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Research Article

Determination of Enterococcus Species and Antibiotic Resistance in Budgerigars

Saniye DOLHAN[1], Göksel ERBAŞ[2]*

[1]Aydın Adnan Menderes University, Institute of Health Sciences, Department of Microbiology, Aydın, TÜRKİYE
[2]Aydın Adnan Menderes University, Faculty of Veterinary, Department of Microbiology, Aydın, TÜRKİYE

ABSTRACT

In this study, it was aimed to determine the resistance profile by examining the prevalence and species distribution of enterococci in rectal samples of healthy budgerigars and their susceptibility to antibiotics. 100 cloacal swab samples were used in the study. Identification and antibiotic resistance of Enterococcal isolates obtained by classical methods were determined with the automated identification system. The results showed that the bacteria isolated in this study were 22 (75.86%) *E. faecalis*, one *S. uberis* (3.44%), two of each (6.90%) *E. faecium* (6.90%), *E. hirae* (6.90%), and *E. casseliflavus/gallinarum*. In general, they were suspectible to amoxicillin clavunate (96.4%), ampicillin (100%), ciproflaxacin (54.2%), levoflaxacin (60.9%), gentamicin (syn) (82.1%), streptomycin (syn) (28.6%), tigecycline (80%), vancomycin (89.3%), teicoplanin (96.4%), linezolide (96.4%), and nitrofurontion (100%), and they showed 100% resistance to cefoxitin, amikacin, gentamicin, tobramycin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole (TMP-SXT), fusidic acid, and quinopuristin-dalfopuristin. The presence of Entrococcal species, which are very important in terms of zoonosis, in healthy budgerigars was revealed. In addition, the different types of antibiotic resistance found in the studies also reveal the necessity of performing antibiotic susceptibility tests in this type of infections. However, it has been demonstrated that which antibiotics will be effective in nosocomial and/or gastrointestinal infections of Enterococcal origin in budgerigars.

Keywords: *Antibiotic resistance, Enterococcus spp, budgerigar*

Muhabbet Kuşlarında Enterokok Türlerinin Dağılımı ve Antibiyotik Dirençliliklerinin Belirlenmesi

ÖZET

Çalışmada sağlıklı muhabbet kuşlarının rektal örneklerinde enterokokların yaygınlığı ve tür dağılımı ile bu türlerin antibiyotiklere duyarlılıkların incelenerek direnç profilinin belirlenmesi amaçlanmıştır. Araştırmada 100 adet kloakal svab örneği kullanılmıştır. Klasik yöntemler ile elde edilen enterokok şüpheli izolatların identifikasyonları ve antibiyotik dirençlilikleri otomatize identifikasyon sistemi yardımı ile saptandı. Çalışma sonuçlarında 22 adet (%75.86) *E. faecalis*, 2'şer adet (%6.90) *E. faecium*, (%6.90) *E. hirae*, (%6.90) ve *E. casseliflavus/gallinarum* ve 1 adet de *S. uberis* (%3.44) elde edilmiştir. İzolatlar; amoksisilin klavunat (%96.4), ampisilin (%100), siproflaksasin (%54.2), levoflaksasin (%60.9), gentamisin (syn) (%82.1), streptomisin (syn) (%28.6), tigesiklin (%80), vankomisin (%89.3), teikoplanin (%96.4), linezolide (%96.4) ve nitrofurontion'a (%100) oranlarında duyarlı, sefositin, amikasin, gentamisin, tobramisin, klindamisin, eritromisin, TMP-SXT, fusidik asid ve quinopuristin-dalfopuristine karşı ise %100 direnç göstermişlerdir. Araştırmada Zoonoz özelliği açısından oldukça önemli olan Entrokok türlerinin sağlıklı Muhabbet kuşlarındaki varlığı ortaya konmuştur. Ayrıca çalışmalarda rastlanan farklı antibiyotik dirençlilik tipleri de bu tip enfeksiyonlarda antibiyotik duyarlılık testlerinin mutlaka yapılması gerekliliğini de ortaya koymaktadır. Bununla birlikte Muhabbet kuşlarında Enterokok kökenli nozokomiyal ve/veya gastrointestinal enfeksiyonlarda hangi antibiyotiklerin kullanılmasının etkili olacağı ortaya konulmuştur.

