GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

The first record of *Paraclostridium benzoelyticum* and *Enterococcus thailandicus* bacteria isolated from the *Eryx jaculus* (Linnaeus, 1758) snake species distributed in Türkiye

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GSC Biological and Pharmaceutical Sciences, 2024, 28(02), 297–304

Publication history: Received on 25 June 2024; revised on 06 August 2024; accepted on 08 August 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.2.0287

Abstract

In this study, we focused on the characterization of ten bacterial isolates obtained from *Eryx jaculus* (oral, skin, feces, and cloaca samples) using cultural methods and determine some virulence factors (antibiotic resistance, VanA, and TetM resistant genes, biofilm, siderophore, and some enzymatic activities, etc.). Multiple antibiotic resistance (MAR) and virulence index (VI) of each strain were also calculated. *Citrobacter freundii* NH10 (EJF1), *Enterococcus thailandicus* 2545 (EJF2), *Enterococcus faecium* MG5232 (EJF3), *Klebsiella pneumoniae* KR56 (EJF4, EJC1, EJS1), *Enterococcus mundtii* AF-1 (EJC2, EJC3), *Paraclostridium benzoelyticum* CM8 (EJO1), *Enterococcus faecalis* MG5206 (EJO2) have been identified and this is the first record of the *P. benzoelyticum* CM8 and *E. thailandicus* 2545 from *E. jaculus* species. It was observed that all isolates produced amylase, while most of them produced siderophore (n = 7), hemolysin (n = 6) and protease (n = 6). It was determined that EJF2 and EJC2 strains produced weak positive (++) biofilm, while the other eight strains produced strong positive (+++) biofilm. Different sensitivity and resistance rates were observed against various antibiotic classes. While AM10 and A10 were determined as the antibiotics with the highest sensitivity, aminoglycoside G120 showed 100% resistance in all isolates. Moreover, EJO1 and EJO2 strains were classified in the high threat category with high virulence and MAR indices. PCR analysis showed the presence of resistance genes VanA in strain EJF1 and TetM in strain EJF2.

Keywords: Multiple antibiotic resistance; Resistance genes; Siderophore; Virulence index

1. Introduction

Animals maintain close and complex relationships with the microbial communities living in their gastrointestinal systems. These gut microbes can influence the host's ecology and evolutionary processes by affecting behavior, immune responses, nutrition, and reproductive isolation. While comprehensive microbial diversity inventory studies have primarily been conducted on mammals, birds, fish, and to a lesser extent amphibians, there are relatively few studies on reptiles in this regard. The approximately 10,000 species of reptiles highlight the necessity of studying the gut microbial ecology of these groups to begin understanding generalized patterns among vertebrate groups [1]. The microbial flora of snake species includes a diverse array of aerobic and anaerobic microorganisms, particularly fecal Gram-negative rods [2]. Research on the bacterial flora in the mouths of snakes has been conducted worldwide [3-5], but specific data for Türkiye are notably lacking.

Eryx jaculus (Linnaeus, 1758). is a non-venomous snake species belonging to the Boidae family, found in arid, sandy, and sparsely vegetated rocky areas. It kills its prey by constriction and then swallows it [5,6]. Due to its subterranean lifestyle, it is rarely seen and may be mistakenly killed because it is thought to be venomous [7]. Research on the

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microbiota of snake species in Türkiye has generally focused on economically important species and microbiological studies specific to the species in question are quite limited [8,9].

Paraclostridium benzoelyticum is a bacterium that falls within the Clostridiaceae family, known for its ability to degrade benzoate and other aromatic compounds. This bacterium is typically found in environments where anaerobic conditions prevail, such as in the gastrointestinal tracts of animals, in soil, and in other organic-rich substrates. Studies involving *P. benzoelyticum* often focus on its role in the degradation of aromatic compounds, its potential applications in bioremediation, and its interactions with other microbial communities [10,11].

