The Risk of Vancomycin Resistant Enterococci Infections from Food Industry

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Abstract Several works of literature research for the contribution of Antibiotic-Resistant Enterococci (ARE) and, especially Vancomycin Resistant Enterococci (VRE) which entered into the food chain has gained importance with the increasing significance of VREs in hospital infections. Various studies conducted in Europe, United States of America (USA) and the Middle East were evaluated in terms of prevalence, epidemiology and risk factors of foodborne enterococci in VRE infections. VRE epidemiology has shown some distinctions in Europe and USA. VRE was generally isolated from animals in Europe, which was connected to the extensive/massive use of "avoparcin" as a growth promoter in animal feed in the agriculture sector. Animals fed with this feed act as reservoirs of transferable vanA type resistance. On the other hand, since "avoparcin" was not used in the USA, VRE could not be isolated in animals and healthy humans. However, hospital-acquired VRE infections are more showed in the USA than in European countries. According to numerous studies, since enterococci are used as starter culture and probiotic culture, they have no relationship genetically with the strains which include vancomycin/resistant to antibiotic/or having resistance and virülence genes. In this chapter, important features of enterococci, the role of food chain for ARE especially, VRE infections in community including strategies for future solutions about the problem are summarised.

Keywords VRE · Food chain · Enterococci · Food industry

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Introduction

Antibiotic resistance of the bacteria has become a major problem all around the world. It has been debated both food and clinical microbiology in terms of antibiotic using in food animal and agricultural process related to gaining antibiotic resistance of bacteria especially *Enterococcus* spp. Genus. They are catalase negative, Gram positive and facultative anaerobe and coc-shaped bacteria found in food natures and used as starter/co-culture microorganisms in dairy and meat products as well as inhabit human and warm-blooded animals; generally, in proximal of intestine flora of gastrointestinal tract and female genital system as a permanent flora component. Therefore, Enterococci are used in drinking water as the indicator bacteria of hygiene and fecal contamination (Gelsomino et al. 2001; Samakupa 2003). Although enterococci are not recognized Generally Recognized as Safe (GRAS) microorganism in food microbiology, they have several beneficial effects in food industry such as proteolytic, esterolytic and aroma producing components in dairy and meat production industry as well as probiotics in humans and food animals (Franz et al. 2011).

VREs have been detected and isolated in many parts of the world (Wegener et al. 1999; Hershberger et al. 2005). The extensive/massive use of antimicrobials in food animal may have undesirable consequences for human health, so it could result in a possible selection of a reservoir of opportunistic human pathogens such as Enterococci may carry antibiotic resistance determinants and including pathogenic virulence genes. Thus, in recent years the increase in VRE acquired nosocomial infections leads the possible relationship between community/food industry-acquired VRE or Glycopeptide Resistant Enterococci (GRE) strains to colonize the intestine through food and VRE/GRE strains isolated from cross infections and colonization to be detected. In medical settings; Some Chemotherapeutic agents and/or endoscopes used for diagnosis and/or treatments and VREs involved in the general circulation through the translocation from the endogenous flora as a result of the tissue damage in gastric mucosa may cause bacteremia and endocarditis which are the difficult-treated disease. Moreover, the strains being disseminated around by colonized patients persist in being in the hospital environments for a long time which results in severe nosocomial infection especially in immunocompromised and intensive care units' patients. In spite of the starter microorganisms' contribution to the food industry, because of the fact that they cause the rapid spoilage of food products stored in unsuitable conditions and, more importantly, the bacteria may gain easily resistance to antimicrobials and can easily spread lots of virulence factors, mobile genetic elements and antibiotic resistance genes to other microorganisms.

Development of new strategies/solutions for prevention of ARE especially VRE, as well as Multidrug-Resistant(MDR) Bacterial infections in the community, depend on appropriate plans and applications in clinical settings, veterinary medicine, food industry, medical-veterinary drug production sectors and international/national legislation. Those preventive strategies may be used such as alternative

antimicrobials producing instead of conventional antimicrobial agents, international standard procedures-applications of vaccination and growth promoting agents for food animals, effective disinfection programmes for hospital settings and hand washing programmes for health workers as well as regular monitoring and analyzing of all data should be considered by international/national authorities in order to prepare preventive/corrective actions. In addition, suitable and safety starter culture selection and preparing standardized regulations about this subject can be beneficial effect in both food industry and public health.

Important Features of Enterococci in Food Industry

In general, *E. faecium* and *E. faecalis* species from enterococcal group which can keep their vitality in in-vitro conditions for a long time as a result of their resistance to harsh physical conditions and their ability to spread around nature with organic elicitation, are important industrial microorganisms in food microbiology, due to both showing activity lipolytic and esterolytic in food and also utilizing citrate and creating volatile aromatic compounds. In addition, they can improve organoleptic properties such as taste, color, and the smell of some foods. Therefore, with lactic acid bacteria, they are used in fermented meat and dairy products as starter cultures (Maschieto et al. 2004).

Enterococci as Starter Microorganism

Enterococci's permitting high temperature can easily enable these bacteria to be an indicator of quality sanitation in food products. In some studies, *E. faecium* could maintain their liveness in spite of a 30-min heat treatment at 68 °C, and reported that the presence of these bacteria is inevitable in many processed and stored foods like dairy products and fermented sausages as well as food consumed raw like fresh vegetables and vegetable materials (Giraffa 2003).

