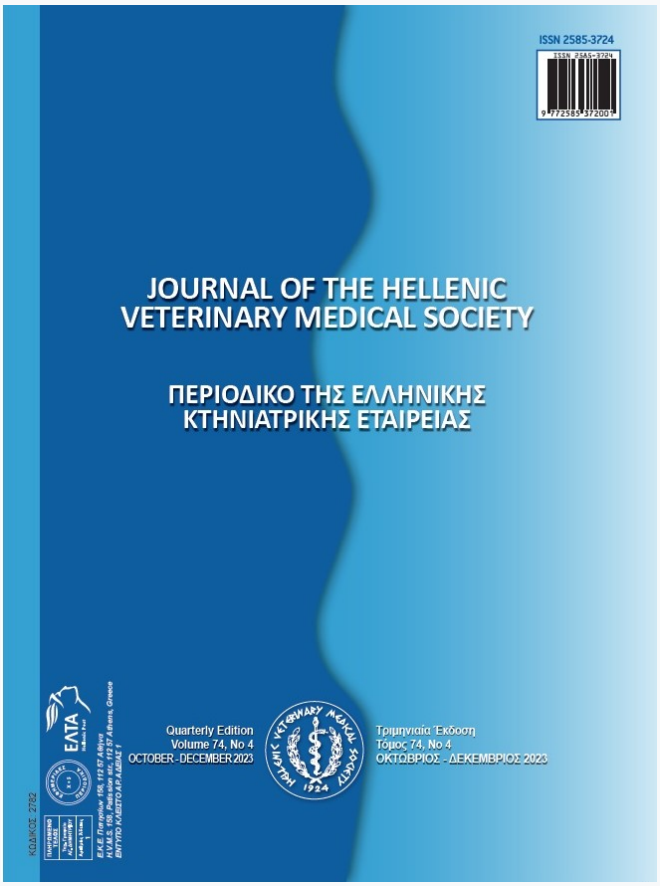
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



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doi: 10.12681/jhvms.34337

To cite this article:

Düztaşlar, S., & Gücükoğlu, A. (2024). Genotypical Determination of Vancomycin-Resistant Enterococcus faecalis and Enterococcus faecium Species Isolates fromBuffalo Milk and Dairy Products: Vancomycin-Resistant Enterococcus faecalis and Enterococcus faecium. *Journal of the Hellenic Veterinary Medical Society*, *75*(1). https://doi.org/10.12681/jhvms.34337

J HELLENIC VET MED SOC 2024, 75 (1): 7135-7144
ΠΕΚΕ 2024, 75 (1): 7135-7144

Research article
Ερευνητικό άρθρο

Genotypical Determination of Vancomycin-Resistant *Enterococcus faecalis* and *Enterococcus faecium* Species Isolates fromBuffalo Milk and Dairy Products

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ABSTRACT: In this study, Enterococcus faecalis and Enterococcus faecium were isolated in raw buffalo milk and dairy products in local markets and supermarkets in the province of Samsun. E. faecalis and E. faecium species and VanA and VanB gene presence were identified from confirmed Enterococcus spp. isolates with PCR method, resistance profile of vancomycin was determined phenotypically and the MIC value was determined with E-test method. Enterococcus spp. was isolated from 60 (40%) of the 150 samples analyzed. In terms of species distribution, 28 (32.9%) isolates were identified as E. faecalis, while 41 (48.2%) were identified as E. faecium. Seven isolates harboured the *VanA or VanB* gene presence. One E. faecium isolates obtained from a milk sample was found to have a vancomycin MIC value of ≥4 mg/l, 2 E. faecalis isolates obtained from cheese samples were found to have MIC values of ≥16 mg/l and ≥32 mg/l, 2 E. faecium isolates obtained from cream samples were found to have MIC values of≥2 mg/l and ≥2 mg/l, and 2 E. faecium isolates obtained from ice cream samples were found to have MIC values of≥4 mg/l and ≥16 mg/l. As a result of this study, it was determined that raising awareness among producers and consumers regarding adherence to cold chain parameters during transportation and storage processes is crucial. Additionally, emphasis should be placed on maintaining milking hygiene and minimizing errors that could lead to contamination due to personnel and technological processes associated with production.

