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Enterococcal strains are important in food and clinical microbiology. The most important factors for the evaluation of enterococcal strains are their resistance to antibiotics and the presence of virulence factors. Hence, they exhibit significant resistance to wide ranging antimicrobial agents. Considerable effort have been committed to comprehending the molecular mechanisms of enterococci resistance. They exhibit intrinsic as well as acquired resistance to most antibiotics used in humans. In addition, they have been found to exhibit increasingly multi-drug resistance in recent years. Besides antibiotic resistance, *Enterococcus* spp. are able to produce potential virulence factors. These include cytolysin (*Cyl*), aggregation substance (*asa1*), enterococcal surface protein (*esp*), hyaluronidase (*hyl*), and gelatinase (*gelE*). Virulence genes are related to pathogenicity of enterococci in the human and animal models. A recognized source of antimicrobial resistance in enterococci is the food chain. Hence, this review focuses on the antibiotic resistance and virulence factors of *Enterococcus* species.

Keywords: *Enterococcus*, antibiotic resistance, virulence factor

1. Introduction

Enterococci are important members of lactic acid bacteria (LAB) found in a different foods and dairy products, plants and water surface. Naturally, they inhabit the gastrointestinal tract of humans and animals [1, 2]. So far, 55 species, and 2 subtypes have been reported based on 16S rDNA sequences [3].

Enterococci, mainly *Enterococcus faecalis* and *Enterococcus faecium,* have been recognized as leading causes of hospital-acquired infections (nosocomial infections) such as endocarditis, bacteraemia and urinary tract infections. As nosocomial pathogens, enterococci cause mortality in 61% of cases [4]. In immuno-compromised patients or those with severe underlying diseases, *E. faecalis* is the most implicated being responsible for about 80-90% of infections, followed by *E. faecium* [1, 5]. However, cases of infection caused by other enterococcal species are quite rare [2]. Like other LABs, some enterococcal strains, especially *E. faecium* also play a beneficial role in food maturation processes as starter or probiotic cultures [6, 7]. Enterococcal strains exhibit numerous biochemical and biotechnological properties such as, enzymatic activities (proteolytic, lipolytic and esterolytic) and citrate utilization [1, 7]. In addition, some enterococci, mainly *E. faecalis* and *E. faecium*, produce inhibitory substances (lactic acid, hydrogen peroxide, and bacteriocins) which have the ability to inhibit the growth of food pathogens and spoilage microorganisms [8]. Contrary to their relevance in the food industry, they are not generally recognized as safe (GRAS) as their presence is often an indicator of faecal contamination [1, 9]. To differentiate between apparently safe and non-safe enterococcal strains is difficult because virulence genes can be easily exchanged between strains [10]. However, there have been no conclusive determination that enterococci isolated from food directly cause clinical infections [2].

About 50 years ago, antibiotics were introduced for the treatment of microbial diseases. However, since that time, the greatest risk to the use of antibiotics for the management of bacterial and other microbial infections remains the development of antimicrobial resistance. Meanwhile, the food chain can be considered as the major route of transmission of antibiotic resistant bacteria between animal and human populations [11]. Enterococci are resistant to a wide variety of antibiotics with isolates showing resistance to one or more antibiotics [12].

The presence of antibiotic resistance and virulence factors of enterococci in foods from animal origin have been reported by several authors including Jamet et al. [1], Jurkovic et al. [13], Bulajic et al. [14], Vrabec et al. [15] and Yılmaz et al. [16] to pose potential risks of transmission to humans.

2. Antibiotic Resistance of Enterococci

Globally, enterococci have become important pathogens, especially with respect to nosocomial infections, particularly due to their antibiotics resistance ability. Antibiotics are usually used as therapeutic agents against bacterial infections for the treatment of clinical diseases [17]. Nowadays, these organisms have been subjected to different antibiotics in hospital settings [18]. A major factor that contributes to antimicrobial resistance in *Enterococcus* species includes the environment such as the intestinal tract of animals or foods from animal sources. Moreover, humans may become infected either by ingesting contaminated food or from the environment [19]. Different studies have identified enterococcal antimicrobial resistance in environmental, food and clinical isolates [7, 12, 14, 16, 20, 21, 22]. The climbing occurrence of antibiotic resistant *Enterococcus* spp. is the result of increased antibiotics use in health care systems and animal growth promoters [23]. Enterococci are naturally resistant to numerous antimicrobial agents, and possess the ability to transfer antibiotic resistance to other strains through plasmids, transposons, chromosomal

