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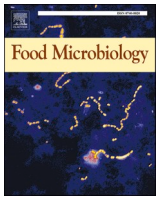

Antibiotic resistance and virulence factors in lactobacilli: something to carefully consider

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ABSTRACT

Lactobacilli are a ubiquitous bacteria, that includes many species commonly found as part of the human microbiota, take part in the natural food fermentation processes, are used as probiotics, and in the food sector as starter cultures or bio-protectors. Their wide use is dictated by a long history of safe employ, which has allowed them to be classified as GRAS (General Recognized As Safe) microorganisms by the US Food and Drug Administration (FDA) and QPS (Qualified Presumption of Safety) by the European Food Safety Authority (EFSA, 2007; EFSA, 2021). Despite their classification as safe microorganisms, several studies show that some members of *Lactobacillus* genus can cause, especially in individuals with previous pathological conditions, problems such as bacteremia, endocarditis, and peritonitis. In other cases, the presence of virulence genes and antibiotic resistance, and its potential transfer to pathogenic microorganisms constitute a risk to be considered. Consequently, their safety status was sometimes questioned, and it is, therefore, essential to carry out appropriate assessments before their use for any purposes. The following review focuses on the state of the art of studies on genes that confer virulence factors, including antibiotic resistance, reported in the literature within the lactobacilli, defining their genetic basis and related functions.

1. Introduction

Antibiotic resistance (AR) is a natural bacterial mechanism. However, the inappropriate and generalized use of antibiotics has increased selective pressure resulting in the adaptation of bacteria to environmental changes and a related increase in resistance rates (Imperial and Ibana, 2016). Indeed, prolonged exposure to different concentrations of antibiotics can decrease the susceptibility of the bacterium, as demonstrated by Drago et al. (2011). Over the years, an increase in resistance rates has therefore been observed not only in pathogens but also in other microorganisms, including lactobacilli, indicating the bacteria previously belonging to the genus *Lactobacillus* given the recent reclassification into new 25 genera made by Zheng et al. (2020). This bacterial group is adapting to the environment by acquiring resistance genes from other resistant bacteria through a horizontal transfer mechanism (Imperial and Ibana, 2016; Lerner et al., 2019; Van Reenen and Dicks, 2011). This phenomenon is heightened by several factors, such as the increasingly selective stresses induced by clinical medication on the lactobacilli that colonize the human gastrointestinal tract (Ma et al., 2017) and from the wide use of antibiotics in the food chain (Willis, 2000), where often lactobacilli are intentionally added as starters. It is important to avoid that food becomes a promoter of new ARs or also a vector of them (Founou et al., 2016; Wang et al., 2006; McDermott et al., 2002; Van Reenen and Dicks, 2011). Therefore, in addition to limiting the use of antibiotics, and monitoring the presence of resistance factors in known pathogens, attention must be paid to the entire microbial population as recently pointed out also by EFSA (EFSA, 2007, EFSA, 2021), including lactobacilli,whose literature studies are lacking. Several authors showed that some lactobacilli can work as reserves of AR genes contributing to their potential transfer to pathogenic microorganisms, making antibiotics treatments ineffective (Egervärn et al., 2010; Gevers et al., 2003b; McDermott et al., 2002; Van Reenen and Dicks, 2011; Yang and Yu, 2019).

However, a specific AR profile in probiotics could be useful for concurrent use with antibiotics in the treatment of certain medical conditions (Anisimova and Yarullina, 2019; Hammad and Shimamoto, 2010). Therefore, it is necessary to highlight which are AR genes most commonly found in lactobacilli and the relative possibility of transfer to assess the safety of these bacteria. The crucial aspect of AR assessment is whether the latter is intrinsic or acquired. Intrinsic resistance is specific

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for a bacterial species or genus. It has minimal possibility of horizontal transfer, while extrinsic resistance, which identifies a strain resistant to a specific antibiotic belonging to a typically sensitive taxonomic unit (EFSA, 2012), has a high possibility of horizontal transfer. In the latter category, it is essential to distinguish resistance induced by chromosomal mutations from acquired genes, due to their higher transmission possibility for their possible collocation on mobile genetic elements, such as plasmids or transposons.

The horizontal transfer can occur through three mechanisms. By transformation, in which foreign genetic material is acquired from the extracellular environment (Lerner et al., 2017); through the transduction mechanism, in which parts of bacterial DNA are included within a bacteriophage during replication, which subsequently infects another bacterial cell causing the transfer (Van Reenen and Dicks, 2011); or through the conjugation process, in which the contact between cells induces the transfer of DNA (Lerner et al., 2017). The latter mechanism, in which plasmids generally transport DNA, is the most commonly encountered in AR gene transfer (Van Reenen and Dicks, 2011), and is linked to the presence of mobile genetic elements such as plasmids, transposons, insertion sequences, bacteriophages (Broaders et al., 2013; Imperial and Ibana, 2016; Van Reenen and Dicks, 2011).

AR genes can be successfully transferred using plasmids from lactobacilli to pathogenic or commensal bacterial strains and vice versa. In his work, Gevers et al. (2003b) highlighted the *in vitro* capacity of four strains of *Lactiplantibacillus plantarum*, two *Companilactobacillus alimentarius* and one *Latilactobacillus sakei* subsp. *sakei* to transfer by conjugation a tetracycline resistance gene to a strain of *Enterococcus faecalis* with a frequency ranging from 10 to 4 to 10–6 transconjugants per recipient. It has been found that transfer can also occur *in vivo* in an animal model (Preethi et al., 2017; Thumu and Halami, 2019). Yang and Yu (2019) demonstrated how tetracycline AR genes have been successfully transferred from *L. plantarum* and *Lactobacillus delbruekii* subsp. *bulgaricus*, isolated from yogurt to the pathogen *Listeria monocytogenes*. Successful plasmid acquisition and stability depend on various factors such as copy number, number of donors, specificity, and growth phase of the recipient organism (Ouoba et al., 2008; Van Reenen and Dicks, 2011; Zonenschain et al., 2009).

Transposons are DNA segments capable of interacting with other elements by recombination or transposition, repeatedly distributed on the chromosome in multiple copies, or associated with plasmids. The most common are the Tn917 and Tn916 families, generally associated with the transportation of the determinants for resistance to tetracycline and erythromycin (Marosevic et al., 2017). In the resistance evaluation, therefore, it is necessary to evaluate the presence of the relative genes and their chromosomal or plasmid positioning and to consider the possible presence of other mobile genetic elements.

The analytical tests for AR include a phenotypic evaluation using various techniques such as E-test, disk diffusion test, and dilution, to determine the MIC value and to compare it with the appropriate species-specific threshold values (Danielsen and Wind, 2003). A strain is defined as resistant if the MIC value is greater than the threshold value, vice versa it is susceptible if the MIC value is lower (EFSA, 2012). However, the phenotypic test alone is only useful as a preliminary test, as it does not differentiate between intrinsic and extrinsic resistance. Furthermore, the MIC cut-off values, especially in lactobacilli, are not standardized, and this can lead to ambiguous results in considering a microorganism resistant or not. Also, a negative phenotypic result does not guarantee the absence of transferable resistance genes (Anisimova and Yarullina, 2019; Aquilanti et al., 2007; Kastner et al., 2006). It is, therefore, useful to combine molecular analysis and genetic sequencing to identify the possible presence of genes related to phenotypic resistance and avoid false assumptions (Arellano et al., 2020). However, the presence of a gene in the genome does not always generate resistance: the possible presence of stop codons, insertions, or deletions could make the gene non-functional. In any case, even if the considered microorganisms are not able to express their relative resistance, the transfer to commensal and pathogenic bacteria cannot be excluded (de Castilho et al., 2019).