Keywords: Buffalo milk; MIC, Van A; Van B; PCR

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Date of initial submission: 02-05-2023
Date of acceptance: 13-07-2023

INTRODUCTION

The number of buffalos is reported as about 204 million in the world. More than 98% of this number is in Asia, 0.8% is in Africa, 0.9% is in South America, and 0.2% is in Europe. The countries with the highest number of buffalos are India, Pakistan, China, Nepal and Egypt (Anonymous, 2021b). There are 184192 buffalos in Turkey and Samsun ranks the city with the highest number of buffalos with 22000 (Anonymous, 2021a). Known as the second largest source of milk in the world, buffalo milk is stated to provide only 13% of world milk production due to its much lower average milk yield (Nasr et al., 2016). With higher milk fat content (8.0%), unsaturated fatty acid content, milk protein composition (4.5%), lower phospolipid and cholesterol levels than cow's milk, buffalo milk has higher nutritional value (Sales et al., 2017). Mozzarella cheese, one of the most well-known cheeses in the world, is produced from buffalo milk, which is the best raw material in the production of dairy products (de Camargo et al., 2015). It has been reported that enterococci, which can be found on the intestinal tract and mucosal surfaces in animals, are also isolated from soil, water, animal products and different food and plants. Enterococci are not essentially specific, some species may also be host-specific (Abouelnaga et al., 2016). Enterococci are considered opportunistic pathogens such as nosocomial infections in humans, and responsible for many infections including nosocomial such as mastitis in cows, diarrhea in pigs and cows and septicemic diseases in poultry (Hollenbeck and Rice, 2012; Maasjost et al., 2015).

In the last two decades, enterococci have been considered a major cause of nosocomial and community-acquired infections, which are difficult to treat as a result of the emergence of antibiotic resistance (Staley et al., 2014). A rapid increase has also been observed in the number of enterococci resistant to vancomycin isolated from farm animals and food (Pesavento et al., 2014; Lozano et al., 2016).

Two main species of enterococci (*E. faecalis* and *E. faecium*) are particularly pathogenic for humans. While *E. faecalis* causes 85-90% of enterococci infections, *E. faecium* causes 5-10%; *E. faecium* has higher antibiotics resistance character and mortality rates than *E. faecalis* (Shenoy et al., 2014; Ulrich et al., 2017). *E. faecalis* and *E. faecium* have a highly advanced ability to acquire resistance genes from the same or different species via transferable plasmids or transposons (Chajęcka-Wierzchowska et al., 2017). Vancomycin's mode of action is to block cell wall formation by targeting building blocks. It binds to the amide bond of the terminal sequences of muramilpentapeptid, that is, to the D alanyl-D-alaninine of the elongated peptidoglycan, thus inhibiting polymerase elongation (Hawkes, 2017). *VanA*gene alone is not responsible for resistance, there are also other genes regulating and expressing resistance. All of these genes are on Tn1549, they are indicated as *VanR*, *VanS*, *VanH*, *VanX*, *VanY* and *VanZ* and they are expressed to synthesize the peptidoglycan precursor to which vancomycin binds with very low affinity (van Harten et al., 2017).*VanA* operon is transported in Tn1546 tipi transposons, which show a high degree of heterogeneity. All of the point mutations, deletions and various insertion sequences have been associated with Tn1546 type transposons (Kuo et al., 2014; Moghimbeigi et al., 2018). In the vancomycin resistance profile, *VanA*type is defined at a higher rate than *VanB*. Despite this, it is emphasized that low-level vancomycin resistance expression is more effective in *VanB* isolates (Howden et al., 2013).