exchange or mutations [24]. Bacteria can, in addition to their natural resistance, develop resistance to antibiotics via several mechanisms; i) minimization of intracellular concentrations of antibiotics as a result of poor penetration into the bacterium or of antibiotic efflux, ii) modification of the antibiotic target due to genetic mutation or post-translational modification of the target, and iii) inactivation of the antibiotic by hydrolysis (Table 1) [25, 26]. The ability of enterococcal strains to transfer antibiotic resistance poses a major challenge to the development of effective antimicrobial therapy in humans [20]. In addition, enterococci are able to exchange antibiotic resistance genes in different environments within a broad-range of other bacteria, including pathogens such as *Staphylococcus aureus* and *Listeria* spp., and non-pathogenic species [1, 27]. The pathogenicity of a strain is not soley indicated by the existence of antibiotic resistance genes but in a combination with virulence factors which may cause the strain to become more dangerous. Particularly, pathogenicity occurs because genes that confer resistance to antibiotics and virulence factors are often situated on the same mobile genetic elements [28]. In comparison with clinical isolates, enterococcal isolates of non-clinical origin show a lower incidence of antibiotic resistance [29].

Table 1 Intrinsic and Acquired mechanisms in Enterococci

Intrinsic Resistance	Acquired Resistance
β-lactams (particularly cephalosporins)	High concentrations of β-lactams
Low concentrations of aminoglycosides	High concentrations of aminoglycosides
Clindamycin	Glycopeptides(vancomycin and teicoplanin)
Fluoroquinolones	Tetracycline
Trimethorprim-sulfamethoxazole (*in vivo*)	Erythromycin
	Fluoroquinolones
	Rifampin
	Chloramphenicol

Source: Gold [30]

Enterococci have also been defined as increasingly resistant to multi-drug (MDR) antibiotics in recent years [31]. MDR enterococci exhibiting high level resistance to penicilin, glycopeptides, fluoroquinolones and aminoglycosides have become known as major causes of nosocomial infections [32]. There are at least three major reasons for the emergence of MDR enterococci: i) baseline intrinsic resistance to several antimicrobial agents, ii) acquired resistance via mobility of the resistance genes on plasmids and transposons, and chromosomal exchange, and iii) the transferability of resistance [33]. Table 2 shows MDR levels of enterococci on country basis.

Table 2 Multidrug Resistance of Enterococci

Multidrug Resistance (%)	Country	Reference
11.5	Italy	[27]
20.27	Slovakia	[15]
64.29	Malaysia	[21]
24.59	Serbia	[14]
59.0	Canada	[34]
70.90	Turkey	[22]
78.0	Egypt	[5]
83.0	Spain	[35]

2.1 Beta-lactam Antibiotics

The basic targets of β-lactam agents are penicillin binding proteins (PBPs). PBPs interact with β-lactam ring, hence are not available for new peptidoglycan production. The disruption in peptidoglycan synthesis leads to bacterial lysis [36]. Usually, *Enterococcus* species exhibit low intrinsic resistance to β-lactam antibiotics such as piperacilin, penicillin, imipenem and ampicillin which exerts a bacteriostatic effect. On the susceptibility/sensitivity of Enterococcus species to β-lactams, *E. faecalis* is about 10 to 100 times less sensitive to penicilin while *E. faecium* shows susceptibility of at least 4 to 16 times less than *E. faecalis* [37]. The most active β-lactams are ampicillin and penicillin. Typically, enterococci, like most bacteria synthesize about 5 PBPs which were initially named by convention on the order of migration. Following genome analysis, *E. faecalis* and *E. faecium* showed six putative PBP genes; three class A (*ponA, pbpF and pbpZ*) and B (*pbp5, pbpA and pbpB*). There is a correlation between increased PBP synthesis and high resistance (MIC ≥128 μg/mL) to ampicillin. Hence, a higher concentration of antibiotic is required to saturate the active site. A different form of ampicillin resistance in *E. faecalis* and *E. faecium* mediated by a β-lactamase that inactivates the antibiotic through the cleavage of the β-lactam ring [38]. *E. faecium* and *E. faecalis* produce low affinity PBPs (PBP4 and PBP5, respectively) that bind weakly to β-lactam antibiotics. Consequently, for *E. faecalis* and *E. faecium*, the MIC for penicillins are typically between 2–8 and 8–16 mg/mL, respectively. Increased enterococci resistance to penicilins may be developed via the acquisition of β-lactamase or PBP4/5 mutations. First described in 1983 were the plasmid-mediated *bla* genes encoding β-lactamases in *E. faecalis* and since then, enterococcal β-lactamase synthesis has been unusual and mostly described in this species. However, in *E. faecium,* increased penicillin

resistance is predominantly linked with the accumulation of point mutations in the penicillin-binding proteins 5 (PBP5). A variety of point point mutations have been described in both *E. faecium* and *E. faecalis* [39].

