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Genotypic and Phenotypic Evaluation of Heavy Metal Resistance of Enterococcal Isolates from Seafood Products for Consumption

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Abstract: The aim of this study was to genotypically and phenotypically evaluate the resistance to heavy metal salts of enterococci isolated from fisheries sold in our country's market for food safety. Using concentrations computed as 1000 ppm of seven heavy metal salts, copper (Cu^{+2}), nickel (Ni^{+2}), cadmium (Cd^{+2}), zinc (Zn^{+2}), lead (Pb^{+2}), chromium (Cr^{+2}), and iron (Fe^{+2}) heavy metal resistance (MIK) tests were conducted on each of the enterococci isolates. As a result of the analyses made in this context, resistance to heavy metal salts such as copper, lead, nickel, and cadmium in many isolates and the presence of copper (*tcrB*) and cadmium (*cadA*) resistance genes in some isolates were determined. It is considered that the isolates and the fishery products from which these isolates were obtained may carry risks in terms of food safety and public health. In addition, genes encoding heavy metal resistance are also effective in antibiotic resistance. For this reason, it is stated that these microorganisms gain importance not only in applications such as bioremediation and biomining but also in the healthcare sector.

Keywords: Bacteria, Enterococcus, fishery products, heavy metal resistance, food safety.

Tüketime Sunulan Su Ürünlerinde Enterokok İzolatlarında Ağır Metal Direncinin Genotipik ve Fenotipik Olarak Değerlendirilmesi

Öz: Bu çalışmanın amacı, ülkemiz piyasasında satışa sunulan su ürünlerinden izole edilen enterokokların gıda güvenliğine uygunluğunun ağır metal tuzlarına dirençlilik özelliklerini genotipik ve fenotipik olarak değerlendirmektedir. Enterokok izolatlarının her biri için bakır (Cu^{+2}), nikel (Ni^{+2}), kadmiyum (Cd^{+2}), çinko (Zn^{+2}), kurşun (Pb^{+2}), krom (Cr^{+2}) ve demir (Fe^{+2}) olmak üzere 7 adet ağır metal tuzları 1000 ppm olarak hesaplanan konsantrasyonlar kullanılarak ağır metal dirençlilik (MIK) testleri yapılmıştır. Bu kapsamda yapılan analizler sonucunda test edilen pek çok izolatlarda bakır, kurşun, nikel, kadmiyum gibi ağır metal tuzlarına karşı direnç özellikleri ve bazı izolatlarda bakır (*tcrB*) ve kadmiyum (*cadA*) direnç genlerinin varlığı da belirlenmiştir. İzolatların ve bu izolatların elde edildiği su ürünü örneklerinin gıda güvenliği ve halk sağlığı yönüyle risk taşıyabileceği düşünülmüştür. Bunun yanında ağır metal dirençliliğini kodlayan genler aynı zamanda antibiyotik dirençliliğinde de etkilidir. Bu nedenle bu mikroorganizmalar sadece biyoremediasyon, biyomadencilik gibi uygulamalar için değil sağlık sektöründe de önem kazandığı belirtilmiştir.

Anahtar kelimeler: Bakteri, enterokok, su ürünü, ağır metal direnci, gıda güvenliği.

1. Introduction

Enterococci are naturally found in the digestive tract of humans and animals, in soil, surface waters, plants, vegetables, and the microflora of insects (Franz et al., 1999, 2003; Foulquie Moreno et al., 2006). Enterococci can also be isolated from different aquatic environments such as wastewaters, seacoasts, shellfish, and fish intestines. Due to their tolerance of high salt environments, the enterococci can survive on seacoasts for a long time (Harwood et al., 2000; Valenzuela et al., 2010; Hammad et al., 2014). Enterococci species, especially *Enterococcus faecalis* and *E. faecium*, have also been isolated from traditional or industrially processed seafood (fish, mussel, etc.) of various countries (Pinto et al., 2009; Françoise, 2010; Valenzuela et al., 2010).