Probiotic Features of Enterococci

Various studies show that enterococcus strains that will be used as probiotics can be economic, or they can be occupied from different sources through conventional methods and/or fermentation. Another issue pointed out in the studies is whether the strains got in this way have antibiotic resistance and virulence genes. *E. faecium* strains are used as probiotics in the growth of food animals, production of animal feed and the prevention of diseases in them (Çakır et al. 2002; Franz et al. 2011). These bacteria contribute to metabolism by preventing the colonization of ordinary

bacteria in intestinal flora and help the maturation and proliferation of enterocytes and muscular mucosa (Yaman and Esendal 2004). Therefore, *E. faecium* is suggested to be used in animal nutrition as an alternative to antibiotics, and it is debated that it can be used particularly in the treatment of mastitis (Marekova et al. 2003). A study reported that 15 *Enterococcus* strains gathered from different foods were identified and analyzed for virulence factors and antibiotic resistance. In another study, three *Enterococcus durans* strains were chosen to study PBMC and Caco2 cells for their immunomodulatory properties. It is reported that the strains modulated gut microbiota increasing *Faecalibacterium prausnitzii*, a functionally essential bacterium. Thus, *E. durans* EP1 not only increased *F. prausnitzii* in some cases of dysbiosis but could also be effective in gut inflammatory disorders therapy (Carasi et al. 2017).

Some studies claimed that these bacteria can be used as probiotics in gastroenteritis in humans (Hugas et al. 2003). For example; it is reported that *E. faecium* 68 (SF68) strain can be used for such purposes as strengthening the immune system, preventing diarrhea, and preventing cancer development by contributing to the consumption of carcinogen in bowels (Rinkinen et al. 2003; Linaje et al. 2004). Diarrhea duration of the patients who had diarrhea reduced when *E. faecium* was used as a probiotic; the best electrophoresis combination for producing probiotic yogurt claimed to be S. *thermophilus* and *E. faecium* species (Crittenden et al. 2003).

A similar study conducted in India and examined antibiotic resistance, biogenic amine production and the presence of *agg*, *esp*, *efa*, *afm*, *gelE*, *cylA*, *cylB*, *clyM*, *cpd*, *ccf*, *ace*, and *hyl* in *E. faecium* KH 24 strain isolated before. It was found that this strain had no antibiotic resistance; virulence genes were not detected except in *efaAfm*, and it did not produce biogenic amine. Besides, it was found that KH 24 strain had a good in vitro tolerance to biological barriers and could easily live in mice bowels. Mice groups fed with this strain had better weight control and the number of *Salmonella enteritidis* in their bowels was reduced by approximately 1 log cfu/g in comparison to the control group. A significant decrease in the coliform count (pb 0.05), lactobacilli count (pb 0.05) was observed in the test group. *E. faecium* KH 24 strain was therefore reported to be a reliable strain and it could be used as a protective culture or as a probiotic in food (Strompfova et al. 2004; Bhardwaj et al. 2010).

Another study conducted in Tunisia and reported that *Enterococcus faecium* strains were isolated from an original biotope, and analyzed for assurance and capacity as probiotics on dried Tunisian meat "Dried Ossban". It is reported that *E. faecium* strains gathered from "Dried Ossban," did not have a health risk on humans, and might be considered as candidates for future use as probiotics and bioprotective cultures for use in the food and/or feed industries (Zommiti et al. 2018).

Enterococci in Dairy Products

In the cheese maturation process, enterococci dependable affect the formation of taste, color, and aroma, in addition, to be a good starter microorganism in cheese production due to good salt tolerance, fast acid production and resistance to high temperatures (Giraffa 2003; Abeıjón et al. 2006). The strains of *E. faecium* K77D, used as a starter culture in fermented dairy products was advised by Advisory Committee on Novel Foods and Processes (ACNFP) in England (El-Din et al. 2002).

According to some studies, in Mediterranean Countries, *E. faecium*, sincerely found in milk during the production of traditional cheese like Misha and Domiatti, provides the desired aroma or contributes to the development of the sensory properties by using citrate metabolism and producing typical flavor compounds such as acetaldehyde, acetone, diacetyl and, this cheese contains nitrogen being able to dissolve in more water and improving organoleptic properties and total free amino acids (De Vuyst et al. 2003; Giraffa 2003). It is observed that *E. faecium*, providing degradation of casein in cheese production by producing proteolytic enzymes, hydrolyzes milk fat with esterases during the production of cheese (El-Din et al. 2002).

In another study, Gelsomino and his colleagues (2001) implied that cheese used in the production of cheddar cheese and *E. casseliflavus* and *E. faecalis* in the tank of milk storage were dominant species and these species described above were dominant in human feces as well.

Enterococci in Meat Products

According to some studies of biochemical activities of sausages, it was shown that the presence of enterococci in fermented meat products, particularly *E. faecium*, contributed glycolytic, proteolytic, and lipolytic activities to the sausage flavor, took on a role in "methmyoglobin reduction reactions" providing formation of red color in fresh meat and, in case of the use of *E. faecium* CTC492 as a starter culture in the production of sausages, this strain maintained its effectiveness through the end of the process (Hugas et al. 2003). On the other hand, it was pointed out that *E. faecium*, producing bacteriocins, has more advantageous than traditional chemical preservatives, impeded the growth of pathogens found in the meat products, besides its contribution to the formation of flavor, *E. faecium* strain has significant as being protective against bacterial contamination (Giraffa 2003). Thus, it was implied that these bacteria contributing to the process of food maturation as a starter retarded bacterial decrease in reproduction and putrefaction in fermented products by showing anti-*Listerial* and anti-*Clostridial* effects with the help of bacteriocins they produced (Sarantinopoulos et al. 2002; Hugas et al. 2003; Ribeiro et al. 2011). It was demonstrated that use of enterococci in industrial applications as starters, they could

