naphthylamide) positive, black colored in Bile Aesculin agar and grew in Brain-Heart Infusion (BHI) broth with 6.5% salt were considered as *Enterococcus* spp. This *Enterococcus* strains were tested for identification by arginine decarboxylation, motility test, pyruvate, mannitol, arabinose, raffinose and lactose fermentation test and pigmentation of grew colony in BHI

agar^{5,14}. *E. faecalis* strains (ATCC 291212) were used as positive control for biochemical test.

In order to determine the antibiotic resistance of *E. coli* strains, the discs (Oxoid) of enrofloxacin (5 µg), gentamicin (10 µg), oxytetracycline (30 µg), ampicillin/sulbactam (10 µg/10 µg), nalidixic acid (30 µg) were used. To determine the antibiotic resistance of *Enterococcus* spp. the discs (Oxoid) of erythromycin (15 µg), enrofloxacin (5 µg), tetracycline (30 µg), quinupristin/dalfopristin (15 µg), penicillin G (10 U), teicoplanin (30 µg), streptomycin (5 µg), gentamicin (120 µg), vancomycin (30 µg) were used. The disc diffusion method in "Mueller-Hinton (MH)" agar was used for determining resistance to these antibiotics, according to Clinical and Laboratory Standards Institute -CLSI criteria¹⁵.

For detecting β-lactamase production in *E. coli* strains, β-lactamase (nitrocefin) identification sticks (Oxoid BR66A) were used. Test was performed according to the manufacturer's recommendations. The colonies that grew in MH agar applied onto sticks. Sticks were evaluated visually, for the color changes that observed in a humid environment at 5th, 15th and 30th. min intervals.

RESULTS

180 isolates were obtained from 215 samples in this study. The isolates were identified as *E. coli* isolates, *Enterococcus* spp., *Enterobacter* spp., *Shigella* spp., *C. diversus*, *K. ozoenae*, *B. dispar*, *P. alcalifaciens*, *S. rubidae* (Table 1).

Table 1. Bacteria isolated from cloacal swabs

Tablo 1. Kloakal svaplardan izole edilen bakteriler

Species of Microorganism	Number of Isolate N (%)
<i>E. coli</i>	83 (46)
<i>Enterococcus</i> spp.	46 (25)
<i>Enterobacter</i> spp.	18 (10)
<i>Shigella</i> spp.	13 (7)
<i>C. diversus</i>	8 (4.5)
<i>K. ozoenae</i>	5 (3)
<i>B. dispar</i>	3 (1.5)
<i>P. alcalifaciens</i>	2 (1)
<i>S. rubidae</i>	2 (1)

According to the identification studies of isolated *Enterococcus* strains, the results of *E. avium*, *E. dispar*, *E. raffinosus*, *E. faecalis asaccharolytic variant* were shown in Table 2.

Enterococcus and *E. coli* strains to some antibiotics resistance patterns have been investigated by disc diffusion method. The resistance to antibiotics has been shown in Table 3 and Table 4. 35 of *E. coli* strains were found as positive (42.3%) in terms of β-lactamase activities.

Table 2. The identification results of *Enterococcus* isolates**Tablo 2.** Enterokok izolatlarının identifikasyon sonuçları

Microorganism	Number of Isolate N (%)
<i>E. avium</i>	22 (47.9)
<i>E. dispar</i>	9 (19.6)
<i>E. raffinosus</i>	6 (13.1)
<i>E. faecalis asaccoharolytic variant</i>	4 (8.7)
<i>E. hirae</i>	2 (4.3)
<i>E. pseudoavium</i>	2 (4.3)
<i>E. gallinarum</i>	1 (2.1)

Table 3. The results of antibiotic susceptibility tests of *E. coli* isolates.**Tablo 3.** *E. coli* izolatlarının antibiyotik duyarlılık testi sonuçları

Antibiotic	Resistance (%)
Ampicillin/sulbactam	70
Oxytetracycline	64
Nalidixic acid	49
Enrofloxacin	0
Gentamicin	0

Table 4. The results of antibiotic susceptibility tests of *Enterococcus* isolates**Tablo 4.** Enterokok izolatlarının antibiyotik duyarlılık testi sonuçları