In addition to the antibiotic resistance factors, there are other important genes to consider. In some cases, these organisms have been associated with diseases such as peritonitis, infectious endocarditis, bacteremia, and urinary tract infections (Aaron et al., 2017; Campagne et al., 2020; Chery et al., 2013; Collins et al., 2012; Darbro et al., 2009; Groga-Bada et al., 2018; Lee et al., 2020; Lerner et al., 2019; Martinez et al., 2014; Naqvi et al., 2018; Patnaik et al., 2015; Recio et al., 2017; Rossi et al., 2019; Stroupe et al., 2017; Tavernese et al., 2020; Tena et al., 2013; Wallet et al., 2002; Zeba et al., 2018) mainly in immunocompromised patients. The most commonly involved and documented species consist of *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei,* and *Lacticaseibacillus casei* (Aaron et al., 2017; Cannon et al., 2005; Harty et al., 1994; Martinez et al., 2014; Rossi et al., 2019), corresponding to the most frequently used species as probiotic cultures.

Generally, lactobacilli isolated from infected clinical samples coincide with lactobacilli of the intestinal microbiota, but cases have been reported in which clinical isolates phylogenetically correspond to lactobacilli administered in the form of probiotics (Aaron et al., 2017; Martinez et al., 2014). Yelin et al. (2019) highlighted a greater risk of contracting *Lactobacillus* bacteremia in intensive care unit (ICU) patients treated with probiotics than in untreated patients, noting a phylogenetic similarity between lactobacilli isolated from infected blood and the administered probiotic *L. rhamnosus GG*, underlining its ability to reach the bloodstream. Therefore, the identification of potential virulence traits among lactobacilli is useful to evaluate the safety of these bacteria before their usage in the food industry and as human probiotics. The virulence factors mainly associated with lactobacilli consist in the ability of some strains to produce specific enzymes as glycosidases and arylamidase proteases (Oakey et al., 1995), and proteins capable of binding fibrinogen, collagen, and fibronectin, inducing bacterial migration determining the subsequent evasion from host defense mechanisms and the possibility of platelet aggregation (Antikainen et al., 2007; Collins et al., 2012; Harty et al., 1993, 1994; Rossi et al., 2019). Some lactobacilli have also shown the presence of virulence genes generally associated with pathogenic microorganisms, such as *Enterococcus* spp. and *Staphylococcus* spp. due to the acquisition by horizontal transfer.

The following review deal with genes that confer virulence factors, including antibiotic resistance, found so far in the literature within lactobacilli, defining their genetic basis and related functions.

2. Antibiotic resistance genes found in lactobacilli

Numerous studies highlighted how lactobacilli can be considered reserves of AR genes. The resistance genes to various antibiotics reported in literature for these bacteria were presented below, defining the resistance mechanism and the possible transfer to pathogens.

2.1. Tetracycline

One of the most evaluated resistances was that referred to tetracycline. Tetracycline is a broad-spectrum antibiotic that exhibits activity against a wide range of Gram-positive and Gram-negative bacteria. Its role is the inhibition of protein synthesis by binding the conserved 16 S rRNA sequences of the 30 S subunit of the ribosome to prevent attachment to A-site by t-RNA (Kapoor et al., 2017).

Resistance is related to the presence of two gene groups. The first group is responsible for the production of membrane-associated proteins capable of mediating the antibiotic efflux outside the cell, reducing its intracellular concentration. Genes *tet*(Z), *tet*(K), *tet*(L), and *tcr*3 belong to this category. A second group is composed of genes that encode cytoplasmic proteins able to protect ribosomes from antibiotic attack: *tet*(M), *tet*(O), *tet*(S), *tet*(W), *tet* (Q), *tet*(T), and *otr*(A) (Roberts and Schwarz, 2009).

A large number of lactobacilli carrying one or more genes related to

tetracycline resistance have been reported (Table 1). *tet*(M) gene represented the most widespread determinant. It was found in numerous strains of *L. plantarum* isolated from animals and products of animal origin (Aquilanti et al., 2007; Chang et al., 2011; Gevers et al., 2003a; Preethi et al., 2017; Zonenschain et al., 2009), silage (Egervärn et al., 2009), and green tea (Arellano et al., 2020). Different strains of *Limosilactobacillus reuteri*, *Ligilactobacillus salivarius,* and *L. sakei* showed the same result (Table 1). Todorov et al. (2019) showed the ribosome protection gene presence in three tested strains of *Latilactobacillus curvatus* isolated from smoked salmon, as well as Yang and Yu (2019) in three strains of *L. delbrueckii* subsp. *bulgaricus* from yogurt samples. These data underlined the possible spread of this determinant in numerous food products, creating concern about the possible transfer within the gastrointestinal tract. *tet*(M) is generally associated with transposons of the Tn916 - Tn1545/Tn917 family identified by the presence of the *int* integrase and *xis* excision genes that mediate its transfer (de Castilho et al., 2019; Devirgiliis et al., 2009; Marosevic et al., 2017; Preethi et al., 2017; Thumu and Halami, 2019; Todorov et al., 2017). Furthermore, several studies highlight the presence of this gene on plasmids, contributing to the possibility of its spread also to pathogenic microorganisms (Aquilanti et al., 2007; Egervärn et al., 2009; Gevers et al., 2003a), such as *Listeria monocytogenes* (Yang and Yu, 2019), while others noted a chromosomal positioning (Gfeller et al., 2003).

Other widespread genes within lactobacilli resulted in the *tet*(W) gene, which encodes a ribosome protection protein, and *tet*(K) and *tet*(L) genes, responsible for the antibiotic efflux (Table 1). Chang et al. (2011) showed that on 146 strains of 11 species of lactobacilli isolated from pig intestine, 82.0% of tetracycline-resistant (TETR) strains had *tet*(W) gene, 22.5% *tet*(M) gene, 14.4% *tet*(L) gene and 8.1% *tet*(K) gene. The same authors were also the first to find the presence of the *tet*(Q) gene in a strain of *L. salivarius*.

Although in some cases *tet*(W) gene has been found on non-conjugative plasmids and the possibility of transfer was not prooved (Egervärn et al., 2009; Guo et al., 2017; Kastner et al., 2006), there are pieces of evidence of its presence on plasmids, and therefore the possibility of transfer cannot be excluded. Thumu and Halami (2019) highlighted the presence of a plasmid containing *tet*(W), *tet*(M), and *tet*(L) genes that were transferred from a strain of *L. salivarius* to the recipient *Enterococcus faecalis* JH2-2, both *in vitro* and *in vivo*. It is therefore possible to find multiple *tet* genes in the same microorganism, with the same (ribosomal protection or efflux) or different action (efflux and ribosomal protection) (Ammor et al., 2008; Thumu and Halami, 2019; Zonenschain et al., 2009).