Anahtar kelimeler: *Antibiyotik dirençlilik, Enterococcus spp, muhabbet kuşu*

***Corresponding Author:** Göksel ERBAŞ, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Aydın, TÜRKİYE, gerbas@adu.edu.tr

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Introduction

Enterococci live commensally in the intestinal tract of animals and humans, soil and water (Nasiri and Hanifian, 2022). These bacteria, which are zoonotic, gain pathogenicity when the immune system of the living thing they live in is suppressed under various conditions.

These bacteria are opportunistic pathogens and part of intestinal microbiota. They may become pathogenic agents when the immune system of hosts is suppressed under various conditions (Huycke et al., 1998; Tran et al., 2022). Enterococci can survive at salt concentrations as high as 6.5%, where Gram-positive streptococci cannot grow (Gaca and Lemos, 2019). Also, they can survive high temperatures; They can survive as high as 50 °C, or even up to 30 minutes at 60 °C. They can grow optimally at 37-40 °C depending on the medium. Enterococci can also survive in environments (with some strains based on pH 10.0) that thrive best at pH 7.5, but at pHs as high as 4.8 and 9.6 (some strains based on pH 10.0) (Fisher and Phillips, 2009). They can live 40% (w/v) in bile salts that can remove other bacteria as *Streptococcus pneumoniae* (Murray et al., 2009; Teixeira et al., 2011). The extreme conditions in which enterococci can survive allow them to colonize a wide variety of sites that may be of relevance to their clinical significance (Vu and Carvalho, 2011). The ability of enterococci to withstand wide pH ranges is due to their membrane durability and impermeability to acids and alkalis, while their temperature resistance is attributed to membrane lipids and fatty acids (Fisher and Phillips, 2009). Enterococci are known to be involved in the infections of poultry, canaries and parrots (Devriese et al., 1996). It is stated that these may be infections triggered by secondary infections, and underlying viral or bacterial infections. Six species of the genus Enterococcus *(E. avium, E. cecorum, E. durans, E. faecalis, E. faecium and E. hirae)* have been associated with diseases in poultry (Christensen and Bisgaard, 2016). Of these species, *Enterococcus faecalis* is the most abundant in humans and animals, including mammals and birds. This species (*E. faecalis*) is part of the normal intestinal microflora of mammals and has likewise been reported to be the predominant intestinal microflora in poultry (Devriese et al., 2006). However, this species *(E. faecalis)* is also recognized as an opportunistic pathogen with the potential to cause clinical infections. They are also resistant to a wide variety of antibiotics and may show zoonotic properties. Today, the genus *Enterococcus* has been studied in detail from humans and animals and 38 species have been identified. Among these, the most important species as human and animal pathogens are *Enterococcus faecalis* and *Enterococcus faecium*. Researchs are also ongoing on *Enterococcus gallinarum* and *Enterococcus casseliflavus* because they are naturally resistant to vancomycin and colonize the intestinal tract (Murray et al., 2009; Wada et al., 2022). Although enterococci are low virulence bacteria, they are important agents in community-acquired and especially hospital-acquired infections. Enterococci are intrinsically resistant to many antibiotics, especially beta-lactams and aminoglycosides. They also develop resistance to some antibiotics very quickly (Franz et al., 2001). Enterococci have intrinsic resistance to many antibiotics. However, they can transfer multiple resistance to other bacteria with resistance genes in plasmids, transposons and chromosomes (Aktaş and Derbentli, 2009). Enterococci are resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole (TMP-SMX) and aminoglycosides at low levels, and to polymyxins, monobactams and quinopristin/dalfopristin (Çetinkaya et al., 2000).

In Türkiye, especially the most preferred cage birds, budgerigars, the rate of being kept at home is increasing day by day. Pets are known to be able to transfer antimicrobial resistant bacteria to humans. Therefore, continuous studies are required to monitor antimicrobial resistance carried by zoonotic bacteria from the animals to human. This study was carried out to determine the resistance profile of enterococci in rectal samples of healthy budgerigars by examining the presence, prevalence and species distribution and the susceptibility of these species to antibiotics.