Enterococcus thailandicus is a species of the genus *Enterococcus*, first isolated from a sample of fermented sausage (mum) in Thailand [12]. This bacterium is a facultative anaerobe and produces L-lactic acid from glucose. Its pathogenic potential is not well-documented [13]. To date, *Enterococcus* species have been found in a variety of animal sources, including ruminants, rabbits, piglets, dogs, horses, turkeys, chickens, ostriches, common pheasants, and Japanese quails [14]. No studies have been found in the literature where these two species were isolated from the microbiota of *E. jaculus* or similar snake species. Therefore, this study is highly significant for elucidating the bacterial flora in *E. jaculus*.

In this study, molecular characterization was performed on a total of 10 bacterial isolates obtained from the mouth, cloaca, skin, and fecal samples of *E. jaculus*. Additionally, the antibiotic resistance, certain enzymatic virulence factors, siderophore production, and biofilm-forming capacities were determined. To further understand antibiotic resistance, the presence of tetM and vanA resistance genes was also investigated. Moreover, the characterization of *P. benzoelyticum* and *E. thailandicus*, which were isolated and recorded in the literature for the first time, was conducted based on the aforementioned data. Our data are particularly notable as the first to describe these two bacteria in this context.

2. Material and methods

2.1. Sampling and identification

10 bacteria isolated from oral (O), skin (S), feces (F), and cloaca (C) samples of *E. jaculus* (n=1), stored in the culture collection, were utilized. Conventionally, isolates were mainly identified via microscopy (gram staining), and biochemical testing techniques (respiratory-fermentation tests, oxidase, catalase, IMVIC, and H_2S production) [15], and confirmation was obtained through the Microgen ID test kit Microgen ID test kits.

Molecular Identifications of Isolates: Genomic DNA isolation from bacterial isolates was performed using the Thermo Scientific GeneJET Genomic DNA Purification Kit (K0721). The qualitative amount of the obtained genomic DNA was assessed using 1% agarose gel electrophoresis, and the gel was stained with 10 mg/ml EtBr for visualization under UV light. For 16S rDNA gene analysis, bidirectional sequencing was conducted using Sentegen Primers (0341f 5'→3' CCTACGGGGGCGCAG, 0785r 5'→3' GACTACGGGTATCTAATCC). The resulting sequences were compared with reference sequences in the NCBI databases [16].

2.2. Assessment of isolate virulence properties

To evaluate the virulence properties of the isolates, standardized enzymatic activity tests were used, including DNAse, hemolysis, protease, lipase, amylase, and siderophore assays [17,18]. The virulence index is a measure that quantitatively assesses the pathogenic potential of the microorganism. Data obtained for each virulence factor were evaluated to calculate the virulence index (VI) [19].

2.3. Measurement of biofilm formation

The biofilm-forming capacity was measured using the method proposed by [20] with microtiter plates. The final optical density in the microtiter plates was measured using a microplate reader at 550 nm. During this process, the average optical density of the control wells, obtained with a spectrophotometer, was determined to be 0.133. Based on this value, the results of the study were classified as "strong positive" (+++), "positive" (++), "weak positive" (+), and "negative" (-).

2.4. Antibiotic sensitivity testing

Antimicrobial resistance was determined by using the disk diffusion method on Mueller-Hinton agar (MHA) according to the [21] recommendations. The isolates were screened for resistance against 14 antibiotics, including trimethoprim (TR10 µg/mL), tobramycin (TB10 µg/mL), kanamycin (K30 µg/mL), amoxicillin (AM10 µg/mL), oxytetracycline (O30

µg/mL), cephalothin (CH30 µg/mL), cefmetazole (CMZ30 µg/mL), gentamicin (G10 µg/mL), furazolidone (FR50 µg/mL), erythromycin (E15 µg/mL), cefoxitin (CN30 µg/mL), ampicillin (A10 µg/mL), cefotaxime (CE30 µg/mL), and chloramphenicol (C30 µg/mL). Intermediate susceptible and sensitive isolates were marked as not resistant.

The Multiple Antibiotic Resistance (MAR) index was calculated and interpreted according to [22] using the formula: *a/b*, where '*a*' represents the number of antibiotics to which an isolate was resistant, and '*b*' represents the total number of antibiotics tested.