Because of their environmental destruction and toxicity, heavy metal contamination is important in terms of human and environmental health. Water and food play a key role in the contamination of heavy metals in biological systems. Aquatic animals, especially fish, are the main source of heavy metal contamination to humans. In addition, heavy metal contamination significantly affects the microbial community of the environment (Bhakta et al., 2012). Genes encoding heavy metal resistance are also effective in antibiotic resistance. For this reason, these microorganisms gain importance not only in applications such as bioremediation and biomining but also in the health sector. Before the discovery of antibiotics, heavy metals were used for centuries as drugs in the treatment of diseases. Therefore, heavy metal resistance has developed in pathogenic microorganisms. Resistance genes in microbial plasmids can be transferred, especially with plasmid transfer, within and between species and cause an increase in pathogenicity (Yavuz & Sarıgül, 2016).

Enterococci, unlike other bacteria of intestinal origin, are resistant to physical and chemical stresses such as heavy metals present in their environment and can survive for a long time in other environments than their intestinal natural environment (De Niederhäusern et al.,

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2013). In the study conducted by Matyar & Dinçer (2010), *E. faecalis* isolates from the sea water of the Iskenderun Gulf showed 100% to 37.8% tolerance to cadmium, manganese, copper, and lead. Authors stated that this tolerance may have resulted from the excessive pollution of the gulf water by hospitals and industrial wastes (Matyar & Dinçer, 2010). In another study conducted by Aktan et al. (2013), enterococcus isolates showing lead resistance were detected from Kırıkkale region of Kızılırmak River.

In addition, these isolates have been observed to be resistant at different rates to aluminum, lithium, barium, chromium, iron, silver, tin, nickel, zinc, and strontium (Aktan et al., 2013). Silveira et al. (2014) reported that copper tolerance and *tcrB* copper tolerance gene were detected in enterococci isolated from the muscle tissue and internal organs of rainbow trout sold in supermarkets in Portugal. In the study conducted by Bhakta et al. (2012), enterococci were identified in lactic acid bacteria isolated from wastewater and ports in India, Japan, and Vietnam. Furthermore, lead and cadmium resistance were determined in the same bacteria (Bhakta et al., 2012). In our country, no study showing the heavy metal resistance properties of enterococci isolated from fishery products was stated.

In this study, heavy metal resistance properties of enterococci isolates isolated from the raw and processed seafood products obtained from Marmara Region were phenotypically and genotypically evaluated.

2. Material and Methods

2.1. Materials

Aquacultural /seafood products' samples

Within the scope of this project, raw fish samples caught daily from the Marmara Region were obtained from Istanbul and Çanakkale fish markets and delivered through a cold chain to the laboratory within a short time. Processed aquaculture samples were obtained from supermarkets and delicatessens in Istanbul and Çanakkale.

2.2. Methods:

Isolation of enterococci from seafood samples

Under aseptic conditions, 10 g of each sample was weighed, transferred to 90 mL of sterile saline water, and homogenized using a Stomacher for 1 minute. A serial dilution from the homogenized solution was prepared. 0.1 mL from each dilution was spread, using Drigalski loop, on the surface of canamycin aesculine azide (CAA) agar medium and incubated for 24-48 hours at 37°C. After the incubation period, for each sample, 3-5 colonies were selected from the typical colonies grown on the CAA agar medium and purified again using the same medium. After purification of colonies, they were stored in 30% glycerol at -20°C for further analysis (Baixas-Nogueras et al., 2003, Karaalioğlu et al., 2019; Çardak et al., 2022).

2.3. Identification of enterococci isolates at genus and species levels

Gram stain, catalase test, esculin hydrolysis, growth tests at pH 9.6, 40% bile salt medium, 10°C and 45°C were applied to the pure cultures (Sinton et al., 1993; Harwood et al., 2004). The isolates that showed typical reactions for Enterococci in the previous tests were identified at the species level by using the VITEK 2 Compact (Bio-Merieux, France) automated identification system and API 20 strep (Bio-Merieux, France) biochemical test kits (Karaalioğlu et al., 2019; Çardak et al., 2022).

