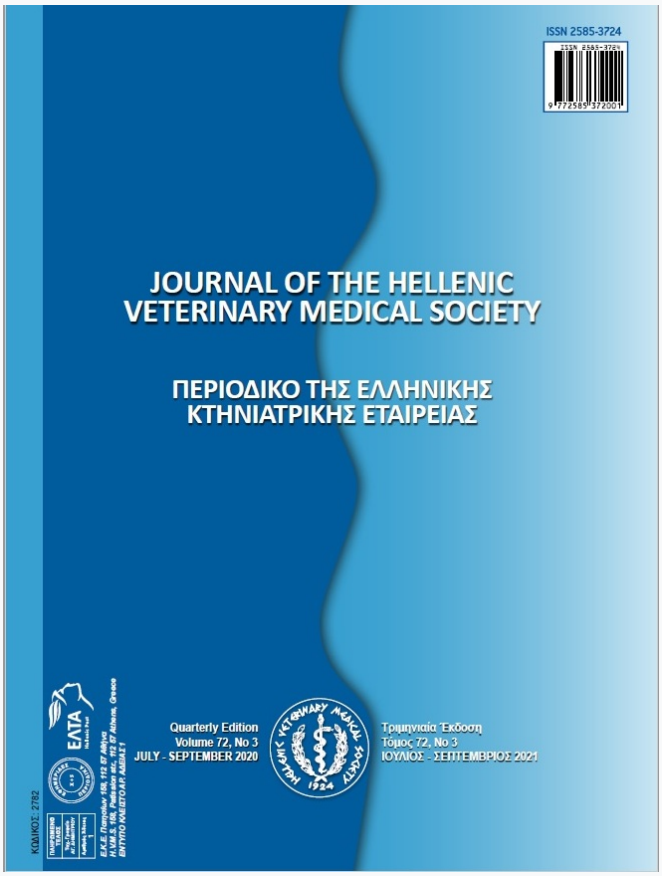
Journal of the Hellenic Veterinary Medical Society

Vol 72, No 3 (2021)



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doi: 10.12681/jhvms.28482

To cite this article:

DIREN SIGIRCI, B., CELIK, B., HALAC, B., BASARAN KAHRAMAN, B., BAGCIGIL, A., & AK, S. (2021). Characterization of Faecal Enterococci from Wild Birds in Turkey and Its Importance in Antimicrobial Resistance. *Journal of the Hellenic Veterinary Medical Society*, *72*(3), 3015–3022. https://doi.org/10.12681/jhvms.28482

J HELLENIC VET MED SOC 2021, 72(3): 3015-3022
ΠΕΚΕ 2021, 72(3): 3015-3022

Research article
Ερευνητικό άρθρο

Characterization of Faecal Enterococci from Wild Birds in Turkey and Its Importance in Antimicrobial Resistance

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SUMMARY: This research aimed to investigate the diversity of faecal enterococci isolated from wild birds, to detect their antibiotic resistance patterns and to determine their distribution of genes related to vancomycin resistance. Additionally, to investigate their virulence factors that are important in the development of the disease. One hundred seven cloacal/rectal samples were inoculated onto Enterococcus Agar, and presumptive colonies were identified and confirmed by PCR. Multiplex PCR assays were used to screen *vanA, vanB, vanC1 and vanC2/3*. The virulence-related genes; *ace, gelE, efa and agg* were determined by PCR. Among the 103 enterococci, 62 *E.faecalis*, 23 *E.faecium* 3 *E.gallinarum*, 2 *E.durans*, 1 *E.casseliflavus* and 12 *Enterococcus* spp. were identified. Of the 103 enterococci, 26 were found to be resistant against to three or more antibiotics. The highest percentages were detected for chloramphenicol (52%), tetracycline (33%) and erythromycin (30%). Two *E.gallinarum* isolates were harboring three virulence factors, and one isolate was carrying a single virulence factor. There is no virulence factor in the *E.casseliflavus* isolate. Also, *vanA and vanB* genes were not found. Forty-two of 103 enterococci were harboring virulence factors, more frequently in *E.faecalis*. Forty-two enterococci carried *efa A*, 31 isolates carried *gel* E, and *ace* was found in 18 isolates. Virulence gene *agg* was not detected. When the results of the study were evaluated in general, multiple drug resistance was described as 25%. Considering the risk of polluting the water resources of wild animals, it is suggested that the continuity of this type of epidemiological study in wildlife animals is necessary. In conclusion, the wild birds may act as substantial reservoirs carrying antimicrobial resistance among enterococci and estimate the potential risk for man, pets and farm animals.