2.5. Determination of antibiotic resistance genes

The presence of isolates vanA and tetM genes, which encode the vancomycin and tetracycline resistance feature, respectively were investigated by the Polymerase Chain Reaction (PCR) method. The sequences of PCR primers of TetM and VanA genes are given in Table 1. Using the literature information on PCR conditions, necessary modifications were made to the PCR components [23].

Table 1 Antibiotic resistance genes primer sequences

Antibiotic resistance genes	Primer sequences	Base pairs
vanA	F-5' GTA CAA TGC GGC CGT TA R-5' GGG ACA GTT ACA ATT GC	732 bp
tetM	F-5' GTT AAA TAG TGT TCT TGG AG R-5' CTA AGA TAT GGC TCT AAC AA	657 bp

3. Results

3.1. Identification of strains

The purity and biochemical characterization of all strains were confirmed using conventional biochemical tests. The 16S rDNA gene sequence analyses of all strains were performed using the NCBI BLAST application, and the genetic typing of each isolate was carried out by comparing sequence similarity ratios in the databases (Table 2; Figure 1).

Table 2 Bioinformatics analysis results of the isolates

Isolate code	Bioinformatic closest match	Similarity Ratio (%)
EJF1	*Citrobacter freundii* NH10	100
EJF2	*Enterococcus thailandicus* 2545	99,76
EJF3	*Enterococcus faecium* MG5232	100
EJF4	*Klebsiella pneumoniae* KR56	100
EJC1	*Klebsiella pneumoniae* KR56	100
EJC2	*Enterococcus mundtii* AF-1	100
EJC3	*Enterococcus mundtii* DE-5	100
EJO1	*Paraclostridium benzoelyticum* CM8	95,35
EJO2	*Enterococcus faecalis* MG5206	100
EJS1	*Klebsiella pneumoniae* KR56	100

EJ: *Eryx jaculus,* F: Feces, C: Cloaca, O: Oral, S: Skin

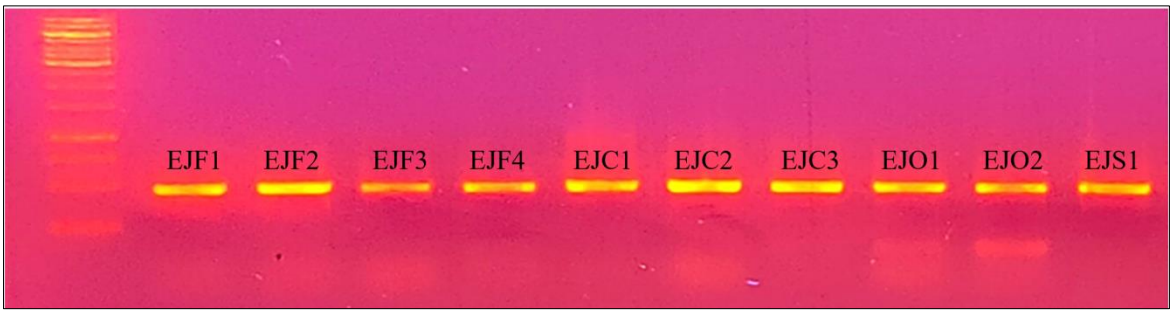

Figure 1 PCR bands of the strains

These results reveal species matches and similarity ratios determined with high accuracy in the genetic typing of the isolates. The findings clearly illustrate the genetic diversity of the bacterial strains and the distribution of the identified species.

3.2. Evaluation of the virulence properties of the isolates

Regarding the characterization of virulence, it was observed that all isolates produced amylase (n = 10), while most produced siderophores (n = 7), hemolysins (n = 6), and proteases (n = 6). Less than half of the isolates produced DNase (n = 2) and lipase (n = 2). Notably, the highest levels of siderophore production were qualitatively detected in the strains EJF4, EJC1, and EJS1 (Figure 2). This strain demonstrated a significant superiority in siderophore production compared to other isolates. In contrast, siderophore production was not detected in the EJF3, EJC3, and EJO1 strains.