Materials and Methods

In our study, 100 cloacal swab samples collected from budgerigars bred or kept in cages as pet animals in Aydın province and Marmaris district of Muğla were used. Of the 100 samples collected, 50 were male and 50 were female birds. The swab samples were brought to the routine diagnosis laboratory of Aydın Adnan Menderes University Veterinary Faculty Microbiology Department under cold chain and used in the study. Since only swab samples were used in the study, an Ethics Committee Decision is not required.

Fenotypic identification

Cloacal swab samples were first subjected to the pre-enrichment procedure at 37 °C for 24 hours in Enterococcosel™ Broth. After the pre-enrichment process, a loopful of broth was taken and inoculated on 7% sheep blood agar medium and incubated at 37 °C for 24 hours. At the end of this period, gram staining was performed on the breeding colonies and catalase test was applied on those with Gram positive cocci. For this purpose, 3-5 colonies from a 24-hour pure bacterial culture grown on blood agar medium were placed on the slide with a loop. A drop of 3% H_2O_2 (hydrogen peroxide) was dropped on the slide. Strains that did not form air bubbles were considered catalase negative (Koneman et al., 1997). Bacteria with negative catalase test results were defined as *Streptococcus* spp. In order to differentiate Enterococci from these samples, they were inoculated on Enterococcocel Agar™ and incubated for 24 hours. Black colonies grown in this medium were selected and passaged on 'Tryptone soy agar' medium and pure cultures were obtained (Bilgehan, 1995). Obtained isolates were stored in Brain Heart Infusion Broth with 20% glycerol (Merck 4094) until and after the study. In order to identify the

isolated bacteria in the automated identification system, they were cultivated on Tryptone soy agar and they were grown purely. Purely obtained Enterococci suspected colonies were identified using the BD Phoenix™ fully automated identification system, Gram positive bacteria identification and PMIC/ID-87 cartridge, in which antibiotic susceptibility was determined. For this purpose, purified 24-hour fresh cultures on Tryptone soy agar were prepared in glass tubes with ID broth and a suspension according to McFarland 0.5 colony density. On-device diagnostics were performed using the BD Phoenix™ PMIC/ID87 panel kit for each sample of Gram-positive bacterial isolates. Biochemical identification data obtained from the device were evaluated.

Antibiotic resistance studies

Antibiotic susceptibility tests were performed using the BD Phoenix™ PMIC/ID87 kit of the isolates that were bacterially identified with an automated device. Purified 24-hour fresh cultures on Tryptic soy agar were suspended in glass tubes with readily available AST Broth at a McFarland colony density of 0.5. Minimal Inhibitory Concentration values were measured. In the antibiogram sensitivity profile made with the device; penicillin (P), oxacillin (OX), tobramycin (NN), cefoxitin (FOX), ciprofloxacin (CIP), clindamycin (CC), streptomycin-synergy (STS), nitrofurantoin (FM), erythromycin (E), vancomycin (V), fosfomycin (FF), gentamicin (GM), levofloxacin (LVX), amoxicillin/clavulanate (AMC), quinupristin/dalfopristin (SYN), linezolid (LZD), rifampin (RA), teicoplanin (TEC), tetracycline (TE), daptomycin (DAP), tigecycline (TGC), ampicillin (AM), fusidic acid (FA), trimethoprim/sulfamethoxazole (SXT) antibiotics were used. These antibiotic strains are available in the packages of BD Phoenix™ kits for the diagnosis of Gram-positive (PMIC/ID87) bacteria and panels for antibiotic susceptibility. Panels containing bacterial suspensions were placed in the device and bacterial diagnosis was made as well as the detection of antibiograms and sensitivity/resistance data (MIC) were obtained via the electronic system.

Results

A total of 100 samples were taken from 100 healthy budgerigars by cloacal route. *Enterococcus* spp. was isolated in 29 (29%) of 100 samples examined of these are, two (6.90%) *E. casseliflavus/gallinarum*, two (6.90%) *E. hirae*, two (6.90%) *E. faecium*, 22 (75.86%) *E. faecalis*, and one *S. uberis* (3.44%).