Keywords: Wild birds, enterococci, antibiotic resistance, vancomycin resistance, virulence factors

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Date of initial submission: 11-07-2019
Date of revised submission: 21-04-2021
Date of acceptance: 27-04-2021

INTRODUCTION

The wildlife can act an essential role in antimicrobial resistance (AMR) dynamics and many wild animal species harbor multidrug-resistant (MDR) bacteria in a wide variety of natural environments (Vittecoq et al., 2016). Even though wild birds have only rare contact with antibiotics, they can acquire and disseminate resistant bacteria (Oravcova et al.,2018).Water contact, livestock, food chain and the soil appears are the main routes to the transmission of resistant bacteria originated from human or veterinary (Radhouani et al.,2012).

Enterococcus spp. is an intestinal flora bacteria of mammals, birds, invertebrates and some reptiles (Aarestrup, 2005) and is also found in various environments such as water, soil and sewage (Werner, 2012). Some species are important human pathogens and they have appeared as the fundamental causes of nosocomial infections worldwide (Poeta et al.,2005). Enterococci is an indicator of faecal contamination of environmental pollution. It is often used to detect AMR in both human and animal populations (Radhouani et al.,2012; Radimersky et al., 2010). In the world, *Enterococcus faecalis,* together with *Enterococcus faecium* are ranked as 3rd and 4thamong the nosocomial pathogens (Werner,2012).

Vancomycin is an antimicrobial agent used for Gram-positive bacterial infections and can moreover apply for preventive treatments in humans. Two types of vancomycin resistance have been defined in enterococci. The first is acquired type (*vanA, vanB, vanD, vanE, vanG, vanL*), which is observed mostly in *E.faecalis* and *E. faecium*species, is inducible resistance. The second one is the intrinsic type (*vanC*) which is related to *Enterococcus casseliflavus/flavescens* and *Enterococcus gallinarum* species, is a low-level resistance to vancomycin (Bagcigil et al.,2016; Silva et al.,2011). The emergence of vancomycin-resistant enterococci (VRE) with acquired mechanisms, exclusively those carrying *vanA, vanB or vanC* genes have been described worldwide in humans and animals with different incidences. Therefore, the significant increasing prevalence in VRE is seen as a worldwide health problem (Yahia et al.,2018). Various studies have described the incidence of VRE in wild birds, besides in other wild animals in many states (Poeta et al.,2005; Radhouani et al., 2014; Silva et al., 2010).

Severe infections due to enterococci are caused by the adhesion and secretory virulence genes. Virulence factors such as aggregation substance (*agg*), gelatinase (*gel*E), collagen-binding cell wall protein (*ace*), enterococcal surface protein (*esp*), hyaluronidase (*hyl*), endocarditis antigen (*efaA*) and cytolysin (*Cyl*) increase the severity of the infection and contribute to the pathogenesis of their infections by adhering to host tissue, colonisation, increasing invasion and modulating the host immune system (Celik et al., 2017).These genes have been demonstrated in wild birds and have been reported to pose a hazard to man (Klibi et al., 2015; Poeta et al.,2005).

The purpose of there search was to examine the diversity of faecal enterococci in wild birds, their antibiotic resistance patterns, their distribution of genes related to vancomycin resistance, and the occurrence of virulence factors.

MATERIALS AND METHODS

Samples

From one hundred and seven wild birds, cloacal swabs were recovered from November 2017 to February 2018 in Istanbul regions. The distribution of samples was as follows: 1- Passeriformes: crow (n=2), starling (n=1); 2- Non-Passeriformes: pigeon (n=26), collared dove (n=11), owl (n=1), little owl (n=2), tawny owl (n=1), long-eared owl (n=1), woodcock(n=2); 3- Waterfowl: white stork (n=1), grey heron (n=1), little gull (n=6), seagull (n=35), bittern (n=1), cormorant (n=2); 4- Birds of prey: kestrel (n=1), common buzzard (n=3), sparrow hawk (n=6), Short-toed snake eagle (n=1), honey buzzard (n=3). The samples were taken from the animals brought to the Wildlife Rehabilitation Centre of Faculty of Veterinary Medicine, Istanbul University Cerrahpasa, before the treatment.