2.4. Phenotypic determination of the resistance status of isolates to heavy metal salts

In order to determine the heavy metal resistance of *Enterococcus* isolate, at a concentration of 1000 ppm, seven heavy metal salts, namely, copper ($CuSO_4.5H_2O$), nickel ($NiCl_2.6H_2O$), cadmium ($CdCl_2.2H_2O$), zinc ($ZnSO_4$), lead ($Pb(NO_3)_2$), chromium ($Cr_2(SO_4)_3$), and iron ($FeSO_4.7H_2O$) were utilized. To perform the microdilution test and determine minimum inhibition concentrations (MIC) of each isolate, McFarland 0.5 turbidity of our bacterial isolates were prepared in Mueller-Hinton broth (Difco). After that, 50 µL of each bacterial solution was placed on microwell plates and incubated at 37°C for 24 hr (Geiselbrecht et al., 1996; Akinbowale et al., 2007). All plates were read at 600nm (A600) absorbance in a plate reader. *Escherichia coli* (ATCC® 25922TM), *Salmonella enterica* (ATCC® 2577TM) and *Staphylococcus epidermidis* (ATCC® 12228TM) were used as reference.

2.5. Genotypic determination of isolates' resistance to heavy metal salts

Within the scope of the study, the presence of copper (*tcrB*) and cadmium resistance (*cadA* and *corA*) genes in the isolates of aquaculture-derived enterococci was also evaluated (Hasman et al., 2006; Di Cesare et al., 2012). Bacterial genomic DNAs of the isolates were obtained using the GeneJET Genomic DNA Purification Kit (K0721, Thermo Scientific) DNA isolation and purification kit. According to the suggested protocol, the isolated genomic DNAs were precipitated with 0.7 mL of 2-propanol and centrifuged at 13000 g for 5 minutes. The pellet was dissolved in 50 µL of Tris-EDTA (pH 8.0) and stored at -20 °C until use (Cancilla et al., 1992).

In this study, heavy metals to which the enterococci isolates were resistant were determined by using the minimum inhibition method. In addition, heavy metal resistance genes were investigated by using PCR technique. The heavy metal resistance primers, amplicon lengths and product dimensions (bp) used in the PCR were given in Table 1. PCR solutions were prepared in 0.2 mL tubes and treated under PCR conditions specific to each resistance gene. The PCR running conditions were shown in Table 2. *Enterococcus hirae* FM 2.16 strain was used as a positive control in the *tcrB* gene analysis (Pasquaroli et al., 2014).

PCR processes were performed on a TurboCycler 2 (Blue-Ray Biotech. Corp., Taipei city, Taiwan) thermal cycler. Gel electrophoresis of the isolated DNA samples and PCR products was carried out at 120 volts for 25-30 minutes using a 0.2% (w/v) agarose gel. The gel was stained in solution containing 3 µL EtBr ethidium bromide (3 mg/mL) (Amresco Inc., Solon, OH, USA) and examined on a UV transilluminator (Vilber Lourmat, ECX-F20.M, France). The gel photo was taken using the Nikon D5100 digital camera (Nikon Corp., Japan).

Table 1. Primers used in polymerase chain reaction applied to the isolates' DNA.