In this study, the distribution of 5 different species *E. faecium*, *S. uberis*, *E. hirae*, *E. faecalis*, *E. casseliflavus/gallinarum*, by species and gender is as seen in Table 1.

E. faceium species; It shows 100% sensitivity to penicillins (amoxicillin clavunate, ampicillin), fluoroquinolones (ciproflaxacin, levofloxocin), aminoglycosides [gentamycin (syn)], and streptomycin (syn) and oxazolidines (linezolid), while from gentamycin, cefhelosporins (cephelosporins), were found to be 100% resistant to macrolides erythromycin, TMP-SXT, and fusidic acid.

E. faecalis was the most common species and constituted 75.86% of the total isolates. From penicillin group antibiotics amoxicillin clavunate 95.5%, ampicillin 100%, fluoroquinolones to ciproflaxacin 50%, levofloxacin 57.1%, gentamicin (syn) 77.3%, streptomycin (syn) 13.6%, tigecycline 80%, vancomycin 95.5%, teicoplanine 95.5%, linezolid 95.5% were found to be 100% sensitive to nitrofurantoin. These *E. faecalis* strains identified at the same time showed 100% resistance against fusidic acid, tobramycin, amikacin, cefoxitin, clindamycin, gentamicin, erythromycin, TMP-SXT, and quinopuristin-dalfopuristin.

E. hirae strains showed 100% sensitivity to amoxicillin clavunate, ampicillin, gentamicin (syn), streptomycin (syn), vancomycin, teicoplanin, and linezolid. It was found to be 100% resistant to TMP-SXT.

E. casseliflavus/gallinorum strains showed 100% sensitivity to amoxicillin clavunate, ampicillin, gentamicin (syn), teicoplanin, linezolid, and 50% sensitivity to streptomycin (syn). They showed 100% resistance against erythromycin, gentamicin, fusidic acid, vancomycin, amikacin, TMP-SXT and clindamycin. *S. uberis* showed 100% resistance to gentamicin, tobramycin, and fusidic acid.

According to the data we obtained in our study, all the isolates were sensitive to levofloxacin (60.9%), streptomycin (syn) (28.6%), ampicillin (100%), ciproflaxacin (54.2%), gentamicin (syn) (82.1%), amoxicillin clavunate

Table1. Distribution by species and gender (n=29).

Factors	Gender/ Female	Gender/ Male	Total	Total %
S. uberis	1	-	1	3.44
E. hirae	2	-	2	6.90
E. faecium	-	2	2	6.90
E.casseliflavus/ gallinarum	1	1	2	6.90
E. faecalis	7	15	22	75.86
Total	11	18	29	100

(96.4%), vancomycin (89.3%), teicoplanin (96.4%), tigecycline (80%), linezolide (96.4%), and nitrofurontion (100%). However, they showed 100% resistance against gentamicin, cefoxitin, amikacin, clindamycin, erythromycin, TMP-SXT, tobramycin, quinopuristin-dalfopuristine, and fusidic acid.

Discussion

In recent years, some studies have been carried out to determine the prevalence and antibiotic resistance of Enterococcal species. In Iran, Soodmand et al. (2018) investigated the prevalence and antibiotic susceptibility of Enterococcal species among poultry and domestic birds, they collected oral and cloacal swabs from 150 caged birds and detected the presence of Enterococci in 56 of these samples. When their rates were examined, 6 Enterococci were isolated from 48 patients and 50 (49%) Enterococci from 102 healthy birds. In this study, 29 (29%) Enterococci species were isolated from 100 healthy budgerigars. It is thought that this difference is due to the presence of commercial poultry in the sample made in Iran. While all oral swabs taken from healthy animals were negative, all Enterococci growths were from cloacal swabs in parallel with our study. They emphasized that the highest Enterococcus species obtained in their research was *Enterococcus faecalis*, similar to our study. However, they identified *E. faecium* at a rate of 6.66%, similar to our study (6.90%). When the antibiotic resistance results of their studies were examined, they found that all isolates were resistant to cephalosporins in parallel with our study. However, they found that all *E. faecalis* and *E. faecium* isolates were resistant to 5 different antibiotic agents. When their sensitivity to amoxicillin was examined, it was reported that 40% of *E. faecalis* isolates and 79% of *E. faecium* isolates were found to be susceptible. However, the sensitivity of *E. faecalis* to vancomycin was reported as 29%. 22 *E. faecalis* isolates obtained in our study were found to be sensitive to vancomycin at a rate of 95.5%.