colonize in products due to the bad hygiene in slaughterhouses and could become dominant by reproducing/proliferating during being hold with the design of fermentation (Giraffa 2002; Papamanoli et al. 2003). In a study of chicken meat produced with modified atmosphere, it was found that dominant microflora was formed by *E. faecalis* with *Lactobacilli* and corinabacter species during the storage of products at 3.5 °C up to 7 days (Barakat et al. 2000). In another study with German and Italian type of fermented sausages, it was reported that at the end of the maturation process, the number of enterococci in all sausages, having or not having enterococci as starter cultures, was between 10^3 to 10^5 cfu/g and enterococci were main contaminants in addition to their industrial usage (Hugas et al. 2003).

Enterococci in Food Preservation

The use of microbial metabolites such as bacteriocins use for food security instead of chemicals called as "green technology" may be considered a new bioprotective method (Ross et al. 2002). It was suggested that *E. faecium* and *E. faecalis* strains might be bacteriocins harboring technological potential in food industry because of enterocins they produced had better physicochemical characteristics and biological actions than the other bacteriocins did (Linaje et al. 2004; Franz et al. 2011). On these subjects, Bacteriocin-Like Inhibitory Substrates (BLIS), having anti-*Listerial* effect and BLIS were identified in *E. faecium* strains isolated from fermented sausages in Mexico (Alvarez et al. 2010). In another study, it has been reported that *E. faecalis* UGRA1 isolated from Spanish sheep cheese produces a 70 kb enterocin called AS-48, and the strain which can create biofilm has the competence to stick to "Caco2" and "HeLa 229" cells and *L. monocytogenes*. Advanced analyses of the strain producing a broad spectrum of bacteriocin have yielded positive results in terms of food security and it has been implied that it has a biotechnological potential like a probiotic as a protective agent in food storage (Cebrıán et al. 2012).

In a similar study conducted in Iran, it was detected that Bacteriocin *Enterococcus faecium* strain C2 (isolated from local cheese) had the most antibacterial activity. The most antibacterial effect was observed against *Listeria monocytogenes* and *Pseudomonas aeruginosa.* Considering that produced bacteriocin has a wide inhibitory range against gram-positive and gram-negative bacteria, especially pathogenic bacteria, it is recommended to use it as a substitute for chemical antibiotics (Khodaee and Nejad 2017).

Enterococci produce considerable antimicrobial compounds such as lactic acid, hydrogen peroxide and bacteriocins. Therefore, they are suggested to be used as bioprotective in the food industry. The lactic acid produced through the metabolization of carbohydrates acidifies peripheral pH and deters bacteria such as coliforms reproducing in neutral pH (Giraffa 2002; Franz et al. 2007). Despite using enterococci in food production processes, generating biogenic amines caused rotting cheese and refined meat when not stored in appropriate conditions and has provoked a dispute. Furthermore, enterococcal strains have been debated as unwanted

organisms in the food industry in terms of human health as they may develop resistance to many antibiotics used in medical treatments. So, *Enterococcus* spp. is not Generally Recognized as Safe (GRAS) (Giraffa 2002; Franz et al. 2011). Thus, the abundance of enterococci present in food is considered as an indicator for the insufficiency of sanitation conditions. According to a study; post-process contamination by *Pseudomonas* spp. and survival of enterococci supports their recommendation as additional indicator bacteria for plant hygiene, product quality, spoilage and possibly safety of High-moisture raw milk mozzarella cheese (RMMC). Thus, *Enterococcus* spp. might be used as the indicator of rot in soft cheese (Meier et al. 2018).

Importance of Enterococci in Terms of Public Health

The use of enterococci in food production sector shows that these bacteria are the part of human nutrition i.e., the body and brain health. However, enterococci have been the focus of debates in medical area lately, due to the hospital infections they have caused. These microorganisms have developed high resistance against reserve medications including vancomycin and teicoplanin that are used increasingly in clinical applications and against all other beta-lactam group antibiotics and they have been started to be isolated in hospital infections where the treatment failure was observed among patients whose immune system has been suppressed (De Fátima Silva Lopes et al. 2005; Franz et al. 2011). Another example of mechanism two may be VRE; The Enterococci, which normally colonize in the gut, might have acquired resistance to multiple antibiotics over time, making the glycopeptide vancomycin which is one of the last therapeutic alternatives.

The epidemiology of VRE shows some differences essentially between the USA and Europe. In Europe, *Enterococcus faecium* carrying the vanA resistance element (which was transferable to other bacteria) and vancomycin resistance was commonly found in the intestinal flora of farm animals as well as healthy people, but carriage of VRE in farm animals and healthy people were absent in the USA (Bonten et al. 2001). This difference has been proposed to be due to the wide spread agricultural use of avoparcin; a glycopeptide used in Europe since the 1970s, but was never approved for use in the USA. Avoparcin, which confers cross-resistance to vancomycin, has been shown to select for VRE in animals (Aarestrup et al. 1996). A large reservoir of VRE in animals presents conveniences for human infection, and the potential for resistant bacteria to colonize the human digestive tract. In addition, molecular epidemiologic studies have found that the VRE strains isolated from animals and humans are difficult to tell apart, as are the resistance elements (Woodford 1998; Jensen et al. 1998); hence at least the potential for transmission exists.