2.2 Glycopeptides

Glycopeptides bind to the D-alanyl-D-alanine portion of the peptide side chain of the precursor peptidoglycan subunit, thus inhibiting cell wall synthesis [36]. Glycopeptide-resistant enterococci have been previously described in France and the UK. However, in recent times, there have been incidences in many parts of the world [40]. Resistance to the most active glycopeptides (vancomycin and teicoplanin), as characterised by vancomycin, can be described as high-level (MIC > 64 μg/mL) or low-level (MIC 4 - 32 μg/mL). High-level resistance is dependent on the modification of the terminal penta-peptide to D-Ala-D-Lac via a substitution that removes one of the five hydrogen bonds necessary for the binding of vancomycin to the peptidoglycan chain, thus, reducing its affinity by about 1000-fold. Alternatively, low-level resistance is conferred by the production of D-Ala-D-Ser-ending precursors, which reduces the binding affinity of the antibiotic by about seven-fold. Vancomycin resistance gene clusters (designated *van*) likely originated from the need for survival. Based on phenotypic and genotypic criteria, so far, nine diffferent vancomycin resistance clusters have been described in enterococci; *vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM* and *vanN* [37, 38]; with *vanA,* and *vanB* showing the most resistance prevalence. *E. faecium* is the reservoir of transferable *vanA* and partly *vanB* resistance gene clusters in humans and other organisms [37]. Over the years, *vanA* containing enterococci have been frequently detected in animal and environmental samples as glycopeptides such as avoparcin are widely used as growth promoters around the world. Avoparcin can induce cross-resistance to vancomycin, consequently, its use was banned in Europe and other countries [41, 42]. Some *Enterococcus* strains have the potential to be reservoirs glicopeptides resistance genes and transfer them to more virulent pathogens such as methicillin-resistent *Staphylococcus aureus* [18].

2.3 Inhibitors of 30S Subunit Aminoglycosides

Aminoglycosides are positively-charged molecules that target bacterial ribosome. There is bacterial synergism among aminoglycosides, β-lactam and glycopeptides. Aminoglycosides interact with the 16S r-RNA of the 30S ribosomal A site RNA through hydrogen bonds. They result in misreading and premature termination of mRNA translation [36]. Enterococci display intrinsic tolerance to aminoglycosides. Many clinical isolates of *Enterococcus* species possess the enzyme APH(3′)-IIIa, which confers resistance to kanamycin and amikacin through its phosphotransferase ability. Furthermore, enterococci have the ability to modify the ribosomal target through ribosomal RNA (rRNA) methyltransferase. Gentamycin and streptomycin are the only aminoglycosides used effectively in clinical practice since they are not readily affected by the intrinsic enzymes synthesized by enterococci. Meanwhile, as determined by the agar dilution method, high-level resistance to aminoglycosides (MIC >2000 μg/mL for streptomycin and 500 μg/mL for gentamicin) eliminates the synergistic effect of these compounds [37, 38]. *E. faecium* and *E. faecalis* show intrinsic resistance to clinically achievable concentrations of aminoglycosides. In *E. faecalis*, there is variation in MIC values for aminoglycosides, with the highest values of resistance of about 500 mg/mL observed in streptomycin. Intrinsic resistance in *E. faecalis* is attributed to the inability of aminoglycoside to enter the cell. Higher concentrations of intracellular aminoglycoside were obtained in the presence of pencillin when enterococci were exposed to radiolabeled aminoglycoside with or without penicillin. In addition, cell wall active agents and aminoglycosides combination (aminoglycoside-penicillin combination therapy) also produced bactericidal activity (bactericidal synergism) [39].