Cabral et al. (2020) investigated the distribution of Enterococcus species, their resistance to gentamicin and vancomycin in their study on Psittacine (parrot-like, curved-billed birds) birds in Brazil. For this purpose, they took samples from 126 birds and isolated *Enterococcus* species (*E. hirae*, *E. faecium*, *E. phoeniculicola*, *E. faecalis*, *E casseliflavus* and *E. gallinarum*) at a rate of 26.9%, similar to our study (29%). It was reported that the most dominant one (41.7%) among the isolated species was *E. faecalis*, similar to our study. They found high-level Gentamsin resistance similar to our study in all *E. faecalis* strains they obtained in their study. Vancomycin susceptibility was reported in two isolates (94.6%), similar to our study (95.8%). Ben Yahia et al. (2018) provide information on the possible roles of Enterococcus species found in wild birds in the spread of VanA/VanB resistance genes in their study in Tunisia. As a result of their research, the most common species in wild birds was *E. faecalis* (67.1%) as in budgerigars (75.86% *E. faecalis*). This was followed by *E. faecium* 24% and *E. casseliflavus* 8.9%. At least one (68%) of the strains of Enterococci obtained were reported to have developed resistance to the antibiotics tested. It was observed that all species obtained in their research were sensitive to ampicillin, linezolid and rifampicin in parallel with our study. The highest resistance level was found to be tetracycline (46.8%) and erythromycin (34.2%) similarly. These were followed by resistance to chloromphenicol (8.8%), gentamicin and streptomycin (2.5-3.8%), ciproflaxacin, trimethoprim sulfamethoxazole and kanamycin (12.7-21%).

Freitas et al. (2018), in a study examining the fecal microbiota of 88 Amazona aestiva parrots found in zoos in Brazil and their antibiotic resistance, *Enterococcus hermanniensis* (0.9%), *Enterococcus gallinarum* (1.7%), *Enterococcus casseliflavus*. (4.8%), *Enterococcus faecalis* (17.3%) and *Enterococcus hirae* (75.3%) species were obtained. All strains obtained were sensitive to linezoid and teicoplanin, similar to our study. However, susceptibility rates to the other 16 tested antimicrobials ranged from 0.4% to 69.3%.

In the study of Akgül et al. (2016) in chickens and seagulls, in chickens; 57.3% *E. faecium*, 4.7% *E. casseliflavus / gallinarum*, 4.1% *E. hirae*, 2.6% *E. durans*, 21.3% *E. fecalis*, in seagulls, 17.6% *E. faecium,* 8.4% *E. hirae*, 5.9% *E. casseliflavus/gallinarum*, 1.7% *E. raffinosus,* 0.8% *E. durans* and 65.5% *E. fecalis* were identified. In the study, it was found that the highest resistance in enterococci was against cefadroxil (99.5%), cefazolin (98.4%) and kanamycin (96.3%), the rate of resistance against tetracycline (18.8%) was lower than in other countries, and streptomycin (83.3%) and gentamicin (64%) determined that the resistance rates were high.

It is reported that the probability of transmission of *E. faecium* from animals to humans is low. However, *E. faecalis* poses a greater risk due to the transfer of resistance genes to virulent enterococci. (Hammerum et al., 2010). *E. faecalis* causes urinary tract infections in humans who consume and/or work with pork or poultry meat (Abat et al., 2016; Larsen et al., 2010; Poulsen et al., 2012).