Enterococci *in Hospital Infections*

In many cases, isolation of enterococci from hospital infections in recent years has brought them into focus invariably medical area. The *Enterococcus* spp. strains causing hospital infections are isolated from hands of healthcare personnel and nursing homes and from the surfaces around the hospital. Enterococci species transported from one patient to another or even from one department in the same hospital to the other either, due to contaminated hands of health care workers or materials such as the shelves, sheets, and bedsteads may cause infections which are so difficult to treat. Particularly in patients being monitored in intensive care units, enterococci may usually cause endogenous or exogenous origin bacteriemia and endocarditis, urinary system infections, intra-abdominal infections, surgical wound infections, perinatal infections and albeit seen less meningitis and pneumonia (Chenoweth and Schaberg 1990; Morrison et al. 1997; Franz et al. 1999; Tenover et al. 2004). According to some studies, 60% of enterococcal infections are hospital originated and half of them are adhered to intensive care units. Enterococci strains were isolated in about 30% of patients in intensive care units in the USA. Commonly, *E. fecalis* is responsible for 80–90% of *Enterococcus* spp. infections, while *E. faecium* is responsible for 5–10% of them. However, the ability of *E. faecium* acquiring more easily the resistance to a broad range of antimicrobials, largely glycopeptide group antibiotics, changes the balance between hospital infections and the bacteria in favor of *E. faecium* species (Murrey 2000; Freitas et al. 2009).

Vancomycin or glycopeptide group antibiotics are preferred in treatment for difficult-to-treat and resistant Gram-positive bacteria infections, particularly in methicillin-resistant *Staphylococcus aureus* infections. Additionally, arising resistance of staphylococci and enterococci which may cause heavy infections to some antibiotics in this group was noticed in the 1980s. Similarly, there has been an increase in the incidence of VRE infections in big hospitals where patients under risk are accepted. This has aroused a series of such problems as the treatment of *Enterococcus* spp. infections and the enterococci's transmitting horizontally these resistance determinants to other vancomycin sensitive species (Pearson 2002; Hasman et al. 2005; Upadhyaya et al. 2009). Some studies in the USA have shown that the horizontal spread of *vanA* gene from VRE in patients in the hospital into Meticillin-Resistant *Staphylococcus aureus* (MRSA) has resulted in high vancomycin resistance among MRSA strains (Chang et al. 2003; Weigel et al. 2003).

The increase in the asymptomatic colonization rate of VRE/GRE strains in the community, both the exogenous and endogenous origin infections raise the importance of these bacteria in terms of hospital settings. Using of invasive instruments for treatment or diagnostics, use of antibiotics, the duration of staying in hospitals, previous stays in risky units, such as the intensive care units are vital in the increase in the prevalence of hospital origin VRE/GRE colonization. However, the importance of the strains acquired in community origin resistant *Enterococcus* spp. colonization and infections through food must not be ignored (Maschieto et al. 2004; De Fátima Silva Lopes et al. 2005; Valenzuela et al. 2009).

Antibiotic Resistance Mechanisms of Enterococci

Antibiotic resistance of *E. faecium* strains is crucial factor of their pathogenicity (Franz et al. 2001). In *E. faecium* species, that most of the virulence factors and resistance genes are encoded on a plasmid that is clear explanation of this relationship (Lukasova and Sustackova 2003). In resistant strains, the colonization of intestine is facilitated and tissue invasion is carried with the help of factors such as cytotoxin and gelatinase encoded in resistance plasmid (Aktaş and Derbentli 2009). Besides, their virulence increases because of their resistance mechanisms.

There are two types of antibiotic resistance can be improved in these bacteria; natural and acquired types (Giraffa 2002; Upadhyaya et al. 2009).

The mechanism of resistance to antibiotics in enterococcal bacteria can be analyzed in two main groups:

1. Natural (intrinsic-chromosomal) Resistance,
2. Acquired (extrinsic) Resistance.

Natural Resistance

Natural resistance of enterococci is based on species-specific. It refers to the chromosomal resistance observed in all the enterococcal species such as they are inherently resistant to penicillins, cephalosporins, lincosamides, trimethoprim-sulfamethoxazole, and aminoglycosides, including low-level resistance to quinupristin/dalfopristin. *E. gallinarum* and *E. casseliflavus* strains naturally have low-level resistance to vancomycin (Klare et al. 2003).

Acquired Resistance

The mechanism of acquired resistance, like DNA mutations or transposon and plasmid or pathogenicity islands, adapts as a result of the transfer of genome of a new DNA segment. It is the most frequent mechanism of conjugation (Murray 1998).

Despite the fact that acquired resistance in enterococci is usually found in *E. faecium* and *E. faecalis strains*, *E. avium*, *E. durans*, and other enterococcus species can also be observed having this trait. In enterococci, this resistance is encoded with *vanA*, *vanB*, *vanC*, *vanD*, *vane* and *vanG* genes and is identified with the name of the related gene. It is reported that *vanA* and *vanB* type resistance genes were firstly identified in *E. faecium* and *E. faecalis*strains (Murray 1998; Çetinkaya et al. 2000; Aktaş and Derbentli 2009).