Name of gene	Primer	Sequence (5´3´)	PCR product (bp)	References
tcrB	tcrB1-F	CAT CAC GGT AGC TTT AAG GAG ATT TTC	663	Hasman et al., 2006
	tcrB1-R	ATA GAG GAC TCC GCC ACC ATT G		
cadA	cadA1-F	TCT TGT TCC ACG CTT GCT G	83	Di Cesare et al., 2012
	cadA1-R	GG GAA ATG ATT CAA ACC TTA C		
corA	corA1-F	CAC ACT GCG GCA AAG AAC C	628	Di Cesare et al., 2012
	corA1-R	AAG TGT TCG GGC TCA CTT GG		

Table 2. Polymerase chain reaction (PCR) conditions applied in the amplification of heavy metal resistance genes (*tcr*B, *cad*A, *cor*A)

Program type	Temperature (°C)	Timing	Cycles
Initial denaturation	95	2´	-
Denaturation	94	30´´	35
Annealing	54	30´´	
Extension	72	45´´	
Final extension	72	5´	-
Waiting	10	10´´	-

3. Results and Discussion

Within the scope of the study, a total of 397 seafood samples (290 raw and 107 processed) were analyzed for enterococcal isolation and 146 isolates selected as enterococcal suspected colonies from the CAA selective media were analyzed with the genus level confirmation tests. A total of 116 isolates were determined to belong to the genus *Enterococcus* and these isolates were identified at the species level (Project Final report). According to the identification results of the isolates at the species level, it was observed that *Enterococcus gallinarum* and *E. casseliflavus* constitute the dominant flora in both raw and processed products followed by *E. durs, E. faecium* and *E. hirae* (Project Final report, Karaalioğlu et al., 2019).

The 116 enterococci isolates from fresh and processed seafood samples showed a resistance development predominantly against chromium, copper, nickel, cadmium, zinc, iron, and lead. In fresh seafood, the highest resistance was against copper (46.73%), nickel (45.65%), and chromium (44.57%) heavy metal salts, while the highest resistance in processed seafood samples was against lead (54.17%), zinc (50%), iron, and chromium (45.83%) heavy metal salts. The levels of heavy metal resistance of the isolates are shown in Table 3.

Table 3. Heavy metal resistance levels of enterococcal isolates isolated from fresh and processed seafood samples (%)

		Concentration of heavy metals (µg ml^{-1})											Isolation		Resistant isolates
Heavy metals	Sources	0.9	1.9	3.9	7.8	15.6	31.5	62.5	125	250	500	>500	n	n	%
Cu^{+2}	Processed	6	-	1	1	-	-	2	-	-	-	-	24	10	41.67
	Fresh	8	3	-	6	5	3	2	3	5	8	-	92	43	46.73
Ni^{+2}	Processed	-	1	-	-	-	-	1	1	1	2	-	24	6	25
	Fresh	9	2	3	5	7	1	2	-	5	8	-	92	42	45.65
Cd^{+2}	Processed	-	-	-	-	-	4	4	1	-	2	-	24	11	45.83
	Fresh	-	-	-	2	5	7	6	4	7	2	-	92	33	35.87
Zn^{+2}	Processed	-	-	1	3	3	2	2	-	-	1	-	24	12	50
	Fresh	-	-	-	14	11	8	4	2	-	-	-	92	39	42.39
Pb^{+2}	Processed	1	-	1	2	6	3	-	-	-	-	-	24	13	54.17
	Fresh	18	-	-	5	10	2	1	3	-	-	-	92	39	42.39
Fe^{+2}	Processed	1	2	1	5	2	-	-	-	-	-	-	24	11	45.83
	Fresh	15	2	8	3	1	1	6	4	-	-	-	92	40	43.48
Cr^{+2}	Processed	8	-	-	-	-	-	-	-	-	-	3	24	11	45.83
	Fresh	19	1	-	5	-	-	7	1	4	2	2	92	41	44.57
Total													116		

In the study conducted in sea water and sediment of the Aegean Sea (Güllük Bay), the heavy metal resistance to chromium (35.3%), copper (33.3%), zinc (30.3%), iron (28.4%), and lead (25.5) was observed in the 158 identified

isolates. It was stated that the highest resistance was found in bacterial isolates isolated from sediment samples. In the study run by Kalkan in the Black Sea sediment in 2022, the heavy metal resistance rates were listed as follows: Fe > Cr > Mo = Sr > Pb = Sb > Ni > Cu > Ba > Sn > Al = Co > Mn > Zn = Hg > Cd. 72% of all isolates were Fe-tolerant and all isolates were sensitive to Cd. Matyar et al. (2009) determined that 60.2%, 50.5%, 8.6%, and 6.5% of gill bacteria isolated from fish were tolerant to cadmium, copper, manganese, chromium, and lead, respectively while 52%, 45.3%, 10.7%, 3%, and 5.3% of the intestinal bacteria from fishes were tolerant to cadmium, copper, chromium, lead, and manganese respectively.