A large number of studies have been conducted all over the world to find out the presence of enterococci in many foods of animal origin (Hosseini et al., 2016; Golob et al., 2019; Nasiri and Hanifian, 2022; Noroozi et al., 2022). However, there are not enough detailed studies on the subject of buffalo milk and products. Within the scope of this study, the aims were i) to isolate *Enterococcus spp*. from the samples with classical culture-based MALDI-TOF technique, ii) to confirm isolates through *tuF*gene with PCR method, iii) to identify *E. faecalis* and *E. faecium* species from confirmed *Enterococcus* spp. isolates with multiplex PCR method, iv) to show the presence of *VanA* and-*VanB* gen in *E. faecalis* and *E. faecium* isolates with PCR method, v) to determine the resistance profile of vancomycin-resistant *E. faecalis* and *E. faecium* isolates to different antibiotics phenotypically and vi) to determine the MIC value of vancomycin resistant isolates with E Test method.

MATERIAL AND METHOD

Buffalo milk yogurt (n:30), buffalo cheese (n:30), buffalo milk cream (n:30) and buffalo milk ice cream (n:30) samples with different production dates or batch numbers were randomly purchased from the producers, local bazaars and markets in Samsun. Raw buffalo milk samples (each sample was obtained from different animal, n:30) were obtained from 10 different farms. Analyzes were performed between Febru-

ary 2019 and March 2021.

***Enterococcus* spp. Isolation**

Methods reported by Reisner et al. (2000); Sparbier et al. (2012) were used in the isolation of *Enterococcus* spp. in the samples with classical culture technique. 25 gr of each sample was weighed into filtered sterile plastic bags under aseptic conditions, diluted with 225 ml VRE Broth (Oxoid-CM0984+Oxoid-Meropenem Supplement, SR0184) for pre-enrichment and then left for incubation for 18-24 hours at 37°C. A loopfull of the pre-enrichment liquid was streaked onto VRE agar(Oxoid-CM0985 + Oxoid-Meropenem Supplement, SR0184 + Vancomycin Supplement, SR0186) and the plates were incubated for 18-24 hours at 37°C. Pale grey-brown and black moire colonies grown in the medium were subculturedto Triptic Soy Agar (TSA) (Merck-1.05458) and identification carried out with MALDI TOF method.

Genomic DNA Extraction

DNA extraction of isolates was carried out according to boiling method; the isolates were incubated for 24 hours at 37 °C in Brain Heart Infusion Broth (BHI-Merck, 1.10493),were taken from here, transferred to sterile Eppendorf tubes and centrifuged for 5 minutes at 10,000×g; later the supernatant was discarded, 500 µl PBS was added and kept in 95°C water bath for 10 minutes; at the end of 10 minutes, it was centrifuged again at 10,000×g (Hettich Universal R320, Germany) for 5 minutes and kept at -20 ºC as supernatant template DNA until PCR (Zhong et al., 2017).

Confirmation of Enterococcus spp. Isolates With PCR.

PCR procedures for isolates which were identified were performed by using primer pairs (F: TACTGACAAACCATTCATGATG; R: AACTTCGTCACCAACGCGAAC) which formed the *tuf*gene sequence according to the method determined by Ke et al. (1999). PCR mixture for *tuf* gene was prepared with a total volume of 50 µl, 1X PCR Buffer, 1,5 mM $MgCl_2$, 100 µ MdNTP, 2 U Taq-Polymerase, and 0.5µM and 10 µl DNA from each primer. t*uF*gene amplification was performed in Thermal Cycler (Bio-Rad MJ mini Gradient CA-USA) under the conditions of 3 minutes of initial denaturation and 35 cycles at 95°C, 30 seconds of denaturation at 95 °C, 30 minutes of primer bonding at 55 °C, 1 minute of primer elongation at 72 °C and 7 minutes of final elongation at 72 °C Ke et al., (1999). Electrophoresis of the obtained amplicons was carried out in 2% agarose at 80 volts in Bio-Rad PowerPac Basic Power Supply (CA-USA) and Bio-RadWide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank. At the end of the electrophoresis, *tuF*gene was visualized on UV-transilluminator at 112 bp.