Vancomycin/Glycopeptide Resistance

Glycopeptide antibiotics are generally prefered against nosocomial Gram-positive aerobic and anaerobic pathogens as a "last resort antibiotics" in clinical area (Morrison et al. 1997).In the mechanism of glycopeptides briefly: These antibiotics binding to "D-alany-D-alanine" dipeptide locating at the end of the pentapeptide chain linking to muramic acid in N-acetyl glucosamine-N acetyl muramic acid disaccharide forming the backbone of peptidoglycan synthesis impede the process of the glucose transglucolisation and transpeptidation required for peptidoglycan synthesis. Thus, bacterial cell wall undergoes lysis because of not being synthesized. The binding ability of glycopeptides is decreased by placing 'D-Ala-D-Lactate' or 'D-Ala-D-Serine'and ligase enzyme in peptidoglycan side chain bacteria, instead of putting 'D-Ala-D-Ala' (Çetinkaya et al. 2000; Aktaş and Derbentli 2009).

Phenotypes of Vancomycin Resistance

Inducible high-level vancomycin (MIC ≥ 64 μg/mL) and teicoplanin resistance (MIC ≥ 16 μg/mL) are defined in VanA type resistance (Murray 1998) *VanA* gene is substantially detected in *E. faecium* species. However, it is especially found in *E. faecalis*, and then *in E. durans*, *E. avium*, *E. mundtii*, *E. gallinarum*, *E. casseliflavus*, *E. raffinosus* and some species not belonging to enterococci. VanA type resistance, providing vancomycin and teicoplanin resistance among food-originated VRE, is the most common in the world wide (Linden 2007).

A moderate level inducible resistance is VanB type resistance [Vancomycin MIC ≥ 32–64 μg/mL (4–1000 μg/mL)] and it does not lead to any resistance to teicoplanin (Murray 1998; Çetinkaya et al. 2000).

VanC type resistance which is identified in both *E. casseliflavus* and *E. gallinarum* and intrinsic low-level resistance to vancomycin (MIC ≥ 4–32 μg/mL) and teicoplanin susceptibility are defined (Murray 1998; Çetinkaya et al. 2000)

VanD type resistance is recently devoloped resistance which is defined in *E. faecium* strains. *VanD* gene has shown a chromosomal localization and is known that it is not transferred due to conjugation. The strains harboring VanD type strains are resistant to both vancomycin (MIC = 64–256 μg/mL) and teicoplanin (MIC = 4–32 μg/mL).

VanE and VanG phenotypes show similar to VanC phenotypes and are only reported in *E. faecalis* strains (Aktaş and Derbentli 2009).

Pathogenicity and Virulence Factors of Enterococci

Virulence genes in enterococci are encoded in Pathogenicity Island (PAI) occupying genome and/or in plasmids. A pathogenicity island of enterococci for the first time was described in MDR *E. faecalis* in 1980 which causes hospital infection. The magnitude of this PAI whose G + C ratio is 32.2%; is about 150 kb and it encodes 129 Open Reading Frame (ORF) (Tendolkar et al. 2003; Shankar et al. 2002; Upadhyaya et al. 2009).

Alike other pathogenic bacteria, enterococci having a similar number of virulence genes and they easily develop resistance against antimicrobial agents and inhabit endogenous flora, which endorses them to become a considerable opportunistic pathogen. Enterococci carry virulence factors, such as adhesin-like antigen A and enterococcal surface protein (esp) in aggregation agents (agg), gelatinase (Gel), hemolysins, collagen adhesin (as), *E. faecalis* and *E. faecium* strains which both facilitate and also damage the colonization in the host tissue (Eaton and Gasson 2001; Franz et al. 2001; Mannu et al. 2003). *Enterococcus* spp. included the variations in the distribution of virulence genes which was reported in the molecular-based studies. In a study, it was found that spread and adhesion of *E. faecalis* and virulence genes such as cytolysinand pheromone production mechanisms were detected in higher rates than *in E. faecium* strains. It was also shown that these genes were encoded at a higher rate in clinical *E. faecalis* species than in food-originated species (Eaton and Gasson 2001). In a study, virulence factors, antibiotic resistance, bacteriocin production and properties of bile hydrolysis in enterococci isolated from raw and pasteurized milk, meat products, cheese and vegetable in Brazil being analyzed, it was found that 67.7% of the isolates hydrolyzed bile salt, 15.2% of them produced bacteriocins, 12.0% were β-hemolytic and 18.2% of them produced gelatinase, but it was failed to show antibiotic resistance (Gomes et al. 2008). However, it was claimed that virulence genes, encoded in plasmids like hemolysin-cytolysin production, adhesion ability and resistance to antibiotics, were transferred by conjugation pathway with resistance genes in enterococci, so, it was claimed that virulence genes were associated with antibiotic resistance in intestinal flora, environment, food and between strains and species (Eaton and Gasson 2001; Tansuphasiri et al. 2006).

The Prominent Virulence Factors of Enterococci

- Enterococcal Surface Protein (Esp),
- Aggregation agent,
- Microbial surface protein component which describes adhesive matrix molecules of enterococci induced collagen (MSCRAMM Ace).
- Capsules, cell-wall polysaccharides
- Lipoteichoic acid
- Superoxide

- Gelatinase,
- Seven to eight amino acids in length, small hydrophobic peptides identified as sex pheromone and encoded in the chromosome
- Hyaluronidase
- Haemolysin or cytolysin,
- *Enterococcus faecalis* antigens A (Efa)
- AS-48 (Devriese et al. 2006; Upadhyaya et al. 2009; Franz et al. 2011).