2.4 Tetracyclines

In order to inhibit t-RNA binding at the A site of the 30S ribosomal subunit, tetracyclines (minocycline, chlortetracycline or doxycycline) act upon conserved sequences of the 16S r-RNA [36]. Tetracycline resistance is one of the classic examples of efflux-mediated resistance. The Tet efflux pumps extrude tetracyclines using proton exchange as the source of energy. Currently, more than 20 different *tet* genes have been described, most of which are harbored in mobile genetic elements [43]. Tetracycline resistance is mediated by multiple genes. Efflux pumps encoded by *tetK* and *tetL* are plasmid-borne determinants. The genes *tetM, tetO* and *tetS* which confer resistance to minocycline, doxycycline and tetracycline are chromosomal resistance determinants that can be transmitted via the Tn916 transposon [14, 38]. *Enterococcus* species resistance to tetracycline is common in animal and clinical isolates [37].

2.5 Inhibitors of 50S Subunit Chloramphenicol

Chloramphenicol, with its interaction on the conserved sequences of the 23S r-RNA peptidyl transferase activity of the 50S subunit prevents protein synthesis by inhibiting t-RNA binding to the A site of the ribosome [36]. This type of resistance mediate plasmid encoded *cat* genes or efflux mechanisms [42]. Thus chloramphenicol shows bacteriostatic activity against enterococci but has never been considered a “first line” of treating this bacteria [44].

2.6 Macrolides

The early phase of protein synthesis (translocation) is affected by macrolides which target the conserved sequences of peptidyl transferase center at the 23S r-RNA of the 50S ribosomal subunit. This causes the premature detachment of incomplete peptide chains. Macrolides, lincosamides, streptogramins A and streptogramins B show a similar mechanism of action against enterococci [36]. However, enterococci is known to show resistance to these antibiotics with varying structures, that is, macrolides, lincosamides, streptogramins B-resistance phenotype (referred to as the MLSB phenotype). *E. faecium* is susceptible to these antibiotics while *E. faecalis* is resistant. This is because *E. faecalis* expresses a chromosomal gene called *lsa* (that is, lincosamide and streptogramin A resistance) which confers this resistance. The *lsa* gene encodes a putative protein with an ATP-binding cassette (ABC) motif of transporter proteins but not the trans-membrane region that would be expected for an efflux pump [38].

2.7 Oxazolidinones

Linezolid, a member of the new class of completely synthetic antibiotic was recently approved for use. This group of antibiotics disrupts protein synthesis at various stages by: (i) inhibiting protein synthesis with the binding to 23Sr RNA of the 50S subunit, (ii) suppressing 70S inhibition and interacting with peptidyl-t-RNA [36, 38]. Thus, 23S ribosomal subunit mutations confers resistance (8 μg/mL) to linezolid which exhibits high antimicrobial activity against Gram-positive bacteria at 4 μg/mL MIC. Strains that show resistance to linezolid may also exhibit co-resistance to more antibiotics including chloramphenicol, gentamicin vancomycin, fluoroquinolones, macrolides, ampicillin, nitrofurantoin, trimethoprim/sulfomethoxazol and rifampin [37].

2.8 Quinolones

Quinolones show moderate activity against enterococci. In clinical settings, the use of this antibiotic group has resulted in increased enterococci resistance [37]. Fluoroquinolones (FQ) target two of the enzymes responsible for this process; DNA gyrase and topoisomerase IV. Both enzymes are tetramers made up of two different subunits: GyrA and GyrB which form the DNA gyrase complex, while topoisomerase IV comprises ParC and ParE [38]. The DNA gyrase consists of two A and B subunits each. FQs bind to the A subunit of the DNA gyrase at high affinity and interfere with two of its functions, that is, strand cutting and resealing [36]. FQ resistance can take place via three different biochemical routes; i) mutations in genes encoding the target site of FQs (DNA gyrase and topoisomerase IV), ii) over-expression of efflux pumps such as EmeA and EfrAB that extrude the drug from the cell, and iii) protection of the FQ target site by a protein designated Qnr family [43]. Nalidixic acid was the first quinolone derived agent [45]. Enterococci exhibit low levels of intrinsic resistance to quinolones, but through various mechanisms, they can develop high-level resistance. Mutations have been described in the target genes (precisely *gyrA* and *parC*) in *E. faecium* and *E. faecalis* but are not present in *E. gallinarum* and *E. casseliflavus* [38].