Determination of *E. faecalis* and *E. faecium* Species From *Enterococcu*s spp. Isolates With Multiplex PCR

For identified isolates, PCR procedures were performed according to the protocol determined by Jackson et al. (2004), by using FL1-2 *(*F: ACTTATGTGACTAACTTAACC, R:TAATGGTGAATCTTGGTTTGG) gene primer pairs for *E. faecalis* and FM1-1 (F: GAAAAAACAATAGAAGAATTAT R:TGCTTTTTTGAATTCTTCTTTA) gene primer pairs for *E. faecium.* For *E. faecium* and *E. faecalis* genes, PCR mixture was prepared with a total volume of 50 µl of1X PCR Buffer, 1,5 mM $MgCl_2$, 100 µMdNTP, 2 U Taq-Polymerase, 0.5µM and 10 µl DNA from each primer. Amplification of *E. faecium* and *E. faecalis* genes was performed at Thermal Cycler (Bio-Rad MJ mini Gradient CA -USA), under the conditions of 4 minutes of initial denaturation and 30 cycles at 95°C, 30 seconds of denaturation at 95 °C, 1 minute of primer bonding at 55 °C, 1 minute of primer elongation at 72 °C and 7 minutes of final elongation at 72 °C (Jackson et al., 2004). Electrophoresis of the obtained amplicons was carried out in 2% agarose at 80 volts. Electrophoresis was performed in Bio-Rad PowerPac Basic Power Supply (CA-USA) and Bio-RadWide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank. At the end of the electrophoresis, *E. faecium* gene was visualized on UV-transilluminator at 215 bp, while *E. faecalis* gene was visualized at 360 bp.

Phenotypic Determination of Resistance Profile of Vancomycin-Resistant *E. faecalis* and *E. faecium* Isolates to Different Antibiotics

Antibiotics resistance of vancomycin-resistant isolates was determined by disc diffusion method on Mueller Hinton agar based on the methods according to the guidelines of EUCAST.(European Committee on Antimicrobial Susceptibility Testing,2022). Antibiotics resistance properties of the isolates obtained in the study (Vancomycin-BIOTESTS151125E, Erythromycin-BIOTESTS160324C, Tetracycline-BIOTESTS151217B, Penicillin-BIOTESTS160418C,

Chloramphenicol-BIOTESTS160427B, Gentamicin-BIOTESTSCT0024B, Streptomycin-BIOTESTSCT0047B, and Ampicillin-BIOTESTSCT0003B) were determined by disc diffusion method. For this purpose, a few loops were taken from fresh cultures in TSA of 24-hour isolates and suspended in sterile tubes containing 5 ml of 0.09% physiological salt water (PSW) and adjusted to 0,5 McFarland (10^8kob/ml) turbidity with McFarland densitometer (Biosan). Next, this suspension was smeared on Mueller-Hinton Agar (Oxoid CM337, Hampshire-England) with a sterile swab. Following this, petri dishes were dried for 5-10 minutes at room temperature and 8 antibiotics discs were placed in each petri dish at equal distance from each other. After placing the antibiotic discs, petri dishes were incubated for 18-24 hours at 37 °C. At the end of incubation, the diameters of the inhibition zones around antibiotic discs were measured. Zone diameters obtained were compared with EUCAST standards and the isolates were classified as sensitive, moderately resistant or resistant to antibiotics.