Akkan stated that 100% of 356 Gram-negative bacteria isolated from seawater of Iskenderun Bay in 2013 showed resistance to cadmium and copper. In addition, these bacteria showed manganese resistance of which 90.7% in the 1st region, 96.9% in the second region, and 100% in the third region. Moreover, these bacteria showed lead resistance of which 67.7% in the first region, 100% in the second region, and 97.96% in the third region. The heavy metal tolerance observed in bacterial isolates, isolated from marine water and sediment, studied for heavy metal resistance levels indicates that the environment from which these bacteria are isolated is contaminated with these metals.

134 Enterobacteriaceae members isolated from fresh marine fish samples, served for consumption, in Giresun province were investigated for their resistances to 3 different heavy metals. It is reported that all the isolates were resistant to copper, while 61.94% and 46.27% of them were resistant to manganese and lead, respectively. In this study, bacteria were isolated from a total of 397 fresh and processed seafood products sold in Turkey. In addition, resistance to 7 different heavy metal salts, with the highest resistance observed against zinc (54.17%) and lead (50%), the important commercial fish and seafood products offered for consumption can pose a great risk to public health.

In the MIC absorbance evaluation done within the scope of our study, it is observed that 5, 9, 4, 9, 5, and 3 enterococcal isolates showed high resistance against cadmium, lead, iron, copper, zinc, and nickel, respectively.

Enterococci, unlike other bacteria of intestinal origin, are resistant to physical and chemical stresses such as heavy metals in their environment and can survive for a long time in the environments outside their natural intestinal environment (De Niederhäusern et al., 2013). In a study conducted by Matyar & Dinçer (2010), *E. faecalis* isolates harbored in the sea water of the Iskenderun Bay showed cadmium, manganese, copper, and lead tolerance rate ranging from 100% to 37.3%. This tolerance is stated to be caused by the excessive pollution of the gulf water by hospital and industrial wastes. In another study run by Aktan et al. (2013), it was found that enterococci isolates showing lead resistance were detected from Kırıkkale region of Kızılırmak River and these isolates were also found to be resistant to aluminum, lithium, barium, chromium, iron, silver, tin, nickel, zinc, and strontium at different rates.

In the study conducted by Silveira et al. (2014), it was observed that copper tolerance and tcrB copper tolerance gene were detected in enterococci isolated from muscle tissue and internal organs of rainbow trout sold in Portugal supermarkets. In the study of Bhakta et al. (2012), it was stated that enterococci were also identified in lactic acid bacteria isolated from wastewater and ports of India, Japan, and Vietnam. Furthermore, lead and cadmium resistance were observed in these bacteria.

In addition, gel images of the isolates carrying the copper (tcrB) and cadmium (cadA) resistance genes (Table 4) are shown in Figures 1 and 2.