The gastrointestinal tract is known as a reservoir for the exchange of genetic material by horizontal gene transfer, and the zoonotic potential of *E. faecalis* has been reported to be associated with horizontal gene transfer of genetic material encoding virulence factors and antimicrobial resistance (Werner et al., 2013). The virulence characteristics of enterococci are very important in resistance to antibiotics. Stępień-Pyśniak et al. (2019) investigated the biofilm formation ability and virulence genes of enterococci in their study with cloacal samples taken from wild birds. In the study, they stated that the increase in the hydrophobicity of enterococci species increases the aggregation substance and the ability to form biofilms accordingly. It has been shown that the hydrophobicity of *E. faecalis* is higher than that of *E. faecium* species, resulting in increased biofilm production and increased pathogenicity. In this case, it is one of the reasons explaining the high antibiotic resistance. Enterococci are intrinsically resistant to many antibiotics,

especially beta-lactams and aminoglycosides. They also develop resistance to some antibiotics very quickly. In addition to the intrinsic resistance of bacteria to many antibiotics, the acquired resistance due to resistance genes in plasmids, transposons and chromosomes and the transfer of resistance from one bacterium to another are effective in the increase of multi-resistance to antimicrobials in enterococci (Aktaş and Derbentli, 2009). Enterococci are low-level resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole (TMP-SMX) and aminoglycosides, and genetically resistant to polymyxins, monobactams, and quinopristin/dalfopristin (Çetinkaya et al., 2000).

Enterococci obtained in our study were also 100% resistant to tobramycin, cefoxitin, TMP-SXT, gentamicin, clindamycin, erythromycin, amikacin, quinopuristin-dalfopuristin and fusidic acid. In 1979, high levels of gentamicin resistance began to appear in clinical isolates. This has created difficulties in the treatment of enterococcal infections. A new form of antibiotic, vancomycin, was developed in the 1990s. However, the incidence of vancomycin-resistant Enterococci (VRE) has been increasing in recent years (Woodford et al., 1998). VRE is responsible for the deaths of approximately 25,000 people each year in the United States. VRE is reported as the second most common cause of nosocomial infections (McKinnell et al., 2012). However, the prevalence of VRE in South America and Turkey is still relatively low. (Çetinkaya et al., 2000; Panesso et al., 2010). In addition to the high gentamicin resistance obtained in our study, gentamicin synergetic sensitivity is also very important. Enterococci are inherently resistant to the inhibitory and bactericidal activities of the most commonly used agents. Therefore, the recommended treatment for serious infections (ie, endocarditis, meningitis, or other possible serious infections in immunocompromised patients) includes a cell wall active substance such as penicillin or vancomycin in combination with an aminoglycoside (usually gentamicin) or sometimes streptomycin. These combinations overcome the intrinsic resistance exhibited by enterococci and achieve synergistic elimination. Therefore, while gentamicin and streptomycin are 100% resistant in many isolates, Synergetic gentamicin and streptomycin (gentamicin (Syn) and streptomycin (Syn)) give high susceptibility results.

Conclusion

The presence of Entrococcal species, which are very important in terms of zoonotic feature, in healthy budgerigars fed as pets in our homes has been revealed. Although they are harmless under normal conditions, it should be kept in mind that these bacteria can cause serious infections such as endocarditis, septicemia, urinary system infections in humans. In addition, the different types of antibiotic resistance found in the studies also reveal the necessity of performing antibiotic susceptibility tests in this type of infections. However, the data obtained in the research is also important in terms of the necessity of choosing which antibiotics in the treatment of nosocomial and/or gastrointestinal infections in budgerigars.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abat, C., Huart, M., Garcia, V., Dubourg, G., & Raoult, D. (2016). *Enterococcus faecalis* urinary-tract infections: do they have a zoonotic origin. *Journal of Infection*, 73 (4) : 305-313. https://doi.org/10.1016/j.jinf.2016.07.012

Akgül, Ö., Gülhan, T., & Güdücüoğlu, H. (2016). Phenotypic and genotypic analysis for antibiotic resistance of *Enterococcus* species with chicken and gull origin. *Veterinary Journal of Ankara University*, 63, 235-244.

Aktaş, G., & Derbentli, Ş. (2009). The importance and epidemiological characteristics of vancomycin-resistant Enterococci. *Turkish Journal of Infection*, 23 (4): 201-209.

Ben Yahia H, Chairat S, Hamdi N, Gharsa H, Ben Sallem R, Ceballos S, Torres C, Ben Slama K. (2018). Antimicrobial resistance and genetic lineages of faecal enterococci of wild birds: Emergence of vanA and vanB2 harbouring *Enterococcus faecalis*. *International Journal of Antimicrobial Agents*. 52 (6), 936-941. https://doi.org/10.1016/j.ijantimicag.2018.05. 005.

