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Presence of virulence factors and antibiotic resistances in Enterococcus sp collected from dairy products and meat

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ABSTRACT

Enterococci are ubiquitous bacteria present in the environment and in the gastrointestinal tract of healthy animals and humans and may be present in soil, surface waters on plants and vegetables and also they can occur in foods, especially in those of animal origin such as meat, fermented sausages and cheeses. The aim of this study was to determine the occurrence of virulence determinants and vancomycin- resistant genes among Enterococcus faecal is and Enterococcus faecium obtained from various clinical sources. The study was performed on the 200samples collected from dairy food and meat in Hamden. Antibiotic susceptibility testing was performed using disk diffusion methods. The presence of vancomycin-resistant genes and virulence genes was investigated using PCR. Of 135 enterococcal isolates, (48.1%) were identified as E. faecalis, (43.7%) as E. faecium, E. avioum (6.6%) and E. gallinarom (1.5%). The results of antibiotic susceptibility testing showed that of the total 135 isolates, 100 (74.1%) were resistance to tetracycline. Lower antibiotic resistance was seen with Nitrofurantoin 2 (1.5%). None of the isolates was found to be resistant to Teicoplanin. Prevalence of esp, hyl, and asa_1 genes were reported 48.9%, 20%, and 89.6, respectively in Enterococcus strains. Totally, van genes were identified in 70 (51.6%) Enterococcus strains. This study indicates a high prevalence of multidrug resistance among enterococci isolated from meat and milk products that may serve as a vehicle to transport these resistant bacteria and genes from food to humans and become a serious threat to public health in the future.

Keywords: Enterococcus faecium; Enterococcus faecalis; Enterococcus avioum; Enterococcus gallinarom; Antibiotic Resistance; Polymerase Chain Reaction.

INTRODUCTION

Bacteria of the genus Enterococcus, or enterococci, are an important group of lactic acid bacteria (LAB), which are ubiquitous bacteria present in the environment and in the gastrointestinal tract of healthy animals and humans. They can also occur in foods, especially in those of animal origin such as meat, fermented sausages and cheeses [1]. In addition, enterococci are also used to extend the shelf life and improve the hygienic safety of foodstuffs because they produce several antimicrobial substances such as lactic acid, hydrogen peroxide and bacteriocins (enterocins). These latter have become the subject of great interest since they are frequently active against several Gram-positive, food-borne pathogens such as Listeria monocytogenes, Staphylococcus aureus and Clostridium botulinum. For this reason, their use as bio preservatives in foods has been suggested [2, 3]. The detrimental activities of enterococci are associated with spoilage of foods, especially meats and, more importantly, certain enterococcal strains can cause

human disease[4].The differentiation of apparently safe and non-safe enterococcal strains is not simple, especially because virulence genes can be easily exchanged between strains[5].

Since the 1980s, enterococci have been documented as being an important source of nosocomial infections such as urinary tract infections, endocarditis, sepsis, and wound infections [6].Their antibiotic resistance and gene transfer mechanisms add to enterococcal pathogenesis. They are able to acquire resistance to macrolides, chloramphenicol, rifampicin, aminoglycosides, and ampicillin and such resistances may be spread to human beings via the food chain [7, 8].

Vancomycin is one of the main antibiotics used to treat enterococci. The presence of van genes result in vancomycin-resistant. Currently, nine types of vancomycin-resistance have been demonstrated in Enterococci, eight of these types are responsible for this resistance, i.e., vanA, vanB,van C, vanD, vanE,vanG,vanL,vanM and vanN; among them vanA and vanB are clinically the most significant ones. The vanA genotype, as the most commonly genotype in vancomycin-resistant Enterococci (VRE) worldwide, is associated with the transfer of high-level vancomycin resistance from Enterococci, particularly, vancomycin-resistant Enterococcus faecium (VREF) to Staphylococcus aureus[9, 10]. According to the above mentioned issues and by considering the fact that these bacteria have become resistant to a great variety of antimicrobials, there would be some difficulties in treating enterococcal clinical infections of immune-compromised patients [11].