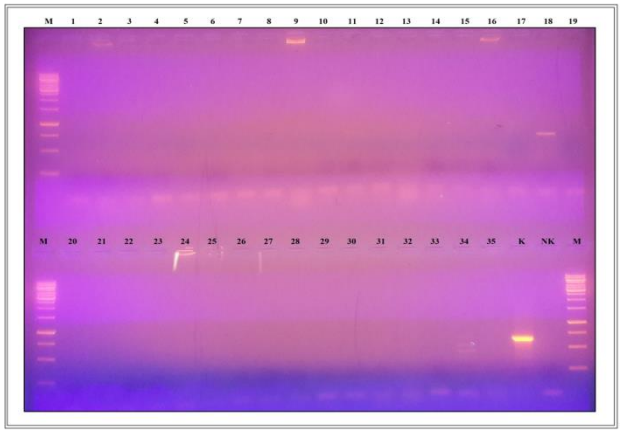

Figure 1. The appearance of PCR (polymerase chain reaction) products of the *tcr*B gene in 2% agarose gel in some enterococci isolates (M: Marker; K: Positive control; NK: Negative control; numbers 1-35 show the wells where PCR products are loaded. The sample in well 18 is positive for the *tcr*B gene. PCR product size is 663 bp (base pair). Marker SM0311 (Thermo Fisher Scientific).

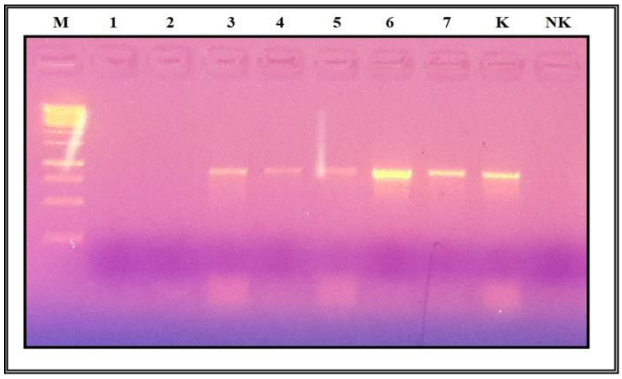

Figure 2. The appearance of PCR (polymerase chain reaction) products of the *cad*A gene in 2% agarose gel in some enterococci isolates (M: Marker; K: Positive control; NK: Negative control; Numbers 1-7 show the wells where PCR products are loaded. Samples loaded in wells 3, 4, 5, 6 and 7 are positive for the *cad*A gene. PCR product size is 883 bp (base pair). Marker SM0311 (Thermo Fisher Scientific).

The presence or absence of various gene regions is also used in the determination of heavy metal resistance in bacterial isolates. To determine arsenic resistance, *ars*A, *ars*B, *ars*C, *ars*D, *ars*R genes regions were amplified using the appropriate primers and visualized. On other hand, the determination of copper resistance was carried out by the amplification of *cop*A, *cop*B, *cop*Y, *copZ pco*A genes regions by using the appropriate primers. Similarly, *pbr*T gene region was amplified to determine the lead resistance while *cad*A, *cad*B, *cad*C genes regions were

amplified for the investigation of cadmium resistance. For chrome resistance analysis, *chr*A, *chr*B gene regions were amplified while the mercury resistance investigation *mer*A, *mer*B, *mer*C, *mer*D, *mer*R, *mer*P, *mer*T, were amplified using the appropriate primers. In addition, *czc*A, *czc*B, *czc*C, *czc*D gene regions were amplified for the determination of the cobalt-zinc-cadmium multiresistance feature while for the determination of nickel-cobalt-cadmium multiresistance characteristics, *ncc*A gene region was amplified and visualized using the appropriate primers (Roosa et al., 2014; Abou-Shanab et al., 2007; Rouch et al., 1995; Bruins et al., 2000). Some gene regions responsible for heavy metal resistance were searched and studied from the NCBI gene bank to determine copper resistance (*tcr*B gene) and cadmium resistance (*cad*A and *cor*A genes).

Table 4. Minimum inhibitory concentration (MIC) values (ppm) of heavy metals resistance of enterococci isolated from seafood products.