Other genes that confer resistance to tetracycline were frequently observed in strains belonging to *L. plantarum* species such as *tet*(S), *tet*(T), and *tet*(O) (Arellano et al., 2020; Ouoba et al., 2008; Yang and Yu, 2019; Zhang and Zhang, 2019; Zonenschain et al., 2009). *tcr*3 genes found mainly in *Streptomyces* spp. (Roberts and Schwarz, 2009) and *otr*A, for resistance to oxytetracycline, were recently found in strains belonging to the species *Limosilactobacillus fermentum*, *L. rhamnosus,* and *L. plantarum* (Zhang and Zhang, 2019). Two tetracycline resistance mosaic genes were also observed, resulting from interclass recombination within the coding regions of the *tet*W and *tet*O genes, such as *tet* (W/O) (Ammor et al., 2007) and *tet* (O/W/32/O/W/O) in a strain of *Lactobacillus johnsonii* isolated from human feces (Van Hoek et al., 2008) (Table 1).

There are numerous data in the literature regarding tetracycline resistance for L. *plantarum* (Table 1). Most of the studies found a *tet* + profile in phenotypically resistant strains (Table 2). However, in a study conducted by Anisimova and Yarullina (2019), on 12 strains of *L. plantarum* tested, all showed susceptibility to the antibiotic, 25% (3/12) of which, however, had a positive response for the gene *tet*(L). Arellano et al. (2020) presented a similar result, in which 11 out of 18 tested strains were susceptible to tetracycline, despite having different resistance genes. These results highlighted how a negative phenotypic result cannot exclude the presence of potentially transmissible genes.

Table 1
Tetracycline resistance genes found in *Lactobacillus* spp.

Ribosomal protection genes

Gene	Species	Reference
tet(W)	*L. amylovorus*	Chang et al. (2011)
	L. kefiri	
	L. parabuchneri	
	L. ruminis	
	L. salivarius	(Chang et al., 2011; Thumu e Halami, 2012)
	L. helveticus	Guo et al. (2017)
	L. paracasei	(Huys et al., 2002)
	L. reuteri	(Chang et al., 2011; Egervärn et al., 2010; Egervärn et al., 2009; Kastner et al., 2006; Thumu e Halami, 2012)
	L. sakei	Zonenschain et al. (2009)
	L. curvatus	
	L. plantarum	(Chang et al., 2011; Thumu e Halami, 2012; Zonenschain et al., 2009)
	L. rhamnosus	(Chang et al., 2011; Thumu e Halami, 2012; Zhang e Zhang et al., 2018; Zonenschain et al., 2009)
	L. fermentum	(Chang et al., 2011; Zhang and Zhang, 2019)
	L. delbrueckii	Campedelli et al. (2019)
	L. reuteri - vaccinostercus	
tet(O)	*L. curvatus*	Todorov et al. (2019)
	L. delbrueckii	
	L. plantarum	(Arellano et al., 2020; Zhang and Zhang, 2019)
	L. rhamnosus	Zhang and Zhang (2019)
	L. salivarius	(Aquilanti et al., 2007; Thumu e Halami, 2012)
tet(Q)	*L. brevis*	Campedelli et al. (2019)
	L. salivarius	Chang et al. (2011)
tet(S)	*L. plantarum*	(Arellano et al., 2020; Yang e Yang and Yu, 2019; Zonenschain et al., 2009)
	L. paraplantarum	Ouoba et al. (2008)
	C. alimentarius	Campedelli et al. (2019)
tet (W/O)	*L. johnsonii*	(Ammor et al., 2008a)
tet(M)	*L. amylovorus*	Chang et al. (2011)
	L. kefiri	
	L. parabuchneri	
	L. pentosus	Preethi et al. (2017)
	L. paracasei	(Devirgiliis et al., 2009; Huys et al., 2002; Zonenschain et al., 2009)
	L. delbrueckii subsp. *bulgaricus*	Yang and Yu (2019)
	L. salivarius	(Aquilanti et al., 2007; Cauwerts et al., 2006a; Chang et al., 2011; Preethi et al., 2017; Thumu e Halami, 2012)
	L. brevis	Zonenschain et al. (2009)
	L. rhamnosus	
	L. sakei	(Chang et al., 2011; Gevers et al., 2003a; Zonenschain et al., 2009)
	L. curvatus	(Todorov et al., 2019; Zonenschain et al., 2009)
	L. reuteri	(Aquilanti et al., 2007; Chang et al., 2011; Zonenschain et al., 2009)
	L. plantarum	(Aquilanti et al., 2007; Arellano et al., 2020; Chang et al., 2011; Egervärn et al., 2009; Gevers et al., 2003a; Preethi et al., 2017; Zonenschain et al., 2009)
	L. casei - maniotivorans	Campedelli et al. (2019)
	L. delbrueckii	
	L. reuteri - vaccinostercus	
*otr*A	*L. fermentum*	Zhang and Zhang (2019)
tet(T)	*L. fermentum*	Zhang and Zhang (2019)
	L. rhamnosus	
	L. plantarum	
tet (O/W/32/O/W/O)	*L. johnsonii*	(Van Hoek et al., 2008)

Efflux proteins genes

Gene	Species	Reference
tet(K)	*L. amylovorus*	Chang et al. (2011)

(*continued on next page*)

Table 1 (*continued*)

	L. buchneri	(Anisimova e Yelin et al., 2019)
	L. curvatus	(de Castilho et al., 2019; Todorov et al., 2019)
	L. delbrueckii	Todorov et al. (2019)
	L. fermentum	(Chang et al., 2011; Thumu e Halami, 2012)
	L. plantarum	(Aquilanti et al., 2007; Arellano et al., 2020; Chang et al., 2011; Todorov et al., 2017)
	L. reuteri	Aquilanti et al. (2007)
	L. ruminis	Chang et al. (2011)
	L. salivarius	(Aquilanti et al., 2007; Chang et al., 2011)
tet (PB)	*L. fermentum*	Zhang and Zhang (2019)
	L. plantarum	
tet(Z)	*L. reuteri*	Cauwerts et al. (2006b)
tet(L)	*L. curvatus*	de Castilho et al. (2019)
	L. plantarum	(Anisimova and Yarullina, 2019; Arellano et al., 2020; Chang et al., 2011; Preethi et al., 2017; Thumu e Halami, 2012)
	L. amylovorus	Chang et al. (2011)
	L. kefiri	
	L. parabuchneri	
	L. reuteri	
	L. ruminis	
	L. sakei	
	L. fermentum	(Thumu e Halami, 2012)
	L. salivarius	(Chang et al., 2011; Preethi et al., 2017; Thumu e Halami, 2012)
tcr3	*L. fermentum*	Zhang and Zhang (2019)
	L. rhamnosus	
	L. plantarum	

Comparing, in Table 2, the number of strains of *L. plantarum* resistant to tetracycline to the corresponding number of resistance genes found, it was noted that the percentage of resistance genes is relatively low (Aquilanti et al., 2007; Campedelli et al., 2019; Preethi et al., 2017; Zonenschain et al., 2009). This finding should not be confused with the possibility of intrinsic resistance, as not all possible genes responsible for resistance were always evaluated. For example, Campedelli et al. (2019) considered only *tet*(W), *tet*(M), *tet*(L), *tet*(P), *tet*(S) e *tet*(Q) determinants.

The ranges of MIC values (Table 2) for *L. plantarum* were found to be relatively broad in all the considered studies, with values higher than 512 μg/mL (Chang et al., 2011; Egervärn et al., 2009; Preethi et al., 2017; Zonenschain et al., 2009).

Other well-documented tetracycline-resistant lactobacilli species were *L. reuteri*, *L. sakei*, and *L. salivarius*. As reported in Table 3, different studies highlighted MIC values even above 32 μg/mL. Chang et al. (2011) observed resistance with MIC values ranging from 16 to 1024 μg/mL for all 60 strains of *L. reuteri* tested, in which the predominantly detected gene was found to be *tet*(W) (43/60 strains tested), and for 100% of the *L. sakei* (6/6) and *L. salivarius* (17/17) strains examined.