Enterococci may carry various genes directly or indirectly contributing to virulence. Genes encoding virulence factors such as aggregation substances, endocarditis antigen, gelatinase, enterococcal surface protein, hyaluronidase or adhesion collagen protein have been described in enterococci isolated from foods [12]. The enterococcal surface protein esp gene hasan association with the increased virulence, colonization and persistence in the urinary tract along with biofilm formation. While aggregation substances encoded by asa1 are responsible for increased bacterial adhesion to renal tubular cells and heart endocardial cell and hyl gene which produces hyaluronidase[13, 14]. Because of the possible role of these bacteria in the transmission of virulence genes and resistance determinants via the food chain, their presence in foods have caused to raise concerns in this area.

Despite the importance of virulence genes, there remains a paucity of study in this area; previous studies have mostly focused on E. faecalis and E. faecium and less attention has been paid to the presence of virulence genes from other enterococcal isolates in foods. Accordingly, this study set out to investigate the distribution of virulence factors, vancomycin-resistant genes and the antibiotic resistance of various enterococci species isolated from foods.

MATERIALS AND METHODS

Sampling, strain isolation and identification

Two hundred food specimens were included raw milk (24 samples), cheeses (6 samples), and meat products (170 samples of raw ground beef, raw chicken meat). Raw bovine milk samples were obtained from a dairy farm located in the Hamadan city/Iran, and the other food samples were purchased from the local retail market from December 2012 to May 2014. Samples were collected by adapting the procedure used by Diego Cariolato et al... Identification of isolates was carried out as described by G. Pesavento et al. and R. H. Olsen et al.[15-17].

DNA extraction

Enterococcus DNA was prepared by suspending a loop of overnight colonies in 1.5 ml tubes containing 500 µl of sterile distilled water, followed by boiling for 10-15 min and then centrifuging at 14000 g for 5 min to pellet cell debris [18].

Detection of Enterococcus spp. by PCR

PCR reaction of ddlgene of E faecalis, faeci of E. faecium, gall of E. gallinarum and avi of E. avium were performed with sequences presented in table 1.The reaction mixture for PCR was composed of 10 µL of 2x Taq premix Mastermix (Parstous Biotech CO. Iran), 5 µL sterile double distilled water, 1 µL of forward primer, 1 µL of reverse primer and 3 µL of DNA sample. The optimum conditions of PCR were as fellow: an initial denaturation step for 5 min at 95 °C followed by 30cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min and a final cycle of 72 °C for 10 min in a Bio-Rad Thermal Cycler. PCR products and 50-bp DNA size marker (Fermentase Co, USA) were

run simultaneously on 1.5% agarose gel stained with DNA safe stain (SinaClon Co, Iran) at 80 V for 1 hour[19]. Finally, the agarose gel was visualized and photographed using UV transilluminator (VilbertLourmat Co, Japan).The E. faecalisATCC29212and E. faeciumBM4147 were used as quality control strains.

Antimicrobial susceptibility Test

The antimicrobial susceptibilities of Enterococcus isolated strains were determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI 2013) guidelines [20], for the antibiotics including Ciprofloxacin (5 µg), vancomycin (30 µg), teicoplanin (30 µg), Tetracycline (30 µg) , erythromycin (15 µg), choloroamphenicol (30 µg), norfloxacin(10 µg), SYN(15 µg), Linezolid (30 µg), ampicillin (10 µg) and Nitrofurantoin(300 µg) (Mast Group Ltd, Merseyside, U.K, ENG). The E. faecalis ATCC 29212(Vancomycin sensitive), E. faecalis ATCC 51299 (Vancomycin resistance), E. faecalis E206 (Vancomycin resistance) were used as quality control strains for performing antimicrobial tests.

Detection of van A, B, C and D genes by PCR

Isolates which were resistant to vancomycin by disk diffusion method were analyzed by PCR for the presence of the genes encoding the vancomycin-resistance determinants vanA, vanB, vanC and vanD using specific primers as described by Depardieu et al (Table 1).The PCR reaction was performed in a volume of 20 µl containing2µltemplate DNA,1µlofeach primer, 10µl of Master Mix, 6µl of sterile distilled water on a Eppendorf and Bioradthermocycler (ASTEC Co., Japan) with an initial denaturation at 94°C for 3 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 7 min [21].