Bacterial isolation

Swabs were inoculated into Enterococ Broth and incubated at 37°C for 24 hours. Cultures with colour change were subcultured onto Enterococ Agar (EA) when the black pinpoint colonies were selected as presumptive enterococci. Catalase negative, growth in 6.5% NaCl positive and esculin hydrolysis positive colonies estimated as presumptive enterococci and established by PCR (Ke et al., 1999). They were then identified by API ID20 Strep system (BioMérieux, France). PCR primers were presented in Table 1.

Antimicrobial susceptibility test

Isolates were tested by standard disk diffusion procedures to eight antimicrobials from 6 different antimicrobial classes (CLSI,2016). The antimicro-

bial agents tested were as follows: ciprofloxacin (5 μg), gentamicin (10 μg), streptomycin (30 μg), chloramphenicol (30 μg),tetracycline (30 μg), ampicillin (10 μg), erythromycin (10 μg), vancomycin (30 μg). MDR was considered as resistant at least three different antimicrobial classes.

Vancomycin Resistance Genes

Multiplex PCR assays were used with specific primers for *vanA*, *vanB, vanC1*and *vanC2/C3* (Kariyama et al.,2000). The multiplex PCR assay was made *E. faecalis* V583 (*vanB*), *E. faecium* BM4147 (*vanA*), *E. casseliflavus* DSMZ 20680 (v*anC2/C3*), *E. gallinarum* BM4174 (*vanC1*) and *E. faecium* CCUG542(vancomycin-susceptible) were used as reference strains (Table 1).

Virulence Genes

The occurrence of genes was established by PCR as described (Mannu et al.,2003). The tested genes were: aggregation substance (*agg*), gelatinase (*gel*E), endocarditis antigen (*efaA*), and collagen-binding cell wall protein (*ace*). *E. faecalis* ATCC 29212, *E. faecium* ATCC 6057 and *E. faecalis* NCDO 581 were used as reference strains for positive and negative controls.

RESULTS

Diversity of isolates

Enterococci were isolated in 96 out of 107 faecal samples (89.7%). One hundred three *Enterococcus* species recovered from 96 wild birds.The predominant species were *E.faecalis* (n=62), followed by *E. faecium* (n=23), *E.gallinarum* (n=3), *E.durans* (n=2) and *E.casseliflavus* (n=1). Twelve isolates could not be recognised to the species level. They were evaluated a s*Enterococcus* spp.

Antibiotic susceptibility testing

MDR was determined in 26 of 103 strains. The highest antibiotic resistance was found against chloramphenicol (53,4%), followed by tetracycline (32%), erythromycin (30%), streptomycin (21.3%), ampicillin (9.7%), ciprofloxacin (6.8%), gentamicin (5.8%).Vancomycin-resistance was not discovered in the isolates. MDR was determined 48% in *E.faecium* and 18% in *E.fecalis* isolates. Antibiotic resistance results of the isolates were presented in Table 2.

Table 1: PCR primers and their products

Gene and primers	Sequence	Product size (bp)	Reference
***Enterococcus* spp.**	5′-TACTGACAAACCATTCATGATG-3′ 5′-AACTTCGTCACCAACGCGAAC-3′	112	Ke et al., 1999
vanA	5’-CATGAATAGAATAAAAGTTGCAATA -3’ 5’-CCCCTTTAACGCTAATACGATCAA -3’	1.030	Devriese and Pot 1995
vanB	5’-AAGCTATGCAAGAAGCCATG -3’ 5’-CCGACAATCAAATCATCCTC -3’	536	Lopez et al. 2011
vanCl	5’-GGTATCAAGGAAACCTC -3’ 5’-CTTCCGCCATCATAGCT -3’	822	Devriese and Pot 1995
vanC2/C3	5’-CGGGGAAGATGGCAGTAT -3’ 5’-CGCAGGGACGGTGATTTT -3’	484	
***rrs* (16S rRNA)**	5’-GGATTAGATACCCTGGTAGTCC -3’ 5’-TCGTTGCGGGACTTAACCCAAC -3’	320	
ace	5’-AAAGTAGAATTAGATCCACAC-3’ 5’-TCTATCACATTCGGTTGCG-3’	320	Mannu et al. 2003
***gel*E**	5’-AGTTCATGTCTATTTTCTTCAC-3’ 5’-CTTCATTATTTACACGTTTG-3’	402	
efaA	5’-CGTGAGAAAGAAATGGAGGA-3’ 5’-CTACTAACACGTCACGAATG-3’	499	
AS	5’-CCAGTAATCAGTCCAGAAACAACC-3’ 5’-TAGCTTTTTTCATTCTTGTGTTTGTT-3’	406	