Bilgehan, H. (1995). "Streptokoklar" Klinik Mikrobiyoloji. Özel Bakteriyoloji ve Bakteri İnfeksiyonları. 9. Basım. İzmir: Şafak Matbaacılık, 248-286.

Cabral, B. G., Davies, Y. M., Menao, M. C., Saidenberg, A. B. S., Gomes, V. T. M., Moreno, L. Z., Sato, M. I. Z., Moreno, A. M., & Knöbl, T. (2020). Companion psittacine birds as reservoir of gentamicin and vancomycin-resistant *Enterococcus* spp. *Pesquisa Veterinaria Brasileira*, 40(2):129-133. https://doi.org/10.1590/1678-5150-PVB-6147.

Christensen, H., & Bisgaard, M. (2016). Members of *Streptococcus* and *Enterococcus* associated with disease in poultry. In A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens. 6th edn. Ed., Williams, S. M., Dufour-Zavala, L., Jackwood, M., Lee, M. W., Lupiani, B., Reed, W. M., Spackman, E. & Woolcock, P. R. 127–137. Jacksonville: American Association of Avian Pathologists.

Çetinkaya, Y., Falk, P., & Mayhall, C. G. (2000). Vancomycin resistant enterococci. *Clinical Microbiology Reviews*, 13, 686-707, 2000. https://doi.org/10.1128/CMR.13.4.686-707.

Devriese L., Baele M., & Butaye P. (2006). The genus *Enterococcus*. *The Procaryotes*, New York: Springer, 163–174.

Devriese, L. A., Ieven, M., Goossens, H., Vandamme, P., Pot, B., Hommez, J., & Haesebrouck F. (1996). Presence of vancomycin-resistant enterococci in farm and pet animals. *Antimicrobial Agents and Chemotherapy*, 40:2285-2287. https://doi.org/10.1128/AAC.40.10.2285

Franz, C. M., Muscholl-Silberhorn, A. B., Yousıf, N. M., Vancanneyt, M., Swings, J., & Holzapfel, W. H. (2001). Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. *Applied and Environmental Microbiology*, 67: 4385– 4389. https://doi.org/10.1128/AEM.67.9.4385-4389.2001.

Fisher, K., & Phillips, C. (2009). The ecology, epidemiology and virulence of Enterococcus. *Microbiology*, 155(Pt 6): 1749–1757. https://doi.org/10.1099/mic.0.026385-0.

Freitas, A. A. R., Faria, A. R., Pinto T. C. A., Merquior, V. L. C., Neves, D. M., Costa, R. C. D., & Teixeira, L. M. (2018). Distribution of spe-

cies and antimicrobial resistance among enterococci isolated from the fecal microbiota of captive blue-fronted parrot (Amazona aestiva) in Rio de Janeiro, Brazil. *Science of the Total Environment,* 615:1428–1437. https://doi.org/10.1016/j.scitotenv.2017.09.004.

Gaca, A. O., & Lemos, J. A. (2019). Adaptation to adversity: the intermingling of stress tolerance and pathogenesis in Enterococci. *Microbiology and Molecular Biology Reviews*, 83(3):1-46. https://doi.org/10.1128/MMBR.00008-19.

Hammerum, A. M., Lester, H., & Heuer, O. E. (2010). Antimicrobial-resistant 205 enterococci in animals and meat: a human health hazard? *Foodborne Pathogens and Disease*, 7(10):1137-1146. https://doi.org/10.1089/fpd.2010.0552.

Huycke, M. M., Sahm, D. F., & Gilmore, M. S. (1998). Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerging Infectious Diseases*, 4(2): 239–249. https://doi.org/10.3201/eid0402.980211.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. JR. (1997). "The Gram Positive Cocci Part II Streptococci, Enterococci and The Streptococci Like Bacteria". In Color Atlas and Textbook of Diagnostic Microbiology. 5 th Ed. Philadelphia: JB Lippincott Company; 577- 629.