Detection of virulence genes esp, hyl, and asa1 by PCR

Multiplex PCR and single PCR were performed for detecting esp, asa1 and hyl virulence determinants using specific primers for each gene with some modification on Vankerckhoven's protocol (Table 1). Briefly, the first 25 µl of PCR mixture contained 3µl of template DNA (1µl of plasmid DNA, 2 µl of chromosome DNA), 1µlofeach primer for genes esp and asa1, 12.5µl of Master Mix, and 5.5µl of twice distilled water; the second 20 µl PCR mixture contained 2µl of template DNA,1µlof each primer forhyl, 10µl of Master Mix, and 6µl of twice distilled water. The PCR reaction were done for both mixtures on a Eppendorf and Bioradthermocycler (ASTEC Co., Japan) with an initial denaturation at 95°C for 10 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min [22]. The E. faecalis ATCC 29212 (asa1 positive), E. faecium C68 (hyl and esp positive) were used as quality control strains.

Statistical analysis

The relationship between various food samples and virulence genes, as well as their relationship with resistance genes resistant to vancomycin (van) was also evaluated. Then the relationship between resistance to different antibiotics and virulence genes and genes resistant to vancomycin (van) was also assessed. All categorical (continuous) variables were compared using the 2-tailed Chi-Square test ($\chi 2$) or Fisher's exact test. p.values of ≤ 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS version 20 software package.

RESULTS

Of 200 samples examined, a total of 135 enterococcal isolates were obtained, 6 isolates (4.4%) were from cheeses, 24 isolates (17.8%) were from raw milk and 105 isolates (77.7%) were from meat products. Of the 135 isolates of enterococci investigated, 65 (48.15%) were identified as E. faecalis, 59(43.7%) E. faecium, 9 (6.65%) E.avium and 2 (1.5%) E.gallinarum. E.faecalis was the most common species isolated from both dairy products and meat products, comprising 14(46.6%) dairy products and 51 (48.8%) meat products. E. faecium was ranked the second most recovered, 15 (50%) from dairy products and 44 (41.9%) from the meat products. E.avium and E. gallinarum were recovered much less often than E. faecalis and E. faecium, accounting for less than 9% of all isolates (table 3).

Antibiotic susceptibility results

Antibiotic susceptibility results revealed that most isolates of E. faecalis from both meat and dairy products were resistant to tetracycline (77 %) and synercid (75.5%). In addition, the rate of resistant to other antibiotics were in the

range of 15.4- 70.8%, with the rate of resistance to being the lowest for Nitrofurantoin and teicoplanin (0%). Antibiotic resistance in isolates of E. faecium showed that most isolates such as E. faecalis were resistant to tetracycline (71.2 %) and synercid (57.6%). The rate of resistance to antibiotics in isolates of E. gallinarum were in the range of (0%) Linezolid, Choloroamphenicol, Teicoplanin, Nitrofurantoin to and were resistant to Synercid and Ampicillin (100%). and in isolates of E.avium the most isolates were resistant to Erythromycin (88.9%) and susceptible to ampicillin (100%). The results of antibiogramare presented in table 2. Antibiotic susceptibility testing showed all enterococcal isolates from both meat and dairy products were susceptible to teicoplanin.

PCR assay for detection of vanA-vanB- vanB–vanDgene

The prevalence of vancomycin resistance genes among vancomycin-resistant Enterococcus isolated strains is shown in Table 3. Totally, van gens were identified in 70 (51.6%) Enterococcus strains, 10 (33.3%) dairy production isolated strains, and 63 (60%) meat production isolated strains. VanA, vanB and vanC genes were identified in 68 (50.4%), 10 (7.4%), and 3 (2.2%) of enterococcus strains, respectively. Moreover, 10(7.4%) samples carried vanA and vanB simultaneously, while both vanA and vanC were identified only in one strain (0.7%).

PCR assay for detection of virulence genes

The prevalence of virulence factors among Enterococcus isolated strains are shown in Table 3. In a total of 135 Enterococci strains, 128 (94.8%) carried virulence genes. The asa_1 gene was the most common virulence factor 121 (89.6%), followed by the esp 66 (48.9%) and hyl genes 27 (20%).

Sequencing

One sample of each of the virulence factors as well as vanA,vanB and vanC PCR products (amplicons) were sequenced by Bioneer Co., Korea mediated by Takapouzist Co., Iran and the data were analyzed using the Chromas software.

Results of statistical analysis

The findings of the present study indicated that there is a significant relationship between the existence of the vanA gene and the resistance to Linezolid, Choloroamphenicol, synercid, Erythromycin, Ciprofloxacin, and Vancomycin antibiotics (p-value <0.05) and also there is no significant relationship between different strains of enterococci with distribution of virulence genes (p>0.05).