Table 2: Antibiotic resistance results of the isolates

	E.faecalis (n=62)	*E.faecium* (n=23)	*E.gallinarum* (n=3)	*E.casseliflavus* (n=2)	*E.durans* (n=1)	*Enterococcus spp* (n=12)
Erythromycin	15	11	-	-	2	3
Ciprofloxacin	2	3	-	1	-	1
Tetracycline	21	8	1	-	1	2
Gentamicin	3	2	-	-	-	1
Streptomycin	10	8	-	-	1	3
Ampicillin	2	6	-	-	-	2
Chloramphenicol	24	19	1	1	2	8
Vancomycin	-	-	-	-	-	-

Table 3: Patterns of antibiotic resistance of the isolates

	E.faecalis	*E.faecium*	*E.gallinarum*	*E.casseliflavus*	*E.durans*	*Enterococcus spp*
E+T+Ch	3	-	-	-	-	-
E+T+S	1	-	-	-	-	-
E+S+ Ch	-	1	-	-	-	1
E+A+ Ch	-	2	-	-	-	-
T+S+ Ch	-	2	-	-	-	-
C+A+ Ch	-	1	-	-	-	-
E+T+S+ Ch	3	3	-	-	1	1
E+C+T+S+ Ch	1	-	-	-	-	-
E+T+G+S+ Ch	2	1	-	-	-	-
C+G+S+A+ Ch	-	-	-	-	-	1
E+T+G+S+A+ Ch	1	-	-	-	-	-
E+C+T+S+A+ Ch	-	1	-	-	-	-
E+C+T+G+S+A+ Ch	-	1	-	-	-	-

C: ciprofloxacin G: gentamicin S: streptomycin T: tetracycline Ch: chloramphenicol A: ampicillin E: erythromycin

Thirteen different resistant patterns were observed among 103 enterococci isolates. Only one isolate (*E. faecium*) was resistant to all antibiotics used. Two of the isolates were resistant to 6 different antibiotic groups and 5 of the strains were resistant to 5 different antibiotic groups. MDR was described in 11 (17.7%) *E. faecalis* isolates and 11 (47.8%) *E. faecium* isolates. The AMR patterns of isolates are shown in Table 3.

Vancomycin Resistance Genes

Four of the 103 isolates had an intrinsic resistance gene. Three *isolates* were harbored the *vanC1* gene. One isolate carried *vanC2* gene. No strains were harbored the *vanA* and the *vanB* genes.

Virulence Genes

Forty-three (41.7%) of 103 enterococci had virulence genes. Forty-two (40.7%) enterococci harbored *efaA*. Besides, the *gel*E gene was discovered in 31 (30.1%) isolates, and the *ace* gene was detected in 18 (17.4%) isolates. None of the isolates was harbored *agg* gene. The multiple virulence factors were determined in 31 enterococci. Seventeen isolates carried three virulence genes. 13 of 14 isolates carrying two genes contained *gel*E and *efaA* genes, while 1 of them carried *ace* and *efaA*genes.The multiple virulence factors were described in 3 of 3 *E. gallinarum*, followed by 27/33 *E.faecalis* isolates.While 60 isolates did not contain any virulence factor.Virulence genes patterns were presented in Table 4.

A total 26 multiple antibiotic-resistant isolates were harbored the *efa A* gene with 38.4%, the *gel*E gene with 26.9 % and the *ace* gene with 19.2 % prevalence.

Two *E.gallinarum* isolates were harboring three virulence factors and one isolate was carrying a single virulence factor. There is no virulence factor in the *E.casseliflavus* isolate.

The results of the diversity of wild birds, isolates, vancomycin resistance genes, virulence genes, and the level of AMR were summarised in Table 5.