Antibiotic Resistance of Foodborne Enterococci

Numerous studies conducted in Europe, it is implied that VRE colonization frequently appears in society, and causes community-acquired asymptomatic VRE colonization due to colonizing in various animals, raw or cooked food and environmental samples and infecting people through contact. Namely, VRE in the food chain is the most critical reason for human gastric colonization (Giraffa 2002; Valenzuela et al. 2009). This view is supported by isolating increasing level *E. faecium* from clinical specimens and presenting sign for food chain whose importance is increasing in incidence, and by associating this species with the ability to live at high temperatures in beef, poultry, pork and other meat products (Wegener et al. 1997; Son et al. 1999; Gambrotto et al. 2001). In a study conducted with meat and chicken products in Spain, enterococcal strains were found in 73% of specimens and it was detected that isolates were resistant to one antibiotic at least or more than one antibiotic such as tetracycline, erythromycin, and vancomycin. Similar results were reported for 90% of pork in Sweden, 55% of chicken and 14% of pork in Denmark (Teuber et al. 1996; Guerrero-Ramos et al. 2016).

AR *E. faecium* strains are shown in dairy products, peculiarly in industrial cheese (Teuber et al. 1999; Giraffa 2002). In some studies, with cheese produced in Europe, *E. faecalis* and *E. faecium* species, resistant to penicillin, tetracycline, chloramphenicol, erythromycin, gentamicin, lincomycin, rifampin, fusidic and vancomycin, in different amounts were isolated (Teuber et al. 1996). Multidrug-resistant enterococcal species were found in both pasteurized cheese and raw milk. ARE's being found in these last products is considered as a serious risk in terms of insertion of antibiotic resistance into the food chain. In some studies, enterococcal species, high level resistant to kanamycin and gentamicin were isolated from French cheese produced from raw milk and patients in hospitals (Bertrand et al. 2000). A similar study conducted in Turkey reported that antibiotic resistance phenotype of 213 *Enterococcus* spp. isolated from traditional Turkish cheese was investigated and kanamycin and gentamicin resistance was found 98.6%, 4.2% respectively (Sanlibaba and Senturk 2018).

VRE Infections and Food Chain

Several works of literature implied that Antibiotic Resistant Enterococci (AREs) and especially VREs which entered into the food chain has gained importance with the increasing significance of VREs in hospital infections. A study conducted in Portugal reported high amounts of AREs in commercial chicken samples (Novais et al. 2005). A similar study conducted in Iran reported that thirty samples of meat, chicken, and cheese were examined for VRE during 2010. Traditional and molecular identification tests showed that all the isolates were *E. faecium* carrying vanA. None of the isolates harbored vanB. The results showed that enterococci are common contaminants in food. Indeed, this study indicates a high prevalence of multidrug-resistant enterococci in the food of animal origin in Iran (Talebi et al. 2015). Another study reported that a total of 160 samples of poultry (80), pork (40), and beef (40) products were evaluated in northwestern Spain in order to find out the prevalence of vancomycin-resistant enterococci (VRE). VRE was detected in 38 (23.8%) samples 37.5% of poultry, 15.0% of pork, and 5.0% of beef samples. It was reported that resistance or intermediate susceptibility to three or more antimicrobials of clinical significance, in addition to vancomycin. Besides, they reported that *vanA*, *vanB*, *vanC*-1, and *vanC*-2/3 genes were identified in various isolates. As a result, they reported that meat products might play a role in the spread through the food chain of VRE including several resistance and virulence genes (Guerrero-Ramos et al. 2016).

The relationship of foodborne enterococci with clinical infections has not been clearly elucidated yet. But, it is argued that foodborne enterococci, with horizontal gene transfer, may play a role in the expansion of virulence genes. Molecular-based studies show that clinical based *E. faecalis* strains had more virulence factors than food originated strains. Moreover, similar studies identified that virulence genes could be transmitted to starter cultures due to clinical type conjugation (Giraffa 2002; Valenzuela et al. 2009). A study conducted in Italy and investigated *Enterococcus* strains isolated from pork meat and stool samples and found, using PCR-RFLP and series analysis methods, that tetracycline resistance gene *tet*(M) and the presence of Tn916 transposon were correlated in all strains; it is emphasized that *Enterococcus* in the food chain can be important in transmitting antibiotic resistance. In a study conducted in Switzerland, Rizzotti et al. (2009) compared food and clinical enterococci with Pulsed Field Gel Electrophoresis (PFGE) method, found a genetically very strong relationship between the isolates, and emphasized that these isolates could spread around through food chain (Giraffa 2002; Leavis et al. 2006; Templer et al. 2008).

Various studies in Europe and the USA have studied the prevalence and epidemiology of VRE, particularly the place of VREs in infection epidemiology, causes, and identification of the risk factors. However, in the Middle East and Asia have less information about this subject (Askarian et al. 2008; Salem-Bekhit et al. 2012).

European countries and the USA show some differences in terms of VREs epidemiology. VRE was usually isolated from food animals in Europe, which was

related to the massive use of "avoparcin" as a growth promoter factor in animal feed in the agriculture sector. If food animals fed with this feed could be play as reservoir role of transferable vanA type resistance. So, VRE was found in farm animal droppings, in raw chicken meat, in fertilizer samples of pigs and poultry, and in wastewater reservoirs in the feeding area. Therefore, using of "avoparcin" was banned in the agriculture sector in 1997 (Bonten et al. 2001). On the other hand, since "avoparcin" was not used in the USA, VRE could not be isolated in animals and healthy humans. However, hospital-acquired VRE infections are shown more common in the USA than Europe (Vehreschild et al. 2018).