Concerning these species, phenotypic resistance is not always correlated to the presence of *tet* genes, probably because all the possible determinants able to induce resistance to tetracycline are not always considered (Campedelli et al., 2019; Cauwerts et al., 2006a; Chang et al., 2011; Zonenschain et al., 2009).

2.2. MLS - macrolides, lincosamides, streptogramins

Another widespread gene in lactobacilli reported in the literature was *erm*(B) (Table 4), which is linked to resistance to erythromycin, a macrolide belonging to the MLS group of antibiotics. The antibiotics present within the group (erythromycin, clindamycin, lincomycin, and streptogramin A) have a different chemical structure but share the same protein synthesis inhibition action. Resistance can occur through three mechanisms: methylation of the target site of the antibiotic, efflux, and inactivation. The methylation mechanism confers a broad spectrum of resistance to macrolides and lincosamides, while antibiotic efflux and inactivation activities are targeted only to certain antibiotics or classes of antibiotics (Leclercq, 2002; Marosevic et al., 2017).

Erythromycin resistance is mediated by genes of the *erm* (*Erythromycin Ribosome Metylase*) class encoding a ribosomal adenine-N6-methyltransferase. These genes can hinder the action of the antibiotic by methylating the 23 S rRNA peptidyltransferase center, thus preventing the antibiotic from attacking the ribosome 50 S subunit (Kapoor et al., 2017). As can be seen in Table 4, which represents the diffusion of erythromycin resistance genes in *Lactobacillus* spp, the most commonly found classes in this group are *erm*(A), *erm*(B), and *erm*(C), detected in several strains of *L. plantarum*, *L. casei*, *L. reuteri*, *L. rhamnosus* and *L. fermentum* (Aquilanti et al., 2007; Anisimova and Yarullina, 2019; Arellano et al., 2020; de Castilho et al., 2019; de Souza et al., 2019; Egervärn et al., 2009; Guo et al., 2017; Hummel et al., 2007; Preethi et al., 2017; Todorov et al., 2017, 2019). It can be noted that, even in the case of erythromycin, several studies have observed numerous strains of *L. plantarum* endowed with at least one of the genes listed above (Table 4). In this case, however, unlike the results obtained for resistance to tetracycline, most of the strains tested in the various studies were found to be susceptible to the antibiotic (Table 5). Generally, lactobacilli are susceptible to antibiotics that inhibit protein synthesis such as erythromycin (Todorov et al., 2017; Yang and Yu, 2019), but the increasing selective pressure has made it possible to adapt and acquire resistance-related genes. Guo et al. (2017) found that a total of 33 lactobacilli tested (11 *Lactobacillus helveticus*, 11 *L. casei*, 11 *L. plantarum*) were sensitive to erythromycin (MIC range 0.016–1 μg/mL), but only 6 strains of *L. helveticus*, 1 strain of *L. casei* and 1 strain of *L. plantarum* reported the presence of the *erm*(B) gene. A comparable result was obtained by Anisimova and Yarullina (2019), who showed that on 20 lactobacilli strains tested with negative phenotype for resistance, 4 strains of *L. plantarum* carried the corresponding resistance genes, highlighting the possible presence of acquired silent genes. In contrast, Thumu and Halami (2012), considering the used cut-off of 1 μg/mL, observed high resistance for all 10 lactobacilli strains tested (MIC range 8–512 μg/mL), each of which carried at least one resistance gene. As reported in Table 5, MIC values for erythromycin in the most studied species (*L. salivarius, L. reuteri*, and *L. plantarum*) were quite different, at both inter-species and intra-species levels. The presence of silent genes with the relative negative phenotypic outcome should cause concern for possible transfer. Although in some cases *erm*(B) gene was found to be positioned at the chromosomal level, reducing the possibility of transfer (Hummel et al., 2007), in others a plasmid positioning was observed, which increase the transfer probability (Egervärn et al., 2009; Gevers et al., 2003a). Indeed, the transmission of erythromycin resistance from strains of *L. reuteri*, *L. plantarum,* and *L. salivarius* to *Enterococcus faecalis JH2-2* has been demonstrated in *vitro* conjugation experiments (Ouoba et al., 2008; Thumu and Halami, 2019). Feld et al. (2009) showed the ability of a strain of *L. plantarum* to transfer, through the plasmid pLFE1, the *erm*(B) gene to *L. rhamnosus*, *Lactococcus lactis*, *Listeria innocua*, to the opportunistic pathogen *Enterococcus faecalis* and the pathogen *Listeria monocytogenes*. Thumu and Halami (2019) demonstrated how the transfer of a plasmid containing the *erm*(B) gene in association with the *tet*(M), *tet*(W), and *tet*(L) genes could also occur *in vivo*. There is also the possibility of finding genes for resistance to erythromycin associated with mobile elements, such as the transposon Tn917, capable of carrying the *erm*(B) gene, or transposons of the Tn916 family able to carry the determinants for resistance to erythromycin associated to the ones to tetracycline (Marosevic et al., 2017).

Although the *erm*(A), *erm*(B), and *erm*(C) genes were the most found and documented determinants in *Lactobacillus* spp, the presence of the *erm*(T) gene was detected in a strain of *L. reuteri* isolated from poultry intestine (Egervärn et al., 2010) and *erm* (LF) gene in a transferable plasmid of a *L. fermentum* strain (Gfeller et al., 2003).

Another mechanism through which resistance to erythromycin can occur is by reducing the intracellular concentration of the antibiotic, thanks to the presence of efflux pumps encoded by the *mef* genes, for example, *mef*(A), observed in *L. plantarum* and *L. salivarius* subsp. *salivarius* (Anisimova and Yarullina, 2019; Cauwerts et al., 2006b), and *mef* (B) and *mef*(E) found in *L. casei and L. delbrueckii* (Campedelli et al.,

Table 2
Resistance to tetracyclin in *L. plantarum*.

Species	Origin	Tested Strains	Phenotype (n° strains/n°tested strains)	MIC range (μg/mL)	Cut-off (μg/mL)	Genotype	n° strains with gene/n°tested strains	Reference
L. plantarum	salami	8	8/8 R	>256 [d]	ND	*tet*(M)	8/8	Gevers et al. (2003a)
	meat products	11	3/3 R**	16 -> 64[c]	16[e]	*tet*(M) *tet*(K)	2/11 2/11	Aquilanti et al. (2007)
	salami	12	11/12 R	16–512[c]	32[f]	*tet*(M) *tet*(W) *tet*(S)	5/11 4/11 1/11	Zonenschain et al. (2009)
	silage	2	2/2 R	>256[d]	ND	tet(M)	2/2	(Egervärn et al., 2009)
	swine colon	11	11/11 R	32–512[c]	32[g]	*tet*(M) *tet*(K) *tet*(L)	4/11 1/11 1/11	Chang et al. (2011)
	ice cream	1	1/1 R	128[c]	32[g]	*tet*(W), *tet*(L)	1/1	(Thumu e Halami, 2012)
	poultry feces and intestines	10	10/10 R	4–1024[c]	ND	*tet*(L) *tet*(M)	4/10 5/10	Preethi et al. (2017)
	salami	1	ND	*ND*	ND	*tet*(K), *tet*(O)	1/1	Todorov et al. (2017)
	silage	12	12/12 S	≥19 mm[b]	≤14 mm	*tet*(L)	3/12	Anisimova and Yarullina (2019)
	green tea, fermented products, insects	18	7/18 R	≤8 ->64[a]	32[h]	*tet*(M) *tet*(K) *tet*(L) *tet*(O) *tet*(S)	2/2 * 2/2 * 2/2 * 2/2 * 2/2 *	(Arellano et al., 2020)
	fermented dairy products	6	1/6 R	1–32[c]	32[g]	*tet*(S)	1/6	Yang and Yu (2019)
	caries	7	1/7 R	16–64[a]	32[g]	*tet*(T).*tet*(L), *tet*(O), *tcr*3, *tet*PB	1/7	Zhang and Zhang (2019)
	Pickles, beer contaminant, fermented vegetables	10	7/10 R	4–64[c]	ND	-	0/10	Campedelli et al. (2019)