Table 4: Virulence genes patterns

	E.faecalis	*E.faecium*	*E.gallinarum*	*E.casseliflavus*	*E.durans*	*Enterococcus spp*
Efa A	5	1	-	-	1	3
***gel*E**	1	-	-	-	-	-
ace	-	-	-	-	-	-
agg	-	-	-	-	-	-
gel*E + *efa A	11	1	1	-	-	-
ace* + *efaA	1	-	-	-	-	-
gel*E+*efa A* +*ace	14	1	2	-	-	-

Table 5: The results of the diversity of wild birds, isolates, vancomycin resistance genes, virulence genes and the level of AMR

Diversity of wild birds	Diversity of isolates						Vancomycin resistance genes				Virulence genes				
	E. spp	*E.f.*	*E.fc.*	*E.g.*	*E.c.*	*E.d.*	*van A*	*van B*	*van* C_1	*van* C_2/C_3	*ace*	*gel*E	*efa A*	*agg*	Level of AMR
Passeriformes															
crow (n=2)	-	2	-	-	-	-	-	-	-	-	1	1	1	-	-
starling (n=1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Non-Passeriformes															
pigeon (n=26)	5	8	13	-	-	2	-	-	-	-	1	5	7	-	26,9% (7/26)
collared dove (n=11)	-	5	2	1	1	-	-	-	C_1	C_2	2	4	4	-	18,2% (2/11)
little owl (n=2)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
owl (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
tawny owl (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
long-eared owl (n=1)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	100% (1/1)
woodcock (n=2)	-	1	-	1	-	-	-	-	C_1	-	-	2	2	-	-
Waterfowl															
white stork (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-
grey heron (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	100% (1/1)
little gull (n=6)	1	5	-	-	-	-	-	-	-	-	1	1	3	-	33,3% (2/6)
seagull (n=35)	2	26	5	-	-	-	-	-	-	-	10	13	17	-	31,4% (11/35)
bittern (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
cormorant (n=2)	1	-	-	1	-	-	-	-	C_1	-	1	1	1	-	50% (1/2)
Birds of prey															
honey buzzard (n=3)	1	2	-	-	-	-	-	-	-	-	-	1	1	-	-
Short-toed snake eagle (n=1)	-	1	-	-	-	-	-	-	-	-	1	1	1	-	-
sparrow hawk (n=6)	2	2	1	-	-	-	-	-	-	-	-	-	1	-	-
common buzzard (n=3)	-	3	-	-	-	-	-	-	-	-	1	2	2	-	-
kestrel (n=1)	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-

E spp: Enterococcus spp E.f : E.faecalis E.fc.: E.faecium E.g.: E.gallinarum E.c.: E.casseliflavus E.d.: E.durans

DISCUSSION

Because of their variety in ecological niches and their ease in obtaining to man and environmental bacteria, wild birds can act as a pre-definitive role for the bacterial load. Although antibiotic treatment is not applied to wild animals, antibiotic-resistant bacteria were reported in them. It is still unclear how the MDR and related genes have spread from man and animals to wildlife. Many researchers emphasised several possible transmission routes, including direct contact with infected individuals, their faeces, food chain, water and soil (Han et al.,2011;Vittecoq et al., 2016; Yahia et al.,2018). Moreover, they underlined that wild mammals and birds could serve as reservoirs of resistance determinants, can spread and facilitate their transfer over large areas through the migration period or seasonal variations (Lozano et al., 2016; Oravcova et al.,2014; Splichalova et al.,2015). Then, wild birds can be regarded as an excellent indicator of the distribution of AMR in wildlife and could cause

a severe public health problem. (Lozano et al., 2016; Radimersky et al. 2010).

The authors reported the isolation of *Enterococcus* spp. from the wild bird's faeces worldwide. Blanco et al. (2006) isolated *E. faecium* and *E. durans* with a prevalence of up to 64 % from the Egyptian Vulture. *E. faecalis* and *E. gallinarum* were detached with 80 % prevalence from raptors and owls (Marrow et al., 2009). Radhouani et al. (2012) identified enterococci with a prevalence of 74 % from the Common Buzzard. Radimersky et al. (2010) determined the prevalence of enterococci as 58 % from feral domestic pigeons and identified the isolates as *E. faecalis, E. faecium, E. gallinarum, E. casseliflavus, E. hirae, E. durans* and *E. mundtii.* Splichalova et al. (2015) detected six different species from Coraciiform birds and determined the prevalence as 74 %. Yahia et al. (2018)recovered enterococci from wild birds with 52% prevalence. In Turkey, Akgul et al. (2016) determined the prevalence of enterococci as 23.8% from the gulls. In our study, the rate of enterococci from wild birds was 89.7%, which is higher than the other reports. The difference between rates could be based on multiple criteria, including geographical differences, sampling techniques and detection methods. Although a limited number of wild bird species were investigated in other studies, a lot of different species studied in this study can cause this difference.