Determination of *VanA* and *VanB* genes in *E. faecalis* and *E. faecium* isolates with PCR method

For identified isolates, PCR procedures were performed according to the protocol determined by Patel et al. (1997), by using the primer pairs constituting the *VanA* (F:CATGACGTATCGGTAAAATC;R: ACCGGGCAGRGTATTGAC) and *VanB* (F:CATGATGTGTCGGTAAAATC; R:ACCGGGCAGRGTATTGAC) gene sequence. For *VanA* and *VanB* genes, the PCR mixture was prepared with a total volume of 50 µl of1X PCR Buffer, 1,5 mM $MgCl_2$, 100 µMdNTP, 2 U Taq-Polymerase, 0.5 µM and 10 µl DNA of each primer. Amplification was performed at Thermal Cycler (Bio-Rad MJ mini Gradient CA -USA), under the conditions of 5 minutes of initial denaturation and 35 cycles at 94°C, 1 minute of denaturation at 94 °C, 1 minute of primer bonding at 54 °C, 1 minute of primer elongation at 72 °C and 10 minutes of final elongation at 72 °C (Patel et al., 1997). Electrophoresis of the obtained amplicons was carried out in 2% agarose at 80 volts in Bio-Rad PowerPac Basic Power Supply (CA-USA) and Bio-RadWide Mini Sub-Cell GT Cell (CA-USA) electrophoresis tank. At the end of the electrophoresis, *VanA* and *VanB*genes were visualized on UV-transilluminator at 885 bp.

Determination of MIC Value of Vancomycin Resistant Isolates with E-test Method

Minimum Inhibition concentration (MIC) of vancomycin resistant isolates was determined with E-test (Epsilometertest) method through disc diffusion method; for this purpose, 24-hour fresh cultures subculturedin TS were adjusted to 0,5 McFarland (108kob/ml) turbidity with McFarland densitometer and this suspension was smeared on Mueller-Hinton Agar (Oxoid CM337) with a sterile swab. Next, the petri dishes were dried for 10-15 minutes at room temperature, vancomycin E-test strips (BIOTESTS EBTest) were placed and Petri dishes were incubated for 18-24 hours at 35°C. At the end of the incubation, inhibition zones were measured and MIC values of the isolates were determined (EUCAST, 2022).

RESULTS

According to the obtained results, 60 (40%) of 150 samples werefound to be contaminated with*Enterococcus* spp. 179 isolates were recovred from 150 positives samples. The 85 recovred isolates inculuded 10 isolates from (11.8%) milk, 21 (24.7%) yogurt, 18 (21.17%) cheese, 22 (26%) cream and 14 (16.5%) ice cream were found to be positive (Table 1).

Genotyping show that 28 (32.9%) of the 85 isolates were *E. faecalis*, while 41 (48.2%) were *E. faecium* (Table 2).

The study revealed that the *E. faecium* isolate obtained from the milk samples contains the *VanA* resistance gene. Two *E. faecalis* isolates obtained from cheese samples were positive for *VanB* gene presence. It was determined that one of the two *E. feacium* isolates detected in the cream sample had the *VanA* gene and the other had the *VanB* gene. One of the *E. faecium* isolates obtained from the ice cream sample was found to carry the *VanB* gene, while the other isolate was found to carry the *VanA* gene. Among the 7 positive isolates, 3 (42.8%) were found to contain the *VanA* gene, while 4 (57.1%) were found to contain the *VanB* gene. These results are shown in Table 3.

The study found that all tested *E. faecalis* strains exhibited 100% resistance to vancomycin, erythromycin, penicillin and chloramphenicol. Additionally, 50% of *E. faecalis* strains were resistant to tetracycline, and 40% were resistant to streptomycin. Among the *E. faecium* strains, 100% exhibited resistance to vancomycin, while 60% were resistant to erythromycin. Additionally, 40% of *E. faecium* strains showed resistance to tetracycline, streptomycin, and penicillin. Only 20% of *E. faecium* strains were resistant to chloramphenicol and gentamicin, and none of the

strains showed resistance to ampicillin.These results are shown in Table 4.