Larsen, J., Schønheyder, H. C., Lester, C. H., Olsen, S. S., Porsbo, L. J., Garcia-Migura, L., Jensen, L. B., Bisgaard, M. & Hammerum A. M. (2010). Porcine-origin gentamicin resistant *Enterococcus faecalis* in humans, Denmark. *Emerging Infectious Diseases*, 16(1):682-684. https://doi.org/10.3201/eid1604.090500

McKinnell, J. A., Kunz, D. F., Chamot, E., Patel, M., Shirley, R., Moser, S., Baddley, J. W., Pappas, P. G. & Miller, L. G. (2012). Association between vancomycin-resistant enterococci bacteremia and ceftriaxone usage. *Infection Control & Hospital Epidemiology*, 33(7):718-724. https://doi.org/10.1086/666331.

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2009). *Enterococcus* and other Gram-positive cocci. *Medical Microbiology,* (6th Ed), 243–246.

Nasiri, M., & Hanifian, S. (2022). *Enterococcus faecalis* and *Enterococcus faecium* in pasteurized milk: Prevalence, genotyping, and characterization of virulence traits. *Lebensmittel-Wissenschaft & Technologie*, 153, art. no. 112452. https://doi.org/ 10.1016/j.lwt.2021.112452.

Panesso, D., Reyes, J., Rincón, S., Díaz, L., Galloway-Peña, J., Zurita, J., Carrillo, C., Merentes, A., Guzmán, M., Adachi, J. A., Murray, B. E. & Arias, C. A. (2010). Molecular epidemiology of vancomycin-resistant *Enterococcus faecium*: a prospective, multicenter study in South American hospitals. *Journal of Clinical Microbiology*, 48(5):1562-1569. https://doi.org/10.1128/JCM.02526-09

Poulsen, L. L., Bisgaard, M., Son, N. T., Trung, N. V., An, H. M. & Dalsgaard, A. (2012). *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. *Emerging Infectious Diseases*, 18(1):1096-1100. https://doi.org/10.3201/eid1807.111754.

Soodmand, J., Zeinali, T., Kalidari, G., Hashemitabar, G., & Razmyar, J. (2018). Antimicrobial Susceptibility Profile of *Enterococcus* Species Isolated from Companion Birds and Poultry in the Northeast of Iran. *Archives of Razi Institute*, Vol. 73, No. 3, 207-213. https://doi.org/10.22092/ari.2017.108332.1089.

Stępień-Pyśniak, D., Hauschild, T., Kosikowska, U., Dec, M. & Urban-Chmiel, R. (2019) Biofilm formation capacity and presence of virulence factors among commensal *Enterococcus* spp. from wild birds. *Scientific Reports*, 9, 1–7. https://doi.org/10.1038/s41598-019-47602-w

Teixeira, L. M., Carvalho, M. G. S., Shewmaker, P. L., & Facklam, R. R. (2011). Manual of Clinical Microbiology. Washington, D.C.: ASM Press, 350–364.

Tran, H. T., Vo, N. N., & Truong, Q. T. (2022). Optimization of medium composition for production of a simulated urine sample containing *Enterococcus faecalis*. *Accreditation and Quality Assurance*, 27, 119–124. https://doi.org/ 10.1007/S00769-022-01493-Z.

Vu, J., & Carvalho, J. (2011). *Enterococcus*: review of its physiology, pathogenesis, diseases and the challenges it poses for clinical microbiology. *Frontiers in Biology*, 6(5): 357–366. https://doi.org/10.1007/s11515-011-1167-x.

Wada, Y., Ahmad, A. I., Rafidah, H. S., Mustapha, W., Hafeez, A. A., Chan, Y. Y., Azian, H., & Abdul, R. Z. (2022). "Prevalence of Vancomycin-Resistant Enterococcus (VRE) in Poultry in Malaysia: The First Meta-Analysis and Systematic Review" *Antibiotics*, 11, 2: 171, 1-16. https://doi.org/10.3390/antibiotics11020171

Werner, G., Coque, T. M., Franz, C. M. A. P., Grohmann, E., Hegstad, K., Jensen, L., van Schaik, W. & Weaver, K. (2013). Antibiotic resistant enterococci-Tales of a drug resistance gene trafficker. *International Journal of Medical Microbiology,* 303, 360–379. https://doi.org/10.1016/j.ijmm.2013.03.001.