Identification of antibiotic resistance and pathogenicity of foodborne enterococci has become much important in the food industry. On this subject, studies conducted in recent years have dealt with these bacteria which can be found in food for a variety of reasons and/or used as starter culture; particularly their antibiotic or glycopeptide/vancomycin resistance, virulence factors and genetic similarities have been subject to much important. Numerious studies reported that some regional differences in the antibiotic resistance and virulence factors of foodborne enterococci. While some studies reported few or no vancomycin and antibiotics resistance, some other studies reported highly resistant strains. For example; a study pointed out that *E. faecium* and *E. faecalis* strains isolated from cheese did not have resistance to vancomycin (Giraffa and Sısto 1997). On the other hand, some studies investigated the presence of VRE in 101 chickens, porks, and turkey meat obtained from 18 different supermarkets and chicken droppings obtained from 50 slaughterhouses. Results implied that VRE was detected in 27.2% of the chicken samples and 16% of the chicken droppings. 11 of the VRE were identified as *E. durans*, 10 of them as *E. faecalis*, and 10 of them as *E. faecium.* Another study which searched a total of 148 *Enterococcus* species resistant to vancomycin in pasteurized meat products and fermented sausages and found that 143 *E. faecium* strains persisted in all meat products, *Enterococcus* species isolated from sausage and raw milk were resistant to tetracycline, chloramphenicol, gentamycin, and erythromycin. Another study reported that *E. faecium* strains isolated from "Dominati" cheese produced in Egypt were found to be resistant to oxacillin, cephazolin and sensitive to ampicillin, amoxicillin, and cefaclor. A similar study examined the presence of hemolysin production and resistance to vancomycin of *Enterococcus* spp. which isolated from 20 sausages and 30 cheese samples and found that there was hemolysin production in *E. durans* strains obtained from cheese and sausage samples but hemolysin production was not found in other strains. Various studies indicate that food of animal origin *Enterococcus* spp. and environment and water originated *Enterococcus* spp. were found in high quantities and they had Multiple Drug Resistance (MDR). It is emphasized that this case can play a potential role in the entrance of antibiotic-resistant bacteria and resistance genes into the food chain as well as the environment. It can also emerge as a potential public-health problem in the future. For instance; a study conducted in Hungary, it was reported that there was VRE in farms even eight years after avoparcin was banned. This finding indicates that VRE can play a reservoir role in its spread, and the technology used in farms in Hungary should be changed so that high rates of VRE can be prevented (Eaton and Gasson

2001; Giraffa 2002; Lukasova and Sustackova 2003; Hauben 2003; Çıtak et al. 2004; Karakaş 2005; Tansuphasiri et al. 2006; Hummel et al. 2007; Ghidan et al. 2008).

A similar study conducted in Turkey that Gram-positive cocciwere isolated from 50 chicken meats and their antibiotic resistance was analysed, it was showed that 50% of the enterococci strains were resistant to vancomycin. Another study which evaluated antibiotic resistance of *Enterococcus* strains isolated from meat and dairy products produced in Çukurova region of Turkey showed that all *Enterococcus* strains were found resistant to vancomycin (Yurdakul et al. 2009; Erginkaya et al. 2010).

The main question of whether foodborne enterococci are reservoirs in the antibiotic resistance and the transmission of virulence genes has made it compulsory the examination of virulence genes and antibiotics resistance of both foodborne and clinical enterococci. Various studies reported the virulence factors of *Enterococcus* spp. strains isolated from food and clinical specimens; it was found that all of *E. faecalis* strains carried virulence factors, but clinical isolates had more virulence factors when compared to the isolates that are used as a starter in foods; no virulence factors were detected in *E. faecium* strains. Another study conducted in Italy shows that the types have the ability to change genetic features through conjugation and *Enterococcus* species isolated from meat products such as salami and raw meat transfer antibiotic resistance determinants to *E. faecalis* JH2–2 species (Cocconcelli et al. 2003; Hugas et al. 2003). Another study conducted in Italy examined bacteriocin, cytolysin, hemolysin and gelatinase production of food, animal and clinic-related VRE. Although no prevalence was given, the necessity of testing antibiotic resistance and virulence features of *Enterococcus* spp. species that will be used as a starter and probiotics in the food industry has been concentrated on (Hummel et al. 2007; Sabia et al. 2008).

Preventive Strategies for VRE/ARB/MDR Bacteria Dissemination

The strategies of prevention/reduction of VRE/ARB/MDR bacteria are based on different sectors. These are clinical/medical settings and all partners of antimicrobial agent production industry, veterinary medicine, food industry as well as international and/national authorities/legislation. Some of these proactive measures are summarized below:

Implementation of Antimicrobial Stewardship Programs (ASPs) in Clinical Settings

Multidisciplinary antimicrobial utilization teams have adequate experience in their fields, including physicians, pharmacists, microbiologists, epidemiologists as well as infectious disease specialists in order to optimize antimicrobial therapy used ASPs. These programs are generally based on education, coupled with the "front-end" treatment (e.g., limiting the availability of chosen antimicrobials) or the "back-end" treatment (e.g., the criticizing of broad-spectrum empirical therapy and then predisposing or stopping therapy based on antimicrobial susceptibility testing (AST) results and clinical reply) (Moehring and Anderson 2012; Paterson 2006). ASPs have also shown a link between antimicrobial usage and the emergence of resistance such as vancomycin using and vancomycin-resistant enterococci (Harbarth et al. 2002). In 2007, the guidelines for ASPs had been suggested by The Infection Diseases Society of America (IDSA) and the Society of Healthcare Epidemiology of America (SHEA) (Dellit et al. 2007). According to various studies, ASPs have the potential to limit the emergence and spread of antibiotic resistance (Drew 2009). ASPs are included such as Antimicrobial Susceptibility Testing (AST), fast and accurate microorganism-identification, development of microbiota, education of all parts of ASPs members related to their routine treatment decisions, appropriate hospital disinfection and personal hygiene of healthcare workers (Lee et al. 2013).