In our study, it was found that *E. faecium* strain in milk sample was resistant to *VanA* gene (≥4 mg/l MIC), *E. faecalis* strain in cheese sample was resistant to *VanB* gene (≥16 mg/l and ≥32 mg/l), *E. faecium* strain in cream sample was resistant to *VanA* gene (≥2 mg/l MIC) and other *E. faecium* strain was resistant to *VanB* gene ((≥2 mg/l MIC), while *E. faecium* strain in ice cream sample was resistant to *VanB* gene (≥4 mg/l), while *E. faecium* strain and *E. faecium* strain in ice cream sample were resistant to *VanA* gene (≥16 mg/l) (Table 5).

Table 1. Prevalence of *Enterococcus* spp. in buffalo milk and dairy products

Samples	Number of positives Samples	Number of *Enterococcus* spp. positive samples (%)	Number of isolated Enterococcus strains
Milk	30	8 (26.6%)	10
Yogurt	30	17 (56.6%)	21
Cheese	30	11 (36.6%)	18
Cream	30	16 (53.3%)	22
Ice cream	30	9 (30%)	14
TOTAL	**150**	**60 (40%)**	**85**

Table 2. Distribution of *Enterococcus* species in buffalo milk and dairy products

Sample origin/ Number	Classical culture+ MALDI-TOF	PCR (*tuf*gene)	Serotype (*FL1-2; FM1-2*)	
	Enterococcus spp. Positive Isolate Number	*Enterococcus* spp. Positive Isolate Number	*E. faecalis*	*E. faecium*
Milk (n:30)	10	10	4	5
Yogurt (n:30)	21	21	7	9
Cheese (n:30)	18	18	6	9
Cream (n:30)	22	22	7	10
Ice cream (n:30)	14	14	4	8
TOTAL (n:150)	85	85	28 (32.9%)	41 (48.2%)

Table 3. Genotypic resistance profile of vancomycin resistant E. faecalis and E. faecium isolates

Sample origin	Isolate code and type	Genotypic Resistance		
		VanA	*VanB*	*VanA* +*VanB*
Milk	*E. faecium*	+		-
Yogurt	-	-	-	-
Cheese	*E. faecalis*	-	+	-
	E. faecalis	-	+	-
Cream	*E. faecium*	+	-	-
	E. faecium	-	+	-
Ice cream	E. faecium		+	-
	E. faecium	+		-
TOTAL (%)		3 (42.8%)	4 (57.1%)	-

Table 4. Antibiotic resistance profil of vancomycin-resistant *E. faecalis* and vancomycin resistant *E. faecium* isolates

Antibiotics	VRE Isolate Phenotypic antibiotics resistance	
	E. faecalis **(n:2)**	E. faecium **(n:5)**
Vancomycin (30 µg/ml)	2 (100%)	5 (100%)
Erythromycin (15 µg/ml)	2 (100%)	3 (60%)
Tetracycline (30 µg/ml)	1 (50%)	2 (40%)
Streptomycin (30 µg/ml)	-	2 (40%)
Penicillin (10 IU)	2 (100%)	1 (20%)
Chloramphenicol (30 µg/ml)	2 (100%)	1 (20%)
Gentamicin (10 µg/ml)	-	1 (20%)
Ampicillin (10 µg/ml)	-	-

Table 5. E-test results of vancomycin resistant E. faecalis and E. faecium isolates

Sample origin	Isolate code and type	Phenotypic resistance	Genotypic resistance	E test/MIC (mg/l)
Milk	12s-a *E. faecium*	+	*Van A*	≥4 mg/l
Yogurt	-	-	-	-
Cheese	14p-a *E. faecalis*	+	*VanB*	≥16 mg/l
	28p-b *E. faecalis*	+	*VanB*	≥32 mg/l
Cream	3k-b *E. faecium*	+	*VanA*	≥2 mg/l
	9k-a *E. faecium*	+	*VanB*	≥2 mg/l
Ice cream	4d-a *E. faecium*	+	*VanB*	≥4 mg/l
	19d-b *E. faecium*	+	*VanA*	≥16 mg/l

DISCUSSION

In the literature review, it was found that studies have been conducted to determine the resistance profile of vancomycin-resistant enterococci and isolates in milk and dairy products of different animal species. However, sufficient numbers of studies were not found in terms of agent and antibiotic resistance profiles in buffalo milk and products, which is the subject of the present study, both in the world and in our study. As a result of the evaluation of studies conducted, studies covering farm animals other than buffalo were also included in the discussion of our study.