Antibiotic	Resistance (%)
Erythromycin	52
Enrofloxacin	37
Tetracycline	32
Quinupristin/Dalfopristin	32
Penicillin	30
Teicoplanin	7
Streptomycin	5
Gentamicin	0
Vancomycin	0

DISCUSSION

Pigeons could be able to carry many pathogenic virus, bacteria, fungus and protozoa for humans and animals without causing any diseases in themselves ^{1,5,7,10,16}. Domestic pigeons are one of the most important vehicle for spreading of zoonotic infections ¹⁶. Although pigeon breeding for hobby purposes are popular worldwide, there are not many researches about their microflora in gastrointestinal system and feces ⁵. Pigeon feces potential risk to act as a reservoir of antimicrobial-resistant bacteria and/or resistant genes that may be transferred to pathogens through the environmental chain ⁸.

In this study, some Gram-negative bacteria profiles and antibiotic resistance were determined in domestic

pigeons' fecal flora. One of the most common type of enteric pathogens is *E. coli* in humans and animals ^{1,6,17}. In accordance with the general opinion, *E. coli* is constituent of Gram-negative flora with 46% as well as the most dominant species of flora in this study. *Enterobacter* spp. and *Shigella* spp. are following it. *Enterococcus* spp. were determined in the rate of 25% in all isolates.

Adesiyun et al.¹, performed an antibiogram by disc diffusion method in 118 of *E. coli* strains with pigeon-origin. In their studies, the resistance of antibiotics determined as 89.8% to cephalothine, 56.7% to ampicillin, 53.4% to streptomycin, 34.7% to neomycin, 24.6% to nalidixic acid, 27.1% to sulfamethoxazole/trimethoprim, 16.1% to chloramphenicol and 8.5% to gentamicin. Kimpe et al.¹¹, determined resistance by antibiogram test to ampicillin at 42%, to gentamicin 8%, to kanamycin 10%, to sulfamethoxazole/trimethoprim 33%, to oxytetracycline 65%, to flumequine 13% and to enrofloxacin 13% in *E. coli* strains with pigeon-origin. In our study, while there were no determination resistance to against enrofloxacin and gentamicin in the isolates of *E. coli* strains, the resistances were determined as 70%, 49% and 64% for Ampicillin/sulbactam, nalidixic acid and oxytetracycline, orderly. Our result of determination for oxytetracycline resistance is consistent with the study of Kimpe et al.¹¹. Silva et al.⁸, were obtained 184 of *E. coli* isolates from 100 fresh urban pigeon feces sampled. In their studies, the resistance of antibiotics determined as 36.8% to amikacin, 7.8% to ampicillin, 2.6% to ampicillin-sulbactam, 2.2% to levofloxacin, 3.9% to trimethoprim-sulfamethoxazole and no resistance were detected to gentamicin, ceftriaxone and ceftazidime. Our result of determination for gentamicin resistance is consistent but Ampicillin/sulbactam resistance is not consistent with the study of Silva et al.⁸. Butaye et al.¹⁸ performed antimicrobial resistance profiles *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus columbae* strains that isolated from racing pigeons. In their studies, the resistance of antibiotics determined as 100% to tetracycline and 100% to tylosin but not resistance to bambamycin, vancomycin, monensin, narasin, virginiamycin, avilamycin and ampicillin this strains. The difference concerning the profiles of antibiotic resistance to other antibiotics is thought to be usage of different antibiotics in the field.

Beta-lactam antibiotics very important role of antibiotic therapy and they are effective against Gram-positive and Gram-negative bacteria. In this study was found 42.3% β -lactamase activities of *E. coli* strains. Our result of determination for β -lactamase activities of *E. coli* strains is consistent with the study of other study ^{19,20}. Fischer et al.¹⁹ were reported bacterial resistance especially *Enterobacteriaceae* to beta-lactamic drugs have increasing.

Pigeon shelters, generally located in very close in human civilization. Pigeon breeders declared that they used various

antibiotics without any veterinary supervision during this study. Overall, the bacteria that isolated from pigeons had high resistance to some antibiotics. Moreover, in this study, it has been concluded that the pigeons have an important role to play in zoonotic transmission of some pathogens and they can pose a risk to human health.

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