2.9 Sulfonamides and Trimethoprim

Each of these antibiotics inhibits specific steps in folic acid metabolism. Folic acid is required to complete a wide range of significant cellular functions such as the synthesis of nucleic acids, specifically thymidine [38]. Sulphonamides and trimethoprim drug combination acts at specific stages in similar biosynthetic pathway that exhibits synergy and decreased mutation rate for resistance. Sulfonamides inhibit dihydropteroate synthase in a competitive manner by exhibiting a higher affinity for the enzyme than the natural substrate, p-amino benzoic acid. On the otherhand, trimethoprim acts at later stages in folic acid synthesis by inhibiting dihydrofolate reductase [36]. Enterococci have the unusual ability to absorb folic acid from the environment by by-passing the effects of trimethoprim-sulfamethoxazole combination. Consequently, *in vitro* evaluation of enterococcal susceptibility to trimethoprim-sulfamethoxazole in a media lacking folate will be positive. Despite the foregoing, the use of trimethoprim-sulfamethoxazole in treating severe *Enterococcus* infections remains ineffective [39].

2.10 Cephalosporin

Enterococci are basically resistant to cephalosporins. Although, this is a well-known characteristic, the molecular basis of this phenotype has not been totally comprehended. However, the general observation is that there is a correlation between natural resistance and decreased binding affinity of cephalosporins for the enterococcal PBPs, particularly Pbp5 [38]. A specific mutation in the *rpoB* gene confers enhanced cephalosporin resistance [37].

2.11 DAP Resistance

DAP, a differerent lipopeptide antibiotic, exhibits anitmicrobial activity by targeting bacterial cell membrane (CM). It is associated with several cationic antimicrobial peptides (CAMPs) synthesized by the natural immune system of eukaryotes. DAP insertion into the CM is dependent on the presence of calcium ions and seemingly, prefers to bind at the division septum. Genome sequence of a clinical strain pair of DAP-resistant *E. faecalis* showed that there are three

genes that confer the resistance phenotype. *E. faecalis* develops various mechanisms to resist DAP. These processes by which changes in the system enable DAP resistance in enterococci are not completely understood hence, further researches are currently underway [38].

2.12 Rifampicin

This antibiotic inhibits mRNA transcription by binding to the β-subunit of enterococcal DNA-dependent RNA polymerase. Rifampicin-resistance is common, despite the fact that it is not generally used to treat enterococcal infections. In the course of treating other bacterial infections with it, commensal microbiota are exposed to this antibiotic hence, resistance develops [37]. Rifampicin resistance results from different mutations in the *rpoB* gene that encodes the β-subunit of the RNA polymerase. Interestingly, a particular mutation in the *rpoB* gene in both *E. faecalis* and *E. faecium* was indicated to improve resistance to broad-spectrum cephalosporins. However, this did not have an effect on other major cell-wall acting antibiotics such as ampicillin and vancomycin. Although, the precise mechanism of Rifampicin-resistance is not understood, it was reversible after incubation with subinhibitory concentrations of daptomycin [38].

3. Virulence Factors of Enterococci

The first investigation of enterococcal virulence was reported in 1899. In 1934, E.W. Todd conducted the first cytolytic or hemolytic study of enterococcal virulence factor [46]. Currently, some *Enterococcus* virulence factors responsible for infections in humans have been characterized [20]. They include: enterococcal surface protein gene (*esp*), aggregation substances (*agg*), cell wall adhesions: *efaAfm* and *efaAfs* by *E. faecium* and *E. faecalis*, respectively, hyaluronidase (*hyl*), gelatinase (*gelE*), collagen adhesion protein (*ace*) and cytolysin (*cyl*) [36, 47, 48]. Current molecular screenings of factors that determine *Enterococcus* virulence showed that medical *E. faecalis* strains had more virulence determinants than the strains found in food [49]. *E. faecalis* strains are also known to contain multiple virulence determinants [29]. It has been shown that several of these enterococcal virulence traits are genetically transmissible with some genes located on specific regions of the genome, specifically in an area called the "pathogenicity islands." [50]. Medical isolates of *Enterococcus* species have been identified to show the highest virulence followed by food isolates and starter strains, respectively. Factors that determine the virulence of enterococcal strains include; i) ability to colonize the gastrointestinal tract, which is its natural habitat, ii) ability to adhere to a range of extracelular matrix proteins, and iii) ability to adhere to human embryo kidney cells, urinary tract and oral cavity epithelia [51].