No	Isolate code	Source	Species	Cu	Ni	Cd	Zn	Pb	Fe	Cr
1	TSM-1	Otocpot salad (Frozen)	*E. faecium*	3.906	250	62.5	31.25	7.813	1.953	1000
2	TSM-59	Mussel meat (Frozen)	*E. gallinarum*	31.25	500	500	62.5	31.25	15.625	0
3	TSM-61	Horse mackerel (Fresh)	*E. gallinarum*	15.625	500	31.25	31.25	31.25	125	0
4	TSM-79	Bream (Fresh)	*E. gallinarum*	31.25	500	250	31.25	7.813	1.953	0
5	TSM-99	Shrimp (Fresh)	*E. gallinarum*	0.977	500	15.625	15.625	0	0	5
6	TSM-103	Picarel(Fresh)	*E. gallinarum*	3.906	3.906	31.25	31.25	3.906	3.906	0
7	TSM-104	Horse mackerel (Fresh)	*E. casseliflavus*	125	250	250	62.5	15.625	3.906	0
8	TSM-130	Picarel (Fresh)	*E. gallinarum*	7.813	500	500	31.25	125	125	1000
9	55-1	Bluefish (Fresh)	*E. casseliflavus*	0	0	31.25	7.813	15.625	62.5	0
10	55-2	Bluefish (Fresh)	*E. casseliflavus*	0	0	31.25	7.813	15.625	62.5	0
11	57-4	Bluefish (Fresh)	*E. gallinarum*	500	15.625	31.25	7.813	0	0	62.5
12	58-2	Bluefish (Fresh)	*E. gallinarum*	500	500	15.625	0	0	0	0
13	58-3	Bluefish (Fresh)	*E. gallinarum*	125	500	125	7.813	0	0	250
14	60-2	Calamari (Fresh)	*E. gallinarium*	0.977	500	15.625	15.625	0	0	5
15	61-2	Stript red mullet (Fresh)	*E. casseliflavus*	125	250	250	62.5	15.625	3.906	0
16	61-4	Stript red mullet (Fresh)	*E. gallinarium*	500	15.625	31.25	7.813	0	0	62.5
17	64-1	Sardine (Fresh)	*E. casseliflavus*	0	0	31.25	7.813	15.625	62.5	0
18	65-3	Sardine (Fresh)	*E. casseliflavus*	3.906	1.953	62.5	15.625	0	0	7.813
19	66-1	Sardine (Fresh)	*E. gallinarum*	500	7.813	31.25	15.625	0	0	62.5
20	66-2	Sardine (Fresh)	*E. gallinarum*	500	15.625	31.25	7.813	0	0	62.5
21	66-4	Sardine (Fresh)	*E. gallinarum*	500	15.625	31.25	7.813	0	0	62.5
22	68-1	Sardine	*E. gallinarium*	15.625	3.906	7.813	7.813	0	0	250
23	68-2	Sardine (Fresh)	*E. casseliflavus*	3.906	1.953	62.5	15.625	0	0	7.813
24	68-3	Sardine (Fresh)	*E. gallinarium*	125	500	125	7.813	0	0	250
25	68-4	Sardine (Fresh)	*E. gallinarium*	500	7.813	31.25	15.625	0	0	62.5
26	70-1	European Anchovy (Fresh)	*E. gallinarium*	500	31.25	62.5	31.25	0	0	0
27	70-2	European Anchovy (Fresh)	*E. gallinarium*	7.813	15.625	125	15.625	31.25	3.906	250
28	70-3	European Anchovy (Fresh)	*E. gallinarium*	0	0	62.5	7.813	15.625	0.977	0
29	70-4	European Anchovy (Fresh)	*E. gallinarium*	0	0	31.25	7.813	15.625	7.813	0.977
30	74-1	Shrimp (Fresh)	*E. gallinarium*	62.5	7.813	7.813	15.625	0	62.5	7.813
31	74-2	Shrimp (Fresh)	*E. gallinarum*	125	7.813	15.625	15.625	0	125	7.813
32	74-4	Shrimp (Fresh)	*E. casseliflavus*	31.25	7.813	15.625	15.625	0	31.25	7.813
33	75-1	Red mullet (Fresh)	*E. gallinarium*	0	0	62.5	31.25	7.813	7.813	0
34	75-2	Red mullet (Fresh)	*E. gallinarum*	0	0	31.25	7.813	15.625	7.813	0
35	75-3	Red mullet (Fresh)	*E. casseliflavus*	0	0	31.25	7.813	15.625	62.5	0
36	77-1	Anchovy (Salted)	*E. casseliflavus*	0	0	31.25	7.813	7.813	7.813	0
37	77-2	Anchovy (Salted)	*E. casseliflavus*	0	0	31.25	7.813	31.25	7.8125	0
38	77-3	Anchovy (Salted)	*E. casseliflavus*	0	0	62.5	15.625	15.625	0	0
39	77-4	Anchovy (Salted)	*E. casseliflavus*	0	0	31.25	15.625	15.625	3.906	0
40	78-1	Lakerda (Salted)	*E. gallinarum*	0	0	31.25	3.906	15.625	7.813	0