R: resistant, S: susceptible, ND: not defined.
*: genotypic analysis performed only on 2 phenotypically susceptible strains.
**: phenotypic analysis carried out on the 3 strains with the related gene.
[a] MIC evaluated with agar dilution method (μg/mL).
[b] MIC evaluated with disk diffusion method (mm).
[c] MIC evaluated with broth microdilution method (μg/mL).
[d] MIC evaluated with E-Test (μg/mL).
[e] Cut-off MIC value for tetracycline defined by CLSI (2008)
[f] Cut-off MIC value for tetracycline defined by EFSA (2005)
[g] Cut-off MIC value for tetracycline defined by EFSA (2008)
[h] Cut-off MIC value for tetracycline defined by EFSA (2012).

2019). Other antibiotic efflux-related genes are *msr* (A/B) found in *L. plantraum* and *L. salivarius,* and the enterococcal gene *msr*(C) discovered in *L. fermentum* and *L. plantarum* (Preethi et al., 2017; Thumu Rao Halami, 2012) (Table 6). This last gene confers resistance to both macrolides and group B streptogramins (Marosevic et al., 2017). Campedelli et al. (2019) mentioned also the *lsa* gene, related to the efflux of the lincosamide clinamycin, which was found in 60 lactobacilli strains, 13 of which resistant to the corresponding antibiotic.

The third mechanism of resistance consists in the inactivation of the antibiotic, mediated by the *lnu*(A) gene encoding a transferase capable of inhibiting the lincosamides action. As showed in Table 7, this gene was observed in strains of *L. reuteri* found to be resistant to clindamycin and lincomycin (Cauwerts et al., 2006b; Kastner et al., 2006). Kastner et al. (2006) noted a 96% similarity between *L. reuteri* SD 2112 *lnu*(A) and *Staphylococcus haemolyticus lin*(A) gene sequence. An acetyltransferase encoded by the *vat*(E) gene, able to inactivate group A streptogramins (e.g. dalfopristin), was observed in strains of *L. curvatus* and *L. fermentum* (Todorov et al., 2019) and in ROT1 isolated from cheese associated with *erm* (LF) gene, placed on the pLME300 plasmid, able to confer high resistance to dalfopristin and erythromycin (Gfeller et al., 2003) (Table 7).

2.3. Aminoglycosides

Aminoglycosides are antibiotics responsible for interfering with protein synthesis by binding to the acceptor (A) site placed on the 16 S rRNA of the 30 S ribosomal subunit, resulting in translation block (Kapoor et al., 2017; Magalhães and Blanchard, 2019). One resistance mechanism is the inactivation of the antibiotic mediated by intracellular enzymes able to modify its structure, inducing the reduction of affinity for A site, and consequently preventing the binding to the 30 S ribosomal subunit. Within this group, there are 4 classes of enzymes: aminoglycosides phosphotransferases (APHs), aminoglycosides nucleotidyltransferases (ANTs), aminoglycosides acetyltransferases (AACs) that confer resistance to antibiotics such as gentamicin, kanamycin, neomycin, amikacin, and adenylyltransferase (AAD) for streptomycin resistance (Kapoor et al., 2017; Magalhães and Blanchard, 2019).

Also, resistance to aminoglycosides among lactobacilli is generally considered as intrinsic resistance. The absorption of this type of antibiotic is connected to the transport of electrons mediated by the cytochrome, a system absent in lactobacilli, which therefore determines the inability of the drug to be absorbed by the cell (Anisimova and Yarullina, 2019; de Castilho et al., 2019; Hummel et al., 2007; Kastner et al., 2006; Ouoba et al., 2008). In fact, in several studies, the genes encoding the enzymes for the inactivation of the target antibiotic weren't identified by molecular methods, although the isolates had high MIC values. For

Table 3
Distribution of tetracycline resistance in *L. reuteri, L. sakei* and *L. salivarius*.

Species	Origin	Tested Strains	Phenotype (n° strains/n°tested strains)	MIC *range* (μg/mL)	*Cut-off* (μg/mL)	Genotype	n° strains with gene/n°tested strains	Reference
L. reuteri	different origins	32	28/32 R	*4 - > 256*[d]	ND	*tet*(W)	24/28	(Egervärn et al., 2009)
	swine colon	60	60/60 R	*16 - 1024*[c]	16[g]	*tet*(M) *tet*(W) *tet*(L)	2/60 43/60 4/60	Chang et al. (2011)
	salami	2	2/2 R	*256- > 512*[c]	16[g]	*tet*(W)	1/2	(Thumu e Halami, 2012)
	meat products	3	2/2 R**	*32 - 64*[c]	16[e]	*tet*(M) *tet*(K)	1/3 1/3	Aquilanti et al. (2007)
	salami	1	1/1 R	*512*[c]	8[f]	tet(M)	1/1	Zonenschain et al. (2009)
	cloaca of broiler poultry	8	8/8 R	≥64[a]	16	*tet*(L) *tet*(W) tet(L)+ *tet* (K) tet(Z)	1/8 3/8 1/8 1/8	Cauwerts et al. (2006a)
	animal feces, vagina, cheese, sour dough, human and pig intestines, human saliva	18	12/18 R	1–128[c]	ND	*tet*(L) *tet*(M) *tet*(W)	2/12 2/12 1/12	(Campedelli et el., 2019)
L. sakei	salami	24	17/24 R	*2 - 512*[c]	8[f]	*tet*(M) *tet*(W)	11/17 1/17	Zonenschain et al. (2009)
	salami	10	10/10 R	*32 - > 256*[d]	ND	*tet*(M)	10/10	Gevers et al. (2003a)
	swine colon	6	6/6 R	*32 - 256*[c]	8[g]	*tet*(M) *tet*(L)	4/6 2/6	Chang et al. (2011)
	fermented meat products, sake starters, rice noodles, silage, milk	5	1/5	*0,5 – 16*[c]	ND	-	0/1	Campedelli et al. (2019)
L. salivarius	cloaca of broiler poultry	31	24/31 R	*2 - > 64*[a]	16	*tet*(M) *tet*(L)+ *tet*(M)	9/24 14/24	Cauwerts et al. (2006a)
	swine colon	17	17/17 R	*16 - 512*[c]	8[g]	*tet*(M) *tet*(W) *tet*(K) *tet*(L) *tet*(Q)	5/17 8/17 1/17 2/17 1/17	Chang et al. (2011)
	salami	3	3/3 R	*256*[c]	16[e]	*tet*(M) *tet*(W) *tet*(O) *tet*(L)	3/3 3/3 1/3 1/3	(Thumu e Halami, 2012)
	meat products	6	6/6 R	*> 64*[c]	16[e]	*tet*(M) *tet*(O) *tet*(K)	4/6 1/6 2/6	Aquilanti et al. (2007)
	intestines and poultry meat, slaughter water	3	3/3 R	*4 - 128*[c]	ND	*tet*(M)	2/3	Preethi et al. (2017)
	cider, must, animal feces, rat and chicken intestines, saliva, slurry, vacuum-packed meat	27	5/27	*0,5 – 128*[c]	ND	*tet*(P)	1/5	Campedelli et al. (2019)