There are two kinds of enterococcal virulence with the following characteristics: i) surface factors that affect colonisation of host cells, and ii) substances secreted by enterococci, which damage tissues. The ability of enterococci to adhere to their host's tissues, in addition to their resistance to low pH and high concentrations of bile salts contribute to their being among the most common bacteria colonising the colon. Adhesins from *Enterococcus* enable these bacteria to bind to receptors on the mucous membrane or proteins of the extracellular matrix, which enable the colonisation of the epithelia. Clearly, colonisation in itself is not a proof of pathogenicity, but, in combination with other virulence factors and with the presence of a number of resistance genes. Virulence factors that stimulate colonisation include: aggregation substance (AS), collagen-binding protein (Ace), cell wall adhesin (Efa A) and enterococcal surface protein (Esp). Virulence factors prodcued by *Entercoccus spp* include: cytolysin (Cyl), gelatinase (GelE) and hyaluronidase (Hyl) [28].

As previously mentioned, many factors are responsible for the virulence of *Enterococcus* spp. However, some of the factors are explained in the following sections.

3.1 Aggregation Substance

The enterococcal aggregation substance (AS) is a surface protein with a molecular weight of about 137 kDa and a hairpin-like structure. AS includes a range of highly homologous adhesins, encoded on large conjugative plasmids transferred in a supposed facilitated conjugation system, facilitated by sex pheromones. These are short, hydrophobic pepdites that penetrate the AS and interact with a particular conjugative plasmid. 20 pheromone-dependent plasmids have been reported to be found in enterococci. Together with genes that encode AS are those that encode antibiotic resistance. The genes responsible AS protein production are strongly preserved and 90% homologous [28]. The *agg* gene is found only in strains *of E. faecalis* but with high incidence among food isolates [52]. Semedo et al. [53] showed that the *agg* gene was found in all (100%) food and clinical enterococcal isolates. In addition, Toğay et al. [31] indicated that it was found in 88 and 57% of *E. faecium* and in *E. faecalis* isolates, respectively, from food sources. Meanwhile, it was also reported by Medeiros et al. [54] that 18.2 and 57.9% of *E. faecalis* strains from food and clinical isolates, respectively contained the *agg* gene.

3.2 Collagen Binding Protein (Ace)

An additional surface protein having adhesive properties is the accessory colonisation factor (Ace) with a molecular weight of approximately 74 kDa encoded by the *ace* gene. This protein was isolated from *E. faecalis* strains in both healthy and infected carriers of *Enterococcus*. Similar to the AS protein, Ace also plays a significant role in colonisation by adhering to proteins of the extracellular matrix in addition to participating in binding types I and IV collagen. Similar to the structure of Ace in *E. faecalis* is the Acm protein (encoded by the *acm* gene) found in *E. faecium.* It should be noted that the *acm* gene is homologous to *ace* and is also in control of binding to collagen [28]. Ace is structurally and functionally associated with staphylococcal Cna adhesion. Ace was detected in the sera samples of 90% enterococcal endocarditis patients [55]. Medeiros et al. [54] also reported that the *ace* genes were identified in 74.5 and 73.7% of *E. faecalis* strains from food and clinical samples, respectively.

3.3 Endocarditis specific antigen - EfaA

Endocardis antigen (EfaA) is a protein with a molecular weight of about 34 kDa encoded by the *efAfs* and *efAfm* genes in *E. faecalis* and *E. faecium* strains, respectively. The *efaA* gene contains a segment of the *afaCBA* operon responsible for encoding the ABC transporter (permease)which is regulated by magnesium ions. There is homology between the EfaA protein and the adhesins that exist in the cell walls of streptococci. Through genetic methods, it has beeen shown that homologous genes *efaA* are present in strains of *E. avium, E. asini, E. durans* and *E. solitarius* [28]. Trivedi et al. [56] also showed that *efaA* gene (22%) were present in enterococci species from food samples.