No	Isolate code	Source	Species	Cu	Ni	Cd	Zn	Pb	Fe	Cr
41	78-2	Lakerda (Salted)	*E. gallinarum*	0	0	62.5	7.813	15.625	1.953	1000
42	80-1	Lakerda (Salted)	*E. gallinarum*	31.25	500	500	31.25	31.25	7.813	0
43	80-3	Lakerda (Salted)	*E. durans*	1.953	125	125	15.625	15.625	7.813	0
44	80-4	Lakerda (Salted)	*E. durans*	31.25	62.5	62.5	62.5	15.625	15.625	1000
45	85-1	Horse mackerel (Fresh)	*E. durans*	15.625	250	250	62.5	62.5	15.625	125
46	85-2	Horse mackerel (Fresh)	*E. durans*	7.813	15.625	500	15.625	125	1.953	62.5
47	85-3	Horse mackerel (Fresh)	*E. durans*	7.813	62.5	62.5	125	125	62.5	1.953
48	87-2	Picarel (Fresh)	*E. gallinarum*	7.813	250	250	31.25	125	125	1000
49	88-1	Picarel (Fresh)	*E. gallinarum*	3.906	15.625	31.25	31.25	15.625	3.906	0.977
50	88-2	Picarel (Fresh)	*E. gallinarum*	3.906	250	250	62.5	7.813	3.906	0.977
51	88-3	Picarel (Fresh)	*E. gallinarum*	1.953	62.5	125	500	7.813	3.906	0
52	88-4	Picarel (Fresh)	*E. faecium*	3.906	3.906	250	125	7.813	3.906	0

4. Conclusion

Tolerance to heavy metals in bacteria indicates these metals contaminate the environment from which these bacteria were isolated. Industrial activities, mining, and fish farming can be counted among the most effective factors that cause changes in the natural ecosystem. These factors create a selective pressure on bacteria. It is stated that the presence of heavy metals in the marine environment negatively impacts the food chain and directly related to public health due to their ability to be accumulated in living organisms (Alonso et al., 2001).

The high level of heavy metal resistance profiles in enterococci isolated from processed aquaculture products in different markets and companies as well as fresh fish species offered for consumption can indicate that fish and different seafood products are exposed to heavy metals in the habitats where they are caught. In developing countries such as Türkiye, domestic and industrial wastes are generated as a result of recreational activities in the sea that reach marine water directly or indirectly. It has been reported that due to different factors like mining operations, industry, industrial wastes, agricultural wastes, food industry wastes, sewage wastes, and natural disasters, chemical pollutants such as heavy metals contaminate the waters in a way that cannot be recycled. They are accumulating in tissues such as liver and gills of aquatic organisms and interfere with the food chain. Transport, dissolution, precipitation, complex formation, adsorption, and bioaccumulation mechanisms of heavy metals in aquatic environments are quite complex processes and are affected by the physicochemical properties of water. In addition, the metals stored in the sediment are directly or indirectly affected by the oxidation and reduction reactions that take place in the sediment.

With a full understanding of heavy metal resistance mechanisms, effective cleaning of the polluted environments can be achieved by developing new products that are effective against bioremediation and biodegradation methods using environment-friendly technologies.

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