Table 1: Primers used for PCR assay in this study

Gene targets	Primer sequences (5' to 3')	amplicon / product size (bp)	References
asa_1	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375	[33]
hyl	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	276	[35]
esp	F: AGATTTCATCTTTGATTCTTGG R: AATTGATTCTTTAGCATCTGG	510	[33]
ddl E. faecalis	F: ATCAAGTACAGTTAGTCTTTATTAG R: ACGATTCAAAGCTAACTGAATCAGT	941	[33]
ddl E. faecium	F: TTGAGGCAGACCAGATTGACG R: TATGACAGCGACTCCGATTCC	658	[33]
Gall E.gallinarum	F: GAAAGACAACAGGAAGACCGC R: TCGCATCACAAGCACCAATC	158	This study
AviE.avium	F: CGGGGAAGATGGCAGTAT R: CGCAGGGACGGTGATTTT	229	This study
vanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732	[35]
vanB	F: ATGGGAAGCCGATAGTC R: GATTTCGTTCCTCGACC	635	[35]
vanC	F: AGCAATAAATCTTTGTGGGTTCGT R: ATTTGCGGCAATGAAAGACAG	158	This study
vanD	F: TGTGGGATGCGATATTCAA R: TGCAGCCAAGTATCCGGTAA	500	[35]

Table 2: Prevalence of antibiotic-resistant enterococci among 135 isolates from meat and milk products

Antibiotic	E. faecalis No=65(%)	E. faecium No=59(%)	E.avium No=9(%)	E. gallinarum No=2(%)	Total No=135(%)
Ciprofloxacin	20 (30.8)	28(47.5)	6(66.6)	1(50)	55 (40.7)
Vancomycin	30 (46.2)	16(27.1)	1(11.1)	1(50)	48 (35.5)
Teicoplanin	0	0	0	0	0
Tetracycline	50 (76.9)	42(71.2)	7(77.8)	1(50)	100 (74.1)
Choloroamphenicol	11 (16.9)	7(11.9)	3(33.3)	0	21 (15.5)
Erythromycin	46 (70.8)	29(49.2)	8(88.9)	1(50)	84 (62.2)
Linezolid	10 (15.4)	1(1.7)	1(11.1)	0	12(8.8)
synercid	49 (75.5)	34(57.6)	6(66.7)	2(100)	91(67.4)
Ampicillin	26 (40)	25(42.4)	0	2(100)	53 (39.25)
Nitrofurantoin	0	1(1.7)	1(11.1)	0	2 (1.5)
Norfloxacin	23 (35.5)	25(42.4)	3(33.3)	1(50)	52(38.5)

Table 3: virulence factor encoding gene in entrococci strains by source and species

Source	Species	Strains tested	Presence of virulence genes (%)			Presence of van genes(%)		
			asa1	**esp**	**hly**	**vanA**	**vanB**	**vanC**
Raw milk (24 isolates)	*E.Faecalis*	13	11 (84.6)	3 (23.7)	2 (15.4)	7 (53.8)	0	0
	E.Faecium	10	10 (100)	7 (70)	1 (10)	1 (10)	0	0
	E.avium	1	1 (100)	0	0	0	0	1
Raw chicken meat (42 isolates)	*E.Faecalis*	15	13 (86.6)	7 (46.6)	6 (40)	14 (93.3)	4 (26.6)	0
	E.Faecium	18	17 (94.4)	9 (50)	2 (11.1)	7 (38.9)	2 (11.1)	0
	E.Avium	8	1 (100)	1 (100)	0	0	0	1 (100)
	E.gallinarum	1	7 (87.5)	4 (50)	0	1 (12.5)	0	0
Raw meat (63 isolates)	*E.Faecalis*	36	33 (91.6)	19 (52.7)	11 (30.5)	23 (63.9)	4 (11.1)	0
	E.Faecium	26	23 (88.4)	13 (50)	4 (15.4)	13 (50)	0	0
	E.gallinarum	1	1 (100)	1 (100)	0	1 (100)	0	1 (100)
Cheese (6 isolates)	*E.Faecalis*	1	1 (100)	0	0	0	0	0
	E.Faecium	5	4 (80)	2 (40)	1 (20)	1 (20)	0	0
Total		135	121(89.6)	66 (48.9)	27 (20)	68 (50.4)	10 (7.4)	3 (2.2)