3.4 Surface Protein -Esp

First described in a clinical isolate of *E. faecalis*, enterococcal surface protein (Esp) has been identified as the largest enterococcal protein with a molecular weight of approximately 200 kDa [52]. The *esp* gene which encodes this protein and others that determine the active outflow of antibiotics are situated on the pathogenicity island (PAI) which is likely due to horizontal gene transfer between *E. faecalis* and *E. faecium*. There are some structural similarity between Esp protein and other proteins present in Gram-positive bacteria. Esp protein participates in the formation of biofilm which play a significant role in the genetic material exchange between cells and improve their antibiotics resistance. In *E. faecium,* the presence of the *esp* gene is correlated with resistance to ampicillin, ciprofloxacin and imipenem [28]. Compared to commensal isolates, the frequency of *esp* gene coding was higher among clinical isolates [55]. Studies of clinical strains of *E. faecium* have indicated that the *esp* gene is present in 83.3% of its vancomycin-resistant strains, with most of the genes showing multi-drug resistance. Additionally, through the plasmid conjugation mechanism, the *esp* gene can be transferred between strains of *E. faecium* and by chromosome-chromosome transposition mechanism between *E. faecalis* strains [28]. Esp contributes to eukaryotic cell adhesion in the urinary tract and resistance to environmental stresses via enterococcal biofilm formation [51]. Medeiros et al. [54] reported undetected *esp* gene in *E. faecalis* strains present in food samples. Meanwhile, in clinical samples, Arshadi et al. [57] observed a high prevalence (68.4%) of *esp* genes, that is, 31.2, 28.5 and 10% of *E. faecium* strains from the clinical isolates, intestine colonizing and environmental isolates, respectively.

3.5 Cytolysin – Cyl

Also known as hemolysin, cytolysin (a bacteriocin-type exotoxin) is one of the most described enterococci virulence factors. This exotoxin exhibits bacteriocidal characteristics towards Gram-negative bacteria and toxic properties towards erythrocytes, leukocytes and macrophages [28, 53]. The operon containing 8 genes is responsible for cytosine synthesis and are: *cylR1, cylR2, cylLl, cylLs, cylM, cylB, cylA* and *cylI*. The operon is situated on highly conserved pheromone-dependent conjugative plasmids or within the island of pathogenicity in bacterial chromosomes at close proximity to other determinants of virulence such as AS and surface protein Esp. Cytolysin encoding genes have been found in *Enterococcus* strains isolated from both infected and commensal microbiota. A number of reports have indicated their common occurrence in strains isolated from both animal and plant origins. Cytolysin encoding genes in the following *Enterococcus* species: *E. malodoratus, E. faecium, E. cecorum, E. casseliflavus, E. durans, E. faecalis E. gallinarum, E. avium* and *E. raffinosus* [28]. Medeiros et al. [54] detected *cyl* genes in 7.2 and 54.4% of food and clinical enterococcal strains, respectively. Banerjee and Anupurba [58] detected haemolysin gene in 40% of *E. faecalis* and 23.22% of *E. faecium* clinical isolates. Haemoysin production was reported by Manavalan et al. [59] in 18.25% enterococcal strains from clinical sources and by Hemalatha et al. [32] in 15% *E. faecalis* and 1% *E. faecium* also from clinical isolates. Upadhyaya et al. [60] showed that 33% clinical isolates of *E. faecalis* and 19% commensal isolates produced haemolysin. In addition, Arshadi et al. [57] reported that 6.2, 7.1 and 0% of *E. faecium* from clinical, intestine colonizing and environmental isolates, respectively, carried the hemolysin gene.

3.6 Gelatinase - GelE

Gelatinase is an extracellular enzyme characterized by a molecular weight of approximately 30 kDa and is a zinc-dependent metalloendopeptidase capable of hydrolysing haemoglobin, elastin, gelatine, collagen, as well as other bioactive peptides such as protein-binding pheromones. The possible impact of enterococcal metalloendopeptidase to virulence was implied for the first time in 1975 while its purification from *E. faecalis* was first described in 1989 [46]. Gelatinase contains the *gel*E gene and is regulated by the transmembrane protein, FsrB. It is controlled by locus fsr which consists of three genes: *fsrA, fsrB and fsrC*. Inspite of carrying the *gel*E gene, deletions within locus fsr produce mutants that do not synthesize gelatinase. Consequently, gelatinase decreases virulence. It has also been indicated that mutations within the *fsrA, fsrB* or *fsrC* genes reduce biofilm production by 28-32%. The presence of the gelE gene is among the determinants of virulence assayed in enterococci. The gene encoding gelatinase production is found in both clinical and food strains of *E. faecalis* and in individual strains of *E. faecium* [28, 55]. Medeiros et al. [54] reported that *gel*E genes were detected in 78.2 and 77.2% of food and clinical isolates, respectively. Banerjee and Anupurba [58] indicated that in clinical isolates, the *gel*E gene was detected in 9.6% of *E. faecalis* isolates and in 8.3% of *E. faecium* isolates. On the otherhand, Manavalan et al. [59] reported that 19.84% enterococcal strains from clinical sources produced gelatinase. Arbabi et al. [61] revealed that *gel*E gene was present in 2% of the clinical isolates. Upadhyaya et al. [60] reported that compared to 39% gelatinase produced by *E. faecalis* clinical isolates, its commensal isolates produced 31%. Meanwhile, Hemalatha et al. [32] showed that 6% of clinical *E. faecalis* and 4% *E. faecium* produced gelatinase.