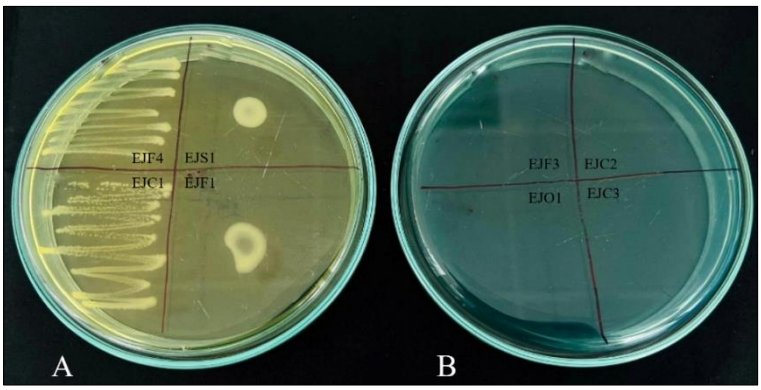

Figure 2. A) Siderophore positive isolates (EJF4, EJC1, EJS1 and EJO1) B) Siderophore negative isolates (EJF3, EJO1, EJC2 and EJC3)

3.3. Measurement of biofilm formation

Values obtained that were below the control value of 0.133 were classified as (-), values between 0.133 and 0.473 were classified as (+), values between 0.474 and 0.946 were classified as (++), and values between 0.946 and 1.892 were classified as (+++). The data were categorized as positive, weak positive, and negative. Biofilm production was determined to be weak positive (++) in the EJF2 and EJC2 strains, while it was identified as strong positive (+++) in the other eight strains.

3.4. Antibiotic resistant profiles

Among the penicillins, it was observed that the antibiotics AM10 and A10 had sensitivity rates of 70% and 60%, respectively. Among the cephalosporins, CE30 showed a sensitivity rate of 40%, while CMZ30 and CN30 were found to be resistant in the majority of isolates (80% and 90%, respectively). From the phenicols group, C30 exhibited 70% sensitivity. The aminoglycoside G120 showed 100% resistance across all isolates. In the potentiated sulfonamides group, TR10 was sensitive in 90% of the isolates. Among the macrolides, E15 had a sensitivity rate of 30%, nitrofurans like FR50 showed 40% sensitivity, and oxazolidinones such as O30 exhibited 30% sensitivity (Figure 3).

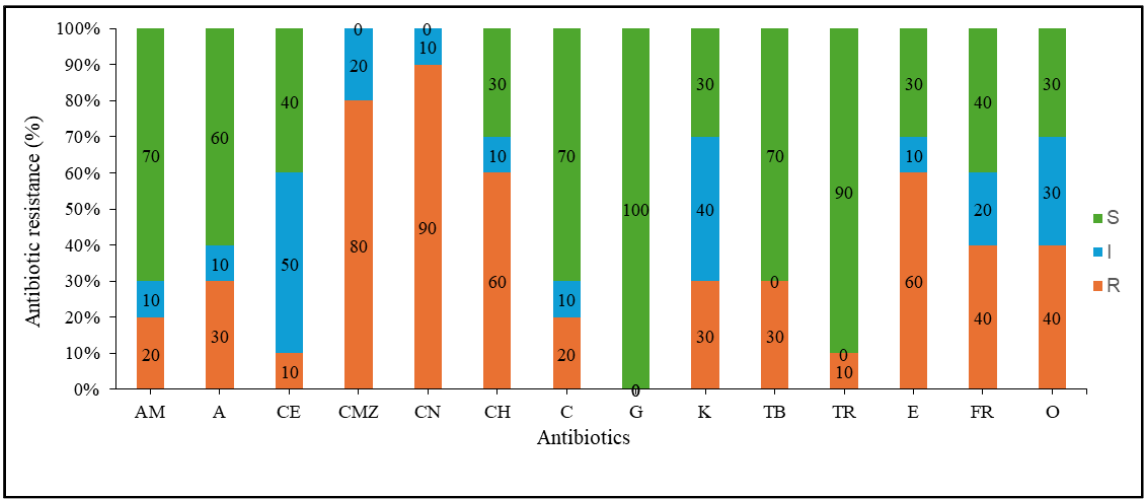

Figure 3 Antibiotic resistant profiles of isolates

In the study, PCR analyses conducted on 10 isolates revealed that two bacteria tested positive for antibiotic resistance genes. According to the analysis results, a positive band formation for the VanA gene was observed in the EJF1 strain. Similarly, a positive band formation for the TetM gene was detected in the EJF2 strain. These results indicate that the bacteria have developed resistance to the respective antibiotics (Figure 4).