Many authors emphasised that *E. faecalis* were the predominant enterococci with 25-67% prevalence (Poeta et al., 2005; Radimersky et al., 2010; Splichalova et al., 2015; Yahia et al., 2018). Conversely, some authors described that *E.faecium* was the main species with 48-82%. In the current research, *E.faecalis* prevalence is 60%, and *E.faecium* is 22%. Since these two species are considered as an emerging human pathogen, the high isolation rate of these species should not be ignored.

Even though infections due to the other enterococci species were much less frequent, the clinical cases have been reported (Cetinkaya et al., 2000; Janoskova and Kmet, 2004). Splichalova et al. (2015) indicated that the prevalence of *E.casseliflavus* was 32% in the coraciiform birds. Radimersky et al. (2010) showed that they identified *E.casseliflavus* and *E.gallinarum* from wild birds with a prevalence of 8% and *E.durans* with 13%. Klibi et al. (2015) reported 4% prevalence for *E.gallinarum, E.casseliflavus,* and 1% for *E.durans*. In the current study, although the isolation rate was low, *E.gallinarum*, *E.durans,* and *E.casseliflavus* were described with 3%, 2% and 1% rate, respectively.

In the list of "Critically Important Antimicrobials for Human Medicine" of the 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" (WHO). According to the"List of Antimicrobials of Veterinary Importance" of the World Organisation for Animal Health (OIE), tetracycline and ampicillin were considered as "essential" agents and erythromycin and streptomycin were regarded as extremely important (OIE). In studies investigating antibiotic resistance profiles in different wild birds, mostly the highest resistance was seen to tetracycline (Han et al.,2011; Radhouani et al.,2012; Radimersky et al.,2010; Yahia et al., 2018). Further, erythromycin, ciprofloxacin, gentamicin, and lincomycin resistance were established in the range of highest to a moderate level (Klibi et al.,2015; Oravcova et al.,2014; Radhouani et al.,2012; Radimersky et al.,2010; Splichalova et al.,2015; Yahia et al.,2018). The prevalence of antimicrobial-resistant enterococci in wild birds investigated in this research was high. The primary resistance was found against chloramphenicol (53.4%). Since chloramphenicol is considered to be critically essential antimicrobials of the WHO, the high resistance detected in our region requires special attention for human health.

High resistance was also shown against tetracycline 32%, and erythromycin 30%. In recent years, interest in wildlife has started to increase. In many cities in our country, there is a veterinary faculty hospital and Wildlife Rehabilitation Center, which is connected to universities or ministries. As there are positive aspects, the release of animals recovered after antibiotic treatment to nature suggests that the increase of AMR in these wild animals may lead to adverse effects. The higher resistance to antibiotics can be attributed to this and also, as noted in the other publications, this may be due to the relatively higher use of these antibiotics in the medicine, in the agriculture and as a growth factor. Depending on these factors, it is possible for bacteria to gain resistance against antibiotics as a result of the pollution of nature and water resources. It is thought that Non-Passeriformes and Waterfowl birds such as pigeons, seagulls, and doves can be more affected by this contamination because they live more closely with the public. In this study, the results of the MDR (33.3% in Waterfowl birds, 25.6% in Non-Passeriformes birds, no resistance in Passeriformes birds and Birds of prey) (data not shown) support this hypothesis.

In enterococci isolated from animals and humans, MDR is widespread because of their natural (intrinsic) resistance mechanisms. They can adapt to the environment and can gain other antimicrobial resistance determinants (Lozano et al., 2016). Consequently, there is a minimal treatment option. In this research, the antibiotic resistance patterns were determined using seven antibiotics from 6 different antibiotic groups. MDR was shown in 26 isolates (25.2%), and our results were similar to the others (Radimersky et al.,2010; Santos et al., 2013). Resistance to three or more antibiotics was revealed 17.7% in *E.faecalis* and 47.8% in *E. faecium*. These results were parallel with Radhouani et al. (2014) indicated that AMR of *E.faecium* was highest than *E.faecalis* isolates.