3.7 Hyaluronidase - Hyl

Hyaluronidase is an *E. faecium* genome protein encoded by *hyl* gene with a molecular weight of about 45 kDa. The *hyl* gene is homologous with hyaluronidases of other enterococci such as *Streptococcus pneumoniae, Streptococcus pyogenes and Staphylococcus aureus*. Hyaluronidase plays a role in destroying mucopolysaccharides in cartilage and connective tissues and, subsequently, in spreading bacteria. Usually, the *hyl* gene in clinical strains of enterococci are found in *E. faecium,* while occurring extremely rarely in *E. faecalis*. In food strains, *Enterococcus* spp. in which the gene is present include, *E. durans, E. mundtii* and *E. casseliflavus*. Clinically orignated strains usually contain more virulence factors compared to those isolated from other sources such as food. Even though –food strains may not be a direct source of infection, they can facilitate the spread of virulence genes. *Enterococcus* spp. possess the ability to exchange genetic material via conjugation which frequently occurs in the gastrointestinal tract. Some virulence factors in *Enterococcus* species such as antibiotic resistance, haemolysin-cytolysin or adhesion can be transferred via the gene exchange mechanism. In most cases, one plasmid contains the genes that encode virulence factors, antibiotic resistance and pheromones. Consequently, it seems logical to check for the presence of virulence factors in *Enterococcus* spp. strains isolated from food, especially considering consumer safety [28].

3.8 Sex Pheromones

Enterococci possess a plasmid accumulation mechanism dependent on the production of genes encoding sex pheromomes such as *cad, ccf, cob and cpd*. Pheromones are small peptides of 7-8 amino acids long, which enable the conjugative transfer of plasmids between cells. Pheromones secreted by recipients are donor-specific and facilitate the expression of conjugative operons in its plasmid. Usually, a strain secretes a number of different pheromones. Apart from pheromones, each pheromone-dependent plasmid encodes peptide secretion which act like inhibitors to the corresponding pheromone. When pheromones bind to receptors on the surface of the donor's cells, the signal is transduced and stimulates gene aggregation [28]. However, Toğay et al. [31] reported that sex pheromone determinanats were present in 87 *Enterococcus* spp. isolated from food sources.

3.9 Capsular Polysaccharide and Cell Wall Carbohydrate

Clinical isolates of *E. faecalis* expresses the operon glyserol phosphate encoding the synthesis of capsular polysaccharide. Similarly, another capsular polysaccharide present on both *E. faecium* and *E. faecalis* surfaces was also characterized. It was shown that the carbohydrate fraction of the cell contained glycerol phosphate, galactose and glucose residues [55].

3.10 Extracellular Superoxide

E. faecalis isolated from the blood stream have the rare ability to produce superoxide and this appears to vary among isolates [55]. Superoxide production enhanced the *in vivo* survival of *E. faecalis* in mixed infections with *Bacteroides fragilis* in a subcutaneous infection model [33]. The exact purpose of extracellular superoxide production remains unknown, however, its biological effect may play a role in the lysis of red blood cells [18]. Most strains of *E. faecalis*

and some *E. faecium* produce substantial extracellular superoxide, with significantly higher production by invasive rather than commensal strains [33].

3.11 AS-48

AS-48 is a peptide with a molecular weight of 7.4 kDa produced by *E. faecalis* which inhibits and lyses a broad spectrum of Gram negative and positive bacteria; *Enterococcus* spp. included. The acitivity of AS-48 against eukaryotic cell membranes has not been reported. Through the generation of pores in the cytoplasmic membranes of target cells, this lytic peptide results in depolarization. AS-48 is encoded by a transmissible plasmid [46].

4. Conclusions

Enterococci play many significant roles in food fermentation processes. However, they are also of major importance in clinical microbiology because they exhibit natural or acquired resistance to many antimicrobial agents and virulence traits. Resistance to antibiotics and the production of virulence factors are generally accepted major risk factors for human and animal health. Therefore, understanding the prevalence rate of virulence factors and monitoring antimicrobial resistance pattern among *Enterococcus* species isolated from various clinical, environment and food samples is important for epidemiological surveillance.

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