The Development of Novel Antibiotics

In order to discover new classes of antibiotics, novel strategies for rational design and screening-based approaches are necessary. New strategies should be presented for the treatment of microbial diseases, such as host defense peptides, bacteriophages, vaccines, immunoglobulins, and probiotics instead of conventional antimicrobial agents (Lloyd 2012).

Veterinary Medicine

If the problem of antibiotic resistance in human medicine is wanted to solve, the first step will be a reduction of antibiotic usage in veterinary medicine, agriculture, and aquaculture. According to various studies, antibiotic-resistant bacteria/genes may be generally transferred to humans by food animals/meat products. So, to prevent the emergence and transfer of antibiotic resistance in food animals, new methods to manage infectious diseases in animal husbandry are extremely essential such as suitable use of existing vaccines, using enzymes, probiotics, prebiotics, and some

organic acids to improve their health (Castanon 2007; Potter et al. 2008; Callaway et al. 2008). In addition, developing hygiene practices in the production steps of food animals, and making use of bacteriocins, antimicrobial peptides, and bacteriophages can be used instead of antibiotics to support growth in food animals. Besides, infectious diseases may also be decreased in them (Joerger 2003; Boklund et al. 2004; Atterbury 2009). Additionally, internationally acceptable standard protocols should be prepared for the use of antibiotics in animal husbandry and about surveillance programs in order to monitor the global emergence of VRE/ARB/MDR bacteria.

Food Industry

Appropriate starter/co-culture selection is very important in the food industry. For example; the usage of enterococci needs to be safe in meat and dairy products, fermented vegetable products as well as probiotic products. Considering the safety, and according to the Qualified Presumption of Safety (QPS) list from the European Food Safety Authority (EFSA), *Enterococcus* spp. is not recommended for the QPS list (EFSA Panel on Biological Hazards et al. 2017). Moreover, these bacteria don't have GRAS status (Ogier and Serror 2008). Lately, some global organizations such as the EFSA, the Advisory Committee on Novel Foods and Processes, (ACNFP), and the Food Standards Agency permitted the use of certain strains of enterococci as food additives and supplements based on a careful case-by-case assessment. Thus, the individual strain must be considered and health risks must be removed for this certain strain (ACNFP 1996; Franz et al. 2011; EFSA 2012). In order to distinguish between safe and potentially harmful strains of *E. faecium* in animal nutrition, a system of methods is also be provided by EFSA guidance (EFSA 2012). It is intended for use as feed additive producers submitting applications to EFSA for safety evaluation.

Conclusion and Recommendation

Antibiotic resistance in bacteria has become one of the most important problems in worldwide. On the other hand, there is a very complex relationship between antimicrobial usage and antimicrobial resistance for many types of pathogen microorganisms and needs more multidisciplinary studies in order to solve this problem.

The use of antimicrobials on farms is linked to (ARG) Antibiotic Resistant Gene emergence but whether food animals are the sources of ARG transfer to human pathogens are the main question and debated human and veterinary medicine researchers as well as public health authorities. Because, ARG exposure is both food safety and public health problem and environmental exposure through air, soil, and water. Furthermore, it is important to establish a diagnostic standard such as

whole genome sequencing for ARG detection such as PFGE which is considered "Gold Standards" for assessing isolate interrelationships and Multilocus Sequence Typing (MLST) methods.

In general, some methods may be used to control antimicrobial resistance in human medicine, veterinary/agricultural area and food industry:

1. Antimicrobial usage should be reduced in both human and veterinary medicine and the use of broad-spectrum antimicrobials which are clinically important should be limited. Alternatives to AGPs, such as good farm practices and use of probiotics, prebiotics, and natural antimicrobial agents must be encouraged in the veterinary sector.
2. Forbidding the dumping of antimicrobial agents to waste into the environment and removing antimicrobial residues that exceed the standard limit in food and food products and water should happen by proper legislation.
3. To limit inappropriate administration of antibiotics, regular consultation with veterinarians can take place. Thus, the awareness/education of food/animal producers is so important.
4. The awareness of farmers regarding the implications of the unnecessary use of antibiotics in food animals has on human health and the environment is also very important, Therefore, orderly education programmes are needed for them. Foodborne enterococci (starter/co-cultures) standards with the view of food security about these subject; food-based enterococci do not have to be genetically related to strains making infection, does not have to include virulence genes which are related to antimicrobial resistant genes. Thus, proper starter culture selection in the food industry and implication of standards about this subject related to international legislation is so important.

The last but not least, in order to solve this problem is needed the cooperation from all sectors that use antimicrobials to control antimicrobial use effectively and to limit the dissemination of antimicrobial-resistant bacteria in the environment (nationally and internationally area).

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