Enterococci exhibit a natural resistance to many antimicrobial agents as well as various acquired AMR genes transmittable to other bacteria (Vittecoq et al.,2016). One of them vancomycin, which has recognised as essential healthcare causes Mondial, is two resistance mechanisms. The first is low-level intrinsic type, which is related with *E.gallinarum* (*vanC*1), *E. casseliflavus* (*vanC*2) and *E.flavescens* (*vanC*3) species. The second one is acquired type (*vanA/B/D/E/G/L/M/N*) and is frequently described in *E.faecium* and *E.faecalis.* (Bagcigil et al.,2016; Silva et al.,2011) The *vanA*-type resistance is highest common resistance factor than *vanB*-type (Werner,2012). The emergence of *vanA*, *van B* or *vanC* genes has been informed worldwide in wild birds with different incidences (1-10.5%) (Oravcova et al.,2014; Poeta et al., 2005; Radhouani et al.,2014; Silva et al., 2011; Yahia et al.,2018)

Acquired-Vancomycin resistance did not appear in this research. While 3.8% of isolates presented intrinsic vancomycin resistance. *E.gallinarum* contained *vanC1*, and one *E.casseliflavus* isolate harbored *vanC2*. Klibi et al. (2015) determined that intrinsic vancomycin resistance was recovered from 5.4% of faecal samples. Some researchers proposed that enterococcal species with low-level vancomycin resistance might frequently be existing in the microbiota of some birds (Lozano et al., 2016; Sellin et al., 2000).

The studies conducted on enterococci' virulence factors focused on wild animals are insufficient (Poeta et al., 2005; Radhouani et al.,2010; Silva et al.,2011). One of these limited studies was underlined *E. faecalis* has at least one virulence genes, and 38% of these isolates were harboured three virulence genes (Poeta et al., 2005). Radhouani et al. (2014) indicated that *E. faecalis* carry more virulence factors than *E.faecium*. In this research, the multiple virulence genes were detected more commonly in *E. faecalis* followed by *E. faecium* with 66.6 % prevalence. Fifty-three point two percent of *E. faecalis* was harbored at least one of the virulence genes, and most of them (81.8 %) carried the multiple virulence factors. In this research, also, the numerous virulence factors in *E. gallinarum* were detected with 100%; however, this high prevalence may be associated with a low number of isolates.

Endocarditis antigen was more frequently detected virulence treat genes coded by *efaA* gene (40.7%). Even though the biological role of *efaA* gene was relatively unknown, our results were similar with the studies which indicated the majority of genes that encode *efaA* in all type of samples (Creti et al.,2004; Eaton and Gasson, 2001).

Gelatinase, encoded by *gel*E gene, detection rate increased endocarditis cases in animals such as 75.3 % (Poeta et al.,2005). Contrarily, Klibi et al. (2015) reported the low prevalence of *gel*E with 12.3 % and Silva et al. (2011) described the absence of virulence factors in wild birds. In this research, the prevalence of the *gel*E was 30.1% and 43.5 % of *E.faecalis* strains had been found positive for gel production.

The *ace* gene which is an accessory colonisation factor encodes a putative protein with similar characteristics to mediate the adherence to collagen. Silva et al. (2011) detected *ace* virulence genes in 4 VRE isolates. Klibi et al. (2015) and Poeta et al. (2005) recognised the gene in nearly 10% of *E. faecalis* isolates. In our research, the prevalence of *ace* gene founded as 17.4%. On the contrary, *E.faecalis* prevalence was 24.1 %. These differences may occur from regional variation.

The *agg* gene, which is an aggregative pheromone, stimulated adherence to extra matrix protein. They have been previously found in enterococci species in a study (Semedo et al., 2003). Silva et al. (2011) reported the absence of *agg* in wild birds and this result supports these findings.

In the present research, the *efa* gene was the frequently carried gene in 26 AMR isolates with 38.4 % prevalence. The increasing virulence discovered in *E. faecalis* may probably conduct the extended of MDR in the environment (Yahia et al., 2018).

CONCLUSION

In our country, to our knowledge, this is a first report from the different species of the wild bird with

a significantly high prevalence. When the results of the study were evaluated in general, MDR was highlighted in a quarter of the isolates. Considering the hazard of polluting the water resources of wild birds, it is suggested that this type of epidemiological study should be maintained in wildlife animals.

ACKNOWLEDGMENT

This research was presented in 2. International Veterinary Microbiology Congress, 16-19 October 2018, Antalya, Turkey.

CONFLICT OF INTEREST

None declared.

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