In a study conducted with raw buffalo milk samples in Brazil, Pereira et al. (2017) reported that of the 79 *Enterococcus* spp. isolates identified, 51(64.56%) were *E. faecalis*, 23(29.11%) were *E. faecium*, 2(2.53%) were *E. durans* and 3(3.80%) were *Enterococcus* spp. Our results are different from the results of Pereira et al., (2017) study. Our results do not agree with those obtained by Pereira et al (2017). Diversity of buffalo breeds living in different geographical regions will be decisive in terms of differences.

In a study conducted by Kirilov et al. (2011) which

included 3 kinds of cheese obtained from buffalo milksamples, 107 lactic acid bacteria were isolated. Of the 58 isolates obtained, 34(99.5%) were identified as *E. faecium*, 22(99.3%) were identified as *E. durans*, 2(99.6%) were identified as *E. faecalis.* It has been reported that *E. faecium* and *E. faecalis* species are dominant in fully ripened cheese and *E. durans* species was reported to a lesser degree. The results of our study were found to be higher than the results of Kirilov et al. (2011) study. The prevalence rate of Enterococcus in our study was found 60 (40%) of 150 samples.This difference between the values can be explained by the higher number of samples obtained due to climatic conditions.

In a study conducted in Morocco by Bouymajane et al. (2018) between May 2015 and April 2017, *Enterococcus* spp. was reported to be isolated in 17(11.3%) of 150 raw cow milk samples. Of these isolates, 11(64.7%) were identified as *E. faecalis,* 3(17.6%) were identified as *E. faecium,* 2(11.8%) were identified as *E. durans* and 1 (5.9%) was identified as *E. hirae.* The results found by Bouymajane et al. (2018) were found to be lower than the results found in our study.

In a study conducted in Poland by Różańska et al. sis cow's milk samples. 360(84.5%) of these isolates were identified as *E. faecalis* and 35(8.21%) were identified as *E. faecium.*

In a study conducted in Poland, Chajęcka-Wierzchowska et al. (2017) reported that 189 strains were isolated and 182 enterococci strains were identified from 320 ready to eat samples which included 75 soft rennet cheese, 46 semi-hard rennet cheese, 91 hard rennet cheese, 88 sour milk cheese (raw, aged) collected from supermarkets, bazaars and local shops. *E. faecium* (53.4%) and *E. faecalis* (34.4%) were identified in the samples analyzed. It was reported that a low rate of *E. gallinarum* (6.3%) and *E. casseliflavus* (2.5%) were identified and *E. durans* and *E. hirae* species were not identified. Výrostková et al. (35) reported that 110 *Enterococcus* spp. were isolated from 10 sheep and 10 goat traditional cheese in Slovakia. It was reported that 12 (8 isolated goat cheese, 4 isolated sheep cheese) of these 110 isolates were identified as *E. faecium*, 28 (13 isolated goat cheese, 15 isolated sheep cheese) were identified as *E. faecalis* and 12 (8 isolated goat cheese, 4 isolated sheep cheese) were identified as *E. durans.*

Pereira et al. (2017) reported that of the 79 isolates obtained from raw buffalo milk, 11(13.9%) were resistant to nitrofurantoin, 10(12.7%) were resistant to tetracycline, 1(1.3%) was resistant to chloramphenicol, 1(1.3%) was resistant to streptomycin, 1(1.3%) was resistant tonorfloxacin, 1(1.3%) was resistant toerythromycin. 47(59.5%) isolates were reported to be moderately resistant to norfloxacin, 46(58.2%) isolates were reported to be moderately resistant tociprofloxacin, 41(51.9%) isolates were reported to be moderately resistant to erythromycin and 5(6.3%) isolates were reported to be moderately resistant tonitrofurantoin. Unlike our study, the results Pereira et al. (31) were found to be high. High values were found in other antibiotics different from the antibiotics in our study. In the study Pereira et al. (2017) was found the highest resistance to enterococci in norfloxacin.