DISCUSSION

Enterococci are ubiquitous in their occurrence, with their habitats ranging from the intestinal tract of man and a variety of farm animals to different forms of food and feed. Several studies demonstrated that enterococci were present almost everywhere in the food chain as well as in the environment [23].In this study, enterococci were isolated from a variety of sources, even foods of animal origin such as meat and dairy products. There is little data on antibiotic resistance among food isolates of enterococci in Hamadan, since most reports on antibiotic susceptibility are for bacteria isolated from patients or from sick and dying animals with few from bacteria isolated from healthy humans, animals or foods of animal origin. Klein, G studied on the food such as Cheese, Meat and etc, and indicated this food contaminated with E.faecalis and E. faecium, while E. gallinarum presence in the meat and fish only(like our study) and none of isolates contaminated by E. Avium and E. Casseliflavus, whereas in our study E. avium presence in the eight sample ofmea[23]. In this study, the most common species found in these types of food products were E. Faecalis 59 (43.7%) and E. faecium 9 (6.65%). The prevalence of these two species in foods has also been reported in Bruna C study[5], that reported the higher prevalence of enterococcal isolates belonged the E. faecium(46.5%),E. faecalis(26.8%),E. gallinarum(2.7%) that similar to, other surveys[4, 24].

The present study showed that the strains of enterococci isolated from meat and milk products had similar susceptibilities to antibiotics. The proportion of enterococci in both meat and milk samples with a high level of resistance was approximately 50%, with a moderate degree of resistance was 15%, and alow degree of resistance was 0.8%. No significant differences in prevalence and rates of resistance between the meat and milk strains were found for any of the drugs ($p > 0.05$) except ampicillin. Rahimi in Tehran showed that the highest level of resistance was observed with erythromycin, tetracycline and ciprofloxacin [25].

Antibiotic susceptibility testing showed most enterococcal isolates from both meat and milk products were generally resistant to two antibiotics commonly used in humans, tetracycline and synercid, while resistance to Linezolid and Nitrofurantoin tested was minimal. Tetracycline and synercid are common drugs used to treat and prevent animal

diseases. We found a significantly greater incidence of resistance to these antibiotics, which is probably related to prolonged exposure. Tansuphasir, also found enterococcal isolates from frozen foods and Environmental water, had a high degree of resistance to Tetracycline and Ciprofloxacin: 52.3% and 48.5%, respectively [26].

The prevalence of vancomycin resistant erococci (VRE) was 35.5% (48 of 135 strains tested). These VRE strains were isolated from nearly all sources studied, 14 and 27 strains from meat chicken and raw ground beef, respectively, six and one strains from milk and cheese, respectively. The rates of vancomycin resistance for strains isolated from meat and milk products food strains were not statistically significant different ($p>0.05$).

Rahimi studied on the total number of 712 enterococci spp. were isolated and the results showed that 56%, 24%, 12%, 4%, 2%, 1% and 1% isolates were E. faecium, E. hirae, E. faecalis, E. gallinarum, E. casseliflavus, E. mundtii and other enterococcal spp, respectively[25]. Author reported the highest level of resistance was observed with erythromycin, tetracycline and ciprofloxacin. E. avium was susceptible to all of the antibiotics tested here (data not shown).Also Author indicated the frequency of VRE strains isolated was 3% VRE from the total enterococcal isolations. Whereas the prevalence of VRE in our study was 35.5% (48 of 135 strains tested), that conflicts with Torres study from isolates of waste water in Spain (0.4%), also in New Zealand, FranceandUSA[27-29], the percent of VRE isolation from broilers, human fecal and farm wastewater has been 6%, 4% and 6%, respectively. In our study resistant to, tetracycline, erythromycinin , norfloxacin and chloramphenicol the isolate of E.faecalis are 77%, 70.8%, 35.5% and 16.9% respectively, that similar to Diego Cariolato study that showed The E.faecalis strains were mostly resistant to tetracycline (65.8%), followed by streptomycin(42.1%), erythromycin(28.9%), norfloxacin(21.1%), chloramphenicol (18.4%) and also reported none of the E. faecalis strains was resistant to ampicillin, while in the our study 40% of isolates were resistant. On the other hand, E. faecium strains were mostly resistant to tetracycline (71.2%), synercid (57.6%), and followed by erythromycin (49.2%), Ciprofloxacin (47.5), ampicillin and norfloxacin (42.4%),vancomycin (27.1%), chloramphenicol (11.9%),and. Only one isolate resulted resistant to Linezolid and Nitrofurantoin. That this results similar to Diego Cariolato and Rahimistudy [15, 25].