R: resistant; S: susceptible; ND: not defined; **: phenotypic analysis carried out on the 2 strains with the related gene.
[b]: MIC evaluated with disk diffusion method (mm).
[a] MIC evaluated with agar dilution method (μg/mL).
[c] MIC evaluated with broth microdilution method (μg/mL).
[d] MIC evaluated with E-Test (μg/mL).
[e] Cut-off MIC value for tetracycline defined by CLSI (2008)
[f] Cut-off MIC value for tetracycline defined by EFSA (2005)
[g] Cut-off MIC value for tetracycline defined by EFSA (2008)

example, Guo et al. (2017) showed that out of 14 lactobacilli strains of the *L. casei*, *L. plantarum*, and *L. helveticus* species found to be resistant to kanamycin (MIC> 64 μg/mL), only one strain of *L. helveticus* carried the correspondent *aph* (3″)-III gene (Table 8). None of the streptomycin-resistant strains presented the corresponding determinants *aad*A, *aad*E, *ant* (6). Similarly, Anisimova and Yarullina (2019) did not observe the presence of *aac* (6′)-Ie-*aph* (2″)-Ia, *ant* (6), *aph* (3)-III and *ant* (2″)-I genes in strains resistant to amikacin, kanamycin, and gentamicin. In contrast, the work of de Souza et al. (2019) showed how antibiotic inactivation genes may be present and not always correlated with phenotypic resistance. In the study, two lactobacilli sensitive to gentamicin, kanamycin, and streptomycin, possessed the genes *aac* (6′)-Ii, *ant* (4′)-Ia and *aph* (2)-Ic responsible for resistance to a broad spectrum of aminoglycosides and aph (3)-III for resistance to kanamycin and neomycin (Table 8), demonstrating how the evaluation can be complicated due to the presence of silent or not expressed genes. However, different studies revealed a correspondence between phenotypic and genotypic results, defining that the mechanism of enzymatic inactivation mediated by acquired genes can also occur in lactobacilli. The *aac* (6′)-Ie-*aph* (2)-Ia gene codifies the bifunctional enzyme 6′-*N*-acetyltransferase-2″-O-phosphotransferase, able to confer a broad spectrum of inactivation of all aminoglycosides, apart from streptomycin, and it was found in highly resistant strains of *L. delbrueckii* subsp. *bulgaricus* and *L. plantarum* (Todorov et al., 2017; Yang and Yu, 2019) (Table 8). *aad*A, *aad*E, and *ant* (6) genes, encoding the adenylyltransferase and nucleotidyltransferase enzymes, endow with resistance

Table 4
Antibiotic resistance genes of the MLS group found in Lactobacillus spp. with methylation action of the target site of the antibiotic.

Gene	Species	Reference
erm(B)	*L. helveticus*	Guo et al. (2017)
	L. pentosus	Preethi et al. (2017)
	L. casei	(de Souza et al., 2019; Guo et al., 2017)
	L. crispatus	Aquilanti et al. (2007)
	L. johnsonii	
	L. sakei	Zonenschain et al. (2009)
	L. curvatus	
	L. paracasei	(Huys et al., 2002; Zonenschain et al., 2009)
	L. brevis	Zonenschain et al. (2009)
	L. rhamnosus	
	L. fermentum	(de Souza et al., 2019; Thumu e Halami, 2012; Todorov et al., 2019)
	L. salivarius	(Aquilanti et al., 2007; Hummel et al., 2007; Preethi et al., 2017; Thumu e Halami, 2012)
	L. plantarum	(Anisimova and Yarullina, 2019; Aquilanti et al., 2007; Arellano et al., 2020; Feld et al., 2009; Guo et al., 2017; Preethi et al., 2017; Thumu e Halami, 2012; Todorov et al., 2017; Zonenschain et al., 2009)
	L. reuteri	(Aquilanti et al., 2007; Egervärn et al., 2009; Ouoba et al., 2008; Thumu e Halami, 2012; Zonenschain et al., 2009)
	L. delbrueckii	Campedelli et al. (2019)
	L. reuteri - vaccinostercus	
erm (A)	*L. curvatus*	Todorov et al. (2019)
	L. delbrueckii	
	L. fermentum	
	L. plantarum	(Arellano et al., 2020)
erm(C)	*L. plantarum*	(Arellano et al., 2020; Todorov et al., 2017; Zonenschain et al., 2009)
	L. brevis	Aquilanti et al. (2007)
	L. johnsonii	
	L. reuteri	(Egervärn et al., 2009)
	L. casei	de Souza et al. (2019)
	L. fermentum	Todorov et al. (2019)
	L. delbrueckii	
	L. curvatus	(de Castilho et al., 2019; Todorov et al., 2019)
erm (LF)	*L. fermentum*	Gfeller et al. (2003)
erm(T)	*L. reuteri*	(Egervärn et al., 2009)

to streptomycin and was found in strains of *L. paracasei*, *L. casei, L. rhamnsosus*, *L. plantarum,* and *L. delbrueckii* subsp. *bulgaricus,* which showed a resistant phenotype (Table 8).

2.4. Vancomycin

Lactobacilli are intrinsically resistant to vancomycin, as reported in a study in which 77% of the analyzed strains (141/182) presented resistance to vancomycin (Campedelli et al., 2019) thanks to their peptidoglican composition (Zhang et al., 2018). This glycopeptide antibiotic can inhibit the synthesis of the cell wall of Gram-positive bacteria by binding to the D-alanyl-D-alanine precursor of the peptidoglycan, compromising the formation of cross-links (Deghorain et al., 2007; Kapoor et al., 2017). Two mechanisms mediate the resistance: by replacing the D-alanine residue at the C-terminus of the peptidoglycan precursor with D-lactate or D-serine, to create a precursor with low affinity for vancomycin, or by preventing synthesis of the D-alanyl-D-alanine bond by eliminating the attack site of the antibiotic (Anisimova and Yarullina, 2019; de Souza et al., 2019). Most lactobacilli possess endogenous enzymes capable of synthesizing D-lactate and binding it to peptidoglycan, thus inducing intrinsic resistance (Deghorain et al., 2007).