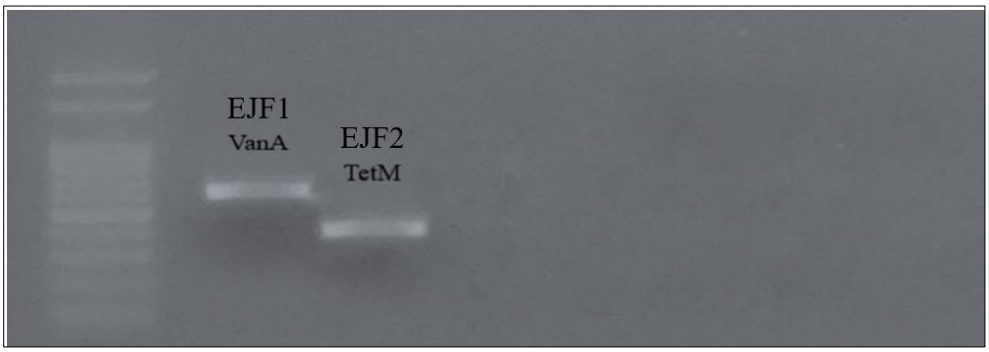

Figure 4 VanA and TetM resistance gene gel image

MAR index revealed that strains with a value greater than 0.2 are considered to have multidrug resistance (Table 3).

E. faecalis MG5206 (EJO1) (VI = 0.83, MAR Index = 0.57) and *P. benzoelyticum* CM8 (EJO2) (VI = 0.66, MAR Index = 0.50) exhibited higher virulence index values. The isolates were classified as high threat (VI ≥ 0.50) (n = 9) and no threat (VI < 0.50) (n = 1). Based on the MAR index data, all isolates were determined to be in the high threat category (Table 3).

Table 3 VI and MAR index findings of isolates

Isolate code	VI	MAR index
EJF1	0.50	0.28
EJF2	0.33	0.21
EJF3	0.50	0.36
EJF4	0.50	0.36
EJC1	0.66	0.43
EJC2	0.50	0.21
EJC3	0.50	0.50
EJO1	0.66	0.50
EJO2	0.83	0.57
EJS1	0.66	0.21

4. Discussion

Wildlife plays a critical role in maintaining ecosystem balance and conserving biological diversity. These animals include species that live freely in their natural habitats and survive without human intervention. The health of wild animals is crucial not only for the sustainability of their own populations but also for the control of pathogens shared with humans and other animal species. In recent years, topics such as microbiota, virulence, and antibiotic resistance have had a significant impact on the health of wildlife.

In this study, the first record of *P. benzoelyticum* and *E. thailandicus* isolated from *E. jaculus* snakes in Türkiye has been established. PCR analyses for VanA and TetM antibiotic resistance genes identified that the EJF1 and ECF2 strains carry these genes. These results indicate the development of resistance to the respective antibiotics and support the presence of antibiotic resistance genes commonly found in environmental and clinical settings.

Hacıoğlu and Tosunoğlu [24], isolated 153 bacteria from various reptile and amphibian species captured in the Kavak Delta. Among these bacteria, species such as *Aeromonas, Plesiomonas, Vibrio, Citrobacter, Enterobacter, Escherichia, Klebsiella, Edwardsiella, Hafnia, Proteus, Providencia*, and *Pseudomonas* were identified. The antibiotic resistance profiles of the isolates showed the highest resistance to cefoxitin (46.40%) and the lowest to gentamicin (6.53%).

Ramos et al [25], isolated and characterized *Escherichia coli, Salmonella* spp., *Clostridium perfringens*, and *Clostridium difficile* from reptiles in Brazil, including 15 lizards, 16 turtles, and 45 snakes. The isolates were found to be resistant to cefalotin and ciprofloxacin antibiotics.