A major concern is the presence of strains harboring multiple antibiotic resistances (from 2 to 7 out of the 11 antibiotics tested). Indeed, most of the vancomycin resistant enterococci showed resistance also to other clinically relevant antibiotics such as ampicillin, erythromycin, Ciprofloxacin, norfloxacin, synercid and streptomycin such as human strain that can be transmitted from those food animals' origins to humans, and thus leaving few therapeutic options [30].

In the present study, the asa1 gene, which encodes aggregation substance, was found in high frequency among E.gallinarum (100%), E.faecium strains (91.5%), E.faecalis (89.2%), and E. avium (88.8%). A high incidence of this gene in E.faecalis was reported in previous studies, as well. Results of studies on E.faecium isolated are contradictory. In some studies, asa1 was not found in E.faecium but in contrast, in some studies this gene was detected in less frequency and in our study and some other studies this gene was detected in higher prevalence among E.faeciumisolates. Ahmed M et al., AKOlawale et al., and R.H.Olsen et al., were detected it among 2.5%, 75% and 78.5% of the studied strains, respectively [16, 31, 32]. DiyoCariolato et al. were not found asa1 gene in 81 entrococci strains[15].The asa 1 were detected significantly from Raw meat and raw poultry meat samples ($p=0.001$).

In the current study, the esp gene was detected in 100% of E. gallinarum, 52.5% of E. faecium , 46.1 % of E. faecalis, and 82% of E. avium isolates, these findings is in accordance with the findings of other studies, which identified the espgene in 42% and 90%of entrococci strains[15, 32].However, this is in contrast to the findings obtained by Ahmed M and R.H.Olsenthat the amount of the gene reported 4.1% and 1.25% respectively[12, 16].

Hyaluronidase, coded by the chromosomal gene hyl, is a degradative enzyme associated with tissue damage that influence the hyaluronic acid (hyaluronate, HA) [33]. Hyaluronidase encoded by the chromosomal gene hyl, indicates homology to the hyaluronidases in Streptococcus pyogenes, Staphylococcus aureus and Streptococcus pneumoniae and contributes to invasion of the nasopharynx and pneumococcal pneumonia [34, 36]. We found the hyl gene amon20% of enterococci strains (table 3).Generally, there is no significant relationship between different strains of enterococci with distribution of virulence genes ($p>0.05$).

Another objective of the study was to evaluate the various genes in the strains that were resistant to vancomycin. The findings of these experiments demonstrated that all the strains that were resistant to vancomycin by disk diffusion method had at least one van gene. Furthermore, some strains that have intermediate phenotypic resistance to vancomycin carried these genes. Totally, 51.9 percent of all isolated strains carried van genes; the most prevalent one was van A identified in 50.4 percent of all strains, following by van B with 7.4 percent and van C with 2.2 percent frequency. Ten strains that composed 7.4 percent of all strains carried both genes vanA and van B simultaneously. The findings of the present study also indicated that there is a significant relationship the existence of the van a gene and the resistance to Linezolid, Choloroamphenicol, synercid, Erythromycin, Ciprofloxacin, and Vancomycin antibiotics (p-value <0.05).

Here, we report for the first time E. faecalis, E. faecium, E.gallinarum and E. avium vancomycin-resistant strains from food of animal origin in Iran. The incidence of vancomycin resistance in all enterococcal isolates is higher than that reported by Franz et al. (2001) and Jamet et al. (2012).

CONCLUSION

This study indicates a high prevalence of multidrug resistance among enterococci isolated from meat and milk products that may serve as a vehicle to transport these resistant bacteria and genes from food to humans and become a serious threat to public health in the future. Then, infections can result that are difficult to cure since the resistant bacteria do not respond to treatment with conventional antimicrobials. The prevalence and level of antibiotic resistance found in the fecal flora of humans and animals are considered to be good indicators of selective pressure caused by antibiotic usage. It is necessary that the use of antibiotics for purposes other than human use, in animal feed and in the treatment of infection in animals, should be eventually reduced.

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