In *Enterococcus* spp. vancomycin-resistant strains, these mechanisms result from the acquisition of a conjugative plasmid, which includes a cluster containing *van*A, *van*H, *van*R, *van*S, *van*X, *van*Y, and *van*Z genes. Of these, *van*A encodes the enzyme D-alanine-D-lactate ligase, conferring a high level of resistance (Deghorain et al., 2007). The vanX gene produces a D-alanyl-D-alanine dipeptidase able to hydrolyze the peptidoglycan dipeptide precursor D-alanyl-D-alanine, eliminating the antibiotic attack site, while *van*H encodes a D-lactate dehydrogenase, which converts pyruvate to D-lactate. The presence of these three genes is necessary to obtain the acquired resistance (Anisimova and Yarullina, 2019; Deghorain et al., 2007; Li et al., 2015). In support of the intrinsic resistance of lactobacilli to vancomycin, several studies highlighted the presence of resistant isolates lacking the characteristic genes described above. Ouoba et al. (2008) showed that on 16 *Lactobacillus* spp. strains belonging to *L. reuteri*, *Lactiplantibacillus paraplantarum*, *L. plantarum*, *L. fermentum*, *L. salivarius*, *Lactobacillus acidophilus*, *L. rhamnosus*, *L. paracasei,* and *L. casei*, only *L. acidophilus* was sensitive to the antibiotic. The MIC value for the resistant strains exceeded 32 μg/mL but no resistance genes were detected. Similarly Kastner et al. (2006) found that more than 50% of their tested strains were phenotypically resistant, but genetic determinants were absent. Although intrinsic resistance has a minimal chance of horizontal transfer, a case in which one *L. plantarum* strain was able to transfer high phenotypic resistance to *Enterococcus faecalis*, both *in vitro* and *in vivo* conjugation experiments has been reported (Preethi et al., 2017). However, the presence of acquired resistance-related genes is not excluded within lactobacilli (Table 9). Several variants of the gene encoding the enzyme ligase (*van*A, *van*B, *van*C1, *van*C2, *van*C2/C3), which substitutes the terminal residue of D-alanine, were observed in *L. plantarum* (Arellano et al., 2020; Todorov et al., 2017), *L. curvatus* (de Castilho et al., 2019; Todorov et al., 2019), *L. fermentum* and *L. delbrueckii* (Todorov et al., 2019) and *L. reuteri* (Dlamini et al., 2019). The transfer of *van*A from enterococci to a probiotic strain of *L. acidophilus* was highlighted *in vitro* and *in vivo* experiments within the digestive tract of mice (Mater et al., 2007), highlighting the problem of possible subsequent re-transfer to commensals or pathogens within the human gastrointestinal tract. The *van*X gene, encoding the dipeptidase enzyme, is considered to guarantee lower resistance values than the *van*A and variant genes. It has been found in several *L. plantarum* strains (Guo et al., 2017; Anisimova and Yarullina, 2019; Liu et al., 2009).

2.5. B-lactam antibiotics

B-lactam antibiotics are drugs with bactericidal action involving the inhibition of cell wall synthesis. Their structure binds to the transpeptidase enzyme (PBPs - Penicillin Binding Protein), making it no longer available for the formation of cross-links within the peptidoglycan structure. In Gram-positive, resistance is generally mediated by a modification of the antibiotic target molecules by reducing the binding affinity, while the production of β-lactamases resulted in the resistance factor for Gram-negative bacteria (Kapoor et al., 2017; Naas et al., 2011).

Although lactobacilli are generally considered susceptible to this antibiotics class, such as ampicillin and penicillin (Anisimova and Yarullina, 2019; Hazlrolan et al., 2019a; Klein, 2011; Saini and Tomar, 2017), some authors reported the presence of ampicillin and cephalosporins resistance in different lactobacilli such as *L. fermentum*, *L. plantarum*, *Levilactobacillus brevis*, *L. salivarius*, *Lactobacillus crispatus* (Anisimova and Yarullina, 2019; Dec et al., 2018; Guo et al., 2017; Khan et al., 2019) and penicillin G in strains of *L. plantarum* (Hummel et al., 2007). In some cases, resistance is linked to the presence of acquired genes encoding broad spectrum β-lactamase (ESBL - Extended Spectrum β-Lactamase) such as *bla*CTX-M, *bla*SHV, *bla*TEM, and *bla*Z (Table 10) (Anisimova and Yarullina, 2019; Aquilanti et al., 2007; Khan et al., 2019). Of these, *bla*CTX-M, which exhibits a high activity spectrum, can be associated with transposons determining its possible spread (Khan et al., 2019).

For the treatment of ESBL producing bacteria, an alternative therapy is the administration of carbapenems (Khan et al., 2019; Naas et al., 2011), although resistance mechanisms also to these antibiotics have been reported, due to the presence of carbapenemase enzymes encoded

Table 5
Distribution of resistance to erythromycin in *L. reuteri, L. salivarius* and *L. plantarum*.

Species	Origin	Tested Strains	Phenotype (n° strains/n°tested strains)	MIC range (μg/mL)	Cut-off (μg/mL)	Genotype	n° strains with gene/n°tested strains	Reference
L. reuteri	meat products	3	1/1 S**	*0,25*[c]	1[e]	*erm*(B)	1/3	Aquilanti et al. (2007)
	swine feces	1	1/1 R	*> 32*[c]	8[f]	*erm*(B)	1/1	Ouoba et al. (2008)
	different origins	32	6/32 R	*0,25 - > 256*[d]	ND	*erm*(B) *erm*(C) *erm*(T)	4/6 1/6 1/6	(Egervärn et al., 2009)
	Salami	1	1/1 R	*512*[c]	4[g]	*erm*(B)	1/1	Zonenschain et al. (2009)
	Salami	2	2/2 R	*256 - 512*[c]	1[h]	*erm*(B)	2/2	(Thumu e Halami, 2012)
	animal feces, vagina, cheese, sourdough, swine and human intestine, human saliva	18	3/18	0,016–16[c]	ND	*erm*(B)	1/3	Campedelli et al. (2019)
L. salivarius	meat products	6	2/3 R**	*0,25 - > 32*[c]	1[e]	*erm*(B)	3/6	Aquilanti et al. (2007)
	Salami	3	3/3 R	*64 - 256*[c]	≥1[l]	*erm*(B)	3/3	(Thumu e Halami, 2012)
	poultry meat and intestine, slaughter water	3	3/3 R	*4 - 128*[c]	ND	*erm*(B) *msrA/B*	2/3 1/3	Preethi et al. (2017)
	Cider, must, animal feces, rat and chicken intestines, saliva, slurry, vacuum-packed meat	27	5/27	*0,06 – 16*[c]	ND	-	0/5	Campedelli et al. (2019)
L. plantarum	green tea, fermented foods, insects	18	1/18 R	0,5 - 2[a]	1[g]	*erm*(A) *erm*(B) *erm*(C)	2/2 * 2/2 * 2/2 *	(Arellano et al., 2020)
	Silage	12	11/12 S 1/12 MS	> 14 mm [b]	≤13 mm	*erm*(B) *mef*(A)	3/12 1/12	(Anisimova e Yelin et al., 2019)
	meat products	11	6/6 S **	*0,125–0,25*[c]	1[e]	*erm*(B)	6/11	Aquilanti et al. (2007)
	Salami	12	6/12 R	*0,25–512*[c]	4[g]	*erm*(B) *erm*(C)	3/6 2/6	Zonenschain et al. (2009)
	ice cream	1	1/1 R	*32*[c]	1[h]	*erm*(B)	1/1	(Thumu e Halami, 2012)
	dairy products	11	11/11 S	*0,0625–0,25*[c]	1[i]	*erm*(B)	1/11	Guo et al. (2017)
	Salami	1	ND	ND	ND	*erm*(B), *erm*(C)	1/1	Todorov et al. (2017)
	poultry meat and intestine	10	10/10 R	*4 - 1024*[c]	ND	*erm*(B) *msr*A/B *msr*C	8/10 3/10 2/10	Preethi et al. (2017)
	pickles, beer contaminant, fermented vegetables	10	2/10	*0,25 – 16*[c]	ND	-	0/2	Campedelli et al. (2019)