Heck et al [26], isolated *Enterococcus* spp. from the oral cavities of healthy snake species in Brazil and evaluated their antimicrobial resistance and virulence properties. They identified 116 enterococci, with *Enterococcus faecalis* being the predominant species across all snake species, followed by *Enterococcus faecium, Enterococcus avium*, and *Enterococcus hirae*. The study found that *E. faecium* (50%) and *E. faecalis* (15.78%) isolates were multidrug-resistant, with 70% carrying the TetM resistance gene.

Brunson et al [27], conducted a study on the siderophore production ability of *E. faecalis* strains and noted that siderophore production enhanced the virulence properties of the bacteria.

The research indicates that *E. jaculus* in the wild may serve as a carrier of various pathogenic and antibiotic-resistant bacterial species, which poses potential threats to both ecosystem health and human health. The antibiotic resistance profiles of bacteria isolated from wildlife underscore the potential for these microorganisms to spread and develop resistance in environmental and clinical settings. In this context, microbiological analysis of wildlife and molecular characterization of isolates are critical for controlling pathogen spread and managing antibiotic resistance.

5. Conclusion

Reptiles are animal species that inhabit various ecosystems and are an important part of biological diversity. These species can serve as carriers of microbiological diversity and potential pathogens. Specifically, the oral flora of reptiles such as snakes contains a range of aerobic and anaerobic microorganisms that may contribute to the spread of potential pathogens relevant to human health. However, there is limited information available about the microbiological flora of reptiles in Türkiye. Therefore, the molecular identification and characterization of virulence properties of microorganisms isolated from reptiles fill an important gap both scientifically and for public health. The findings reveal the complexity and diversity of bacterial communities found in reptiles' natural habitats. Besides the environmental impacts of reptiles, the potential pathogenic characteristics of the microorganisms they carry are also significant for public health. This data is expected to be a valuable resource for future research, public health strategies, and the conservation of natural habitats. Additionally, this study provides the first record of *P. benzoelyticum* and *E. thailandicus* isolated from *E. jaculus* snakes in Türkiye. Isolating *P. benzoelyticum* from a reptile such as *E. jaculus* could provide insights into the microbial diversity of the reptile's gut and the potential ecological roles of this bacterium. This finding also represents a significant step in understanding Türkiye's microbiota diversity and ecosystem dynamics. Particularly, the presence of such a bacterium in local snake species like *E. jaculus* broadens the microbiological profile of regional fauna and offers a new perspective on understanding ecosystem interactions.

Compliance with ethical standards

Acknowledgments

This study was financially supported by the Scientific Research Project Coordination Unit of Çanakkale Onsekiz Mart University, Turkey (FHD-2019-2802).

Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

[1] Kohl KD, Brun A, Magallanes M, Brinkerhoff J, Laspiur A, Acosta JC, Bordenstein SR. Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. Molecular Ecology. 2017; 26(4): 1175-1189.

[2] Garg A, Sujatha S, Garg J, Acharya NS, Parija SC. Wound infections secondary to snakebite. The Journal of Infection in Developing Countries. 2009; 3(03): 221-223.

[3] Blaylock RS. Normal oral bacterial flora from some southern African snakes. Onderstepoort Journal of Veterinary Research. 2001; 68: 175-182.

[4] Shek KC, Tsui KL, Lam KK, Crow P, Ng KH, Ades G, Kam CW. Oral bacterial flora of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*) in Hong Kong SAR, China. Hong Kong Medical Journal. 2009; 15(3): 183.

[5] Dipineto L, Rinaldi L, Bosco A, Russo TP, Fioretti A, Cringoli G. Co-infection by *Escherichia coli* O157 and gastrointestinal strongyles in sheep. The Veterinary Journal. 2013; 197(3): 884-885.

[6] Insacco G, Spadola F, Russotto S, Scaravelli D. (2015). *Eryx jaculus* (Linnaeus, 1758): a new species for the Italian herpetofauna (Squamata: Erycidae). Acta Herpetologica, 2015; 10(2): 149-153.

[7] Hacıoğlu Doğru N, Gül Ç, Tosunoğlu M. First record of the two snakes microbiota from Çanakkale (Turkey). Mathematics and Natural Sciences Research and Theory. 2021; 21-32, Lyon: Livre.net.