In their antibiotic resistance study, Bouymajane et al. (2018) reported that 100%(17/17) of *Enterococcus* spp. isolates were resistant to at least one antimicrobial, 82.3%(14/17) were resistant to two or more antimicrobials and 17.6%(3/17) were resistant to three or more antimicrobials.

According to the results of Różańska et al. (2019) study on 2000 mastitis cow's milk samples, the highest *Enterococcus* spp. resistance was found with lincomycin 82.16%, followed by tetracycline 61.5%, synercid 60.8%, erythromycin 48.83%, kanamycin 47.42%, streptomycin 46.48%, chloramphenicol 44.83% and tylosin 42.49%.

Hammad et al. (2022) stated that antibiotics resistance genes *VanB,* tet(M), tet(L) and erm(B) were identified in 29.1%, 16.6%, 8.3% and 4.1% of the isolates, respectively. *VanB* gene rate found in the study Hammad et al. (2022) was lower than the results we found in our study.

In a study conducted Abdeltawab et al. (2019) in Egypt, in a total of 200 milk and milk products including mastitis milk, karish cheese, white cheese, yogurt and ice cream, 120(60%) *Enterococcus* spp. isolates were identified in raw milk with a rate of 77.3%, in mastitis milk with a rate of 72%, in karish cheese with a rate of 80%, in white cheese with a rate of 24%, in yogurt with a rate of 28% and in ice cream with a rate of 44%. Three isolates were reported to have vancomycin resistant gene (*VanA*).

In a study conducted Furlaneto-Maia et al. (2014), 58.33% *E. faecium* was found, while the rate of *E. faecalis* was 27.77%, the rate of *E. casseliflavus* was

11.11% and the rate of *E. gallinarum* was 2.7%. It was reported that the presence of *VanA* gene was found as 23(42.8%) in *E. faecalis* isolate and as 54(96.6%) in *E. faecium* isolate. It was reported that *VanA* gene was found in 100% of the vancomycin resistant isolates and in 5 isolates that showed multiple antibiotics resistance. In a study conducted by Furlaneto-Maia et al. (2014), vancomycin resistance and *VanA* gene results were approximately similar to the results we obtained in our study.

CONCLUSIONS

In conclusion as a result of our study, it was found that contamination rates were high in buffalo milk and products. It was found that *E. feacium* species was dominant and contamination was high in cream and yogurt samples when compared with other samples. It was found that *E. faecalis* and *E. faecium* strains showed 100% resistance to vancomycin antibiotics. Although a significant part of the samples was provided from local market and sales points, the fact that contamination value was high in the study brings to mind that hygiene rules are not followed enough during the production process of buffalo milk and products, cold chain is broken during the transportation process and also that contamination may be staff-related. Unnecessary and unsuitable antibiotics should not be used in animals raised for both meat and milk production in animal food supply, which has an important place in human nutrition, antibiotics should be used under the control of vegetarians and legislation should be followed. In production, animals should not be exposed to any antibiotics other than in case of disease. However, a large number of studies conducted have found that different agents develop resistance to different antibiotics. It is thought that this is due to environmental factors, and due to the fact that the agent resistant to antibiotics which is in the flora of the enterprise with the feed and water used in feeding animals is always present in the environment. It was concluded that it is important to increase inspections at food production and sales points, to give training to producers about personnel hygiene and to carry out activities to increase awareness in issues such as agents, pathogenicity and antibiotics resistance.

CONFLICT OF INTEREST

This study was summarized from the thesis of the first author.

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