R: resistant, S: susceptible, MS: moderatlely susceptible ND: not defined.
*: genotypic analysis performed only on 2 phenotypically susceptible strains.
**: phenotypic analysis carried out on the strains showing the related gene.
[a] MIC evaluated with agar dilution method (μg/mL).
[b] MIC evaluated with disk diffusion method (mm).
[c] MIC evaluated with broth microdilution method (μg/mL).
[d] MIC evaluated with E-Test (μg/mL).
[e] Cut-off MIC value for tetracycline defined by Danielsen and Wind (2003).
[f] Cut-off MIC value for tetracycline defined by Ouoba et al. (2008)..
[g] Cut-off MIC value for tetracycline defined by EFSA (2005)
[h] Cut-off MIC value for tetracycline defined by EFSA (2008)
[i] Cut-off MIC value for tetracycline defined by EFSA (2012).
[l] Cut-off MIC value for tetracycline defined by CLSI (2008)

by genes such as *bla*OXA-48 observed for the first time in *L. rhamnosus* (Hazlrolan et al., 2019), and *bla*OXA-1 in *L. brevis* subsp. *gravesensis*, *Lentilactobacillus buchneri*, and *L. fermentum* (Anisimova and Yarullina, 2019) (Table 10).

Resistance is not always linked to the presence of corresponding genes or β-lactam activity, with uncertainty about the mechanism that leads to the reduction of susceptibility in this microbial genus (Dec et al., 2018).

There is a lack of data in the literature regarding the spread of these determinants from lactobacilli to pathogenic or commensal microorganisms, stressing that further investigation would be desirable.

2.6. Ciprofloxacin

About 70% of the tested lactobacilli strains were reported to be resistant to ciprofloxacin, an antibiotic belonging to the fluoroquinolone family whose activity consists in the inhibition of DNA gyrase and DNA topoisomerase IV enzymes, interfering with DNA replication and subsequent microbial growth (Kapoor et al., 2017; Petersen and Jensen, 2004; Guo et al., 2017; Hummel et al., 2007; Yang and Yu, 2019). Moreover, the percentage grows up to 95% when the strains belong to the species *L. plantarum*, *L. reuteri*, *L. salivarius*, *L. brevis*, *L. fermentum,* and *L. rhamnosus* (Anisimova and Yarullina, 2019; Klein, 2011; Ouoba et al., 2008).

Table 6
Antibiotic resistance genes of the MLS group found in *Lactobacillus* spp. with efflux action.

Gene	Antibiotic	Species	Reference
mef(A)	Erythromycin	*L. salivarius* subsp. *salivarius*	Cauwerts et al. (2006b)
		L. plantarum	Anisimova and Yarullina (2019)
mef(B)	Erythromycin	*L. casei - maniotivorans*	Campedelli et al. (2019)
mef(E)	Erythromycin	*L. delbrueckii*	
msr(A/B)	Erythromycin	*L. plantarum*	Preethi et al. (2017)
		L. salivarius	
msr(C)	Erythromycin + Streptogramin B	*L. fermentum*	(Thumu e Halami, 2012)
		L. plantarum	Preethi et al. (2017)
lsa	Clindamycin	*L. delbrueckii*	Campedelli et al. (2019)
		L. reuteri	
		L. sakei	
		L. brevis	
		L. buchneri	
		L. casei	
		L. collinoides	
		L. coryniformis	
		C. alimentarius	

Table 7
Antibiotic resistance genes of the MLS group found in *Lactobacillus* spp. with an antibiotic inactivation action.

Gene	Antibiotic	Species	Reference
lnu (A)	clindamycin, lincomycin	*L. reuteri*	(Cauwerts et al., 2006b; Kastner et al., 2006)
vat (E)	streptogramin A, dalfopristin	*L. curvatus*	Todorov et al. (2019)
		L. fermentum	(Gfeller et al., 2003; Todorov et al., 2019)

Within the *Enterococcus* genus, resistance is mediated by the presence of amino acid modifications within the A subunit of the DNA gyrase encoded by the *gyr*A gene, and the C subunit of the topoisomerase IV encoded by the *par*C gene, to reduce the quinolone affinity for the enzymes. The mutations corresponding to this resistance consisted mainly in the substitutions of serine-83 with arginine (*Ser83-to-Arg*), glutamic acid-87 with glycine or lysine (*Glu87-to-Gly* or *Lys*) within the QRDR region (Quinolone Resistance-Determining Region) of the GyrA subunit, and the replacement of serine-80 with leucine or isoleucine (*Ser80-to-Leu* or *Ile*) in the ParC subunit (Petersen and Jensen, 2004). Several authors reported the presence of *gyr*A and *par*C genes within resistant lactobacilli (Table 11), but in none of the cases, the mutations corresponded to the typical ones described above (Anisimova and Yarullina, 2019; Ouoba et al., 2008), giving evidence of the presence of other amino acid substitutions in the DNA gyrase gene (Hummel et al., 2007; Li et al., 2015). However, it is uncertain whether these substitutions were the cause of the resistance. Consequently, the mechanism that controls resistance to ciprofloxacin in lactobacilli is still unclear.

Table 9
Vancomycin resistance genes found in *Lactobacillus* spp.

Gene	Coded Enzyme	*Species*	Reference
*van*A	D-alanine-D-lactate ligase	*L. curvatus*	(de Castilho et al., 2019; Todorov et al., 2019)
		L. fermentum	Todorov et al. (2019)
		L. reuteri	Dlamini et al. (2019)
		L. plantarum	(Arellano et al., 2020)
		L. garvieae	de Castilho et al. (2019)
vanB	D-alanine-D-lactate ligase	*L. curvatus*	(de Castilho et al., 2019; Todorov et al., 2019)
		L. plantarum	(Arellano et al., 2020)
vanC1	D-alanine-D-lactate ligase	*L. plantarum*	(Arellano et al., 2020; Todorov et al., 2017)
vanC2	D-alanine-D-lactate ligase	*L. curvatus*	(de Castilho et al., 2019; Todorov et al., 2019)
		L. delbrueckii	Todorov et al. (2019)
		L. fermentum	
		L. plantarum	(Arellano et al., 2020)
vanC2/C3	D-alanine-D-lactate ligase	*L. plantarum*	(Arellano et al., 2020)
vanX	D-alanyl-D-alanine dipeptidase	*L. brevis*	(Anisimova e Yelin et al., 2019)
		L. fermentum	
		L. casei	Guo et al. (2017)
		L. helveticus	
		L. plantarum	(Anisimova and Yarullina, 2019; Guo et al., 2017; Liu et al., 2009)

Table 8
Aminoglycosides resistance genes found in *Lactobacillus* spp.

Gene	Antibiotic	Phenotype	Species	Reference
aac(6′)-Ii	Aminoglycosides	*S*	*L. casei*	de Souza et al. (2019)
ant(4′)-Ia	Aminoglycosides	*S*	*L. casei*	de Souza et al. (2019)
		S	*L. fermentum*	
aph(2)-Ib	Amminoglicosidi	*R*	*L. plantarum*	Todorov et al. (2017)
		nd	*L. curvatus*	de Castilho et al. (2019)
aph(2″)-Ic	Aminoglycosides	*nd, R*	*L. curvatus*	(de Castilho et al., 2019; Todorov et al., 2019)
		R	*L. delbrueckii*	Todorov et al. (2019)