[8] Şeker E, Sarıeyyüpoğlu M. Examination of the stomach and intestines of freshwater mussels (*Unio elongatulus eucirrus*, Bourguignat, 1860) collected from Koçkale region of Keban dam lake in terms of fecal coliforms. Fırat Univ. Science and Engineering Journal of Sciences. 1998; 10(1): 45–53.

[9] Matyar F. Typing of gram-negative bacteria isolated from hospital sewage and determination of multiple antibiotic resistance. Turkish Journal of Agriculture - Food Science and Technology. 2016; 4(10): 845-849.

[10] Švec P, Devriese LA. Genus I. Enterococcus. In Bergeys Manual of Systematic Bacteriology, 2nd edn, Vol. 3, pp. 594-607. Edited by De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB. New York: Springer, 2009.

[11] Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Whitman WB. (Eds.). Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes (Vol. 3). Springer Science & Business Media. 2011.

[12] Tanasupawat S, Sukontasing S, Lee JS. *Enterococcus thailandicus* sp. nov. isolated from fermented sausage (mum) in Thailand. Int. J. Syst. Evol. Microbiol. 2008; 58: 1630-1634.

[13] Abis Encyklopedia. *Enterococcus thailnadicus*. www.tgw1916.net. 2013.

[14] Lauková A, Simonová M, Strompfová V, Štyriak I, Ouwehand AC, Várady M. Potential of enterococci isolated from horses. Anaer. 2013; 14: 234-236.

[15] Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. Clinical Microbiology. (one). Ankara/Türkiye: Atlas Bookselling. 2009; 734-748.

[16] Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing based diversity studies. Nucleic Acids Res. 2013; 41:e1. doi: 10.1093/nar/gks808.

[17] Collins YF, McSweeney PLH, Wilkinson PLH. Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. Lnt Dairy Journal. 2003; 13: 841–866.

[18] Perez E, Sulbaran M, Ball MM, Yarzabal LA. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol. Biochem. 2007; 39: 2905–2914.

[19] Karakaş İ, Doğru NH, Gül Ç, Tosunoğlu M. Determination of pathogenicity of bacteria ısolated from some aquatic amphibian and reptile species. Biology Bulletin. 2024; 1-10.

[20] Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms, Curr. Prot. Microbiol. 2011; 22(1): 1B-1.

[21] Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests. NCCLS Document M2-A7. National Committee for Clinical Laboratory Standards. 2009; 27(1), Wayne.

[22] Krumpermann PH. Multiple antibiotic resistances indexing of *E. coli* to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology. 1983; 46(1): 165–170.

[23] Pasquaroli S, Di Cesare A, Vignaroli C, Conti G, Citterio B, Biavasco F. Erythromycin- and copper-resistant *Enterococcus hirae* from marine sediment and co-transfer of erm(B) and tcrB to human *Enterococcus faecalis*. Diagnostic Microbiology and Infectious Disease. 2014; 80: 26–28.

[24] Hacıoğlu N. Tosunoglu M. Determination of antimicrobial and heavy metal resistance profiles of some bacteria isolated from aquatic amphibian and reptile species. Environmental Monitoring and Assessment. 2014; 186: 407-413.

[25] Ramos CP, Santana JA, Morcatti Coura F, Xavier RGC, Leal CAG, Oliveira Junior CA, Silva ROS. Identification and characterization of *Escherichia coli, Salmonella* spp., *Clostridium perfringens*, and *C. difficile* isolates from reptiles in Brazil. BioMed Research International. 2019; (1): 9530732.

[26] da Silva Heck JM, Prichula J, Huff R, de Oliveira RB, Silva-Soares T, Frazzon J, Frazzon APG. Captive snakes from Brazil as carriers of multidrug-resistant enterococci. International Journal of Plant, Animal and Environmental Sciences. 2021; 11(3): 503-523.

[27] Brunson DN, Colomer-Winter C, Lam LN, Lemos JA. Identification of multiple ıron uptake mechanisms in *Enterococcus faecalis* and their relationship to virulence. Infection and Immunity. 2023; 91(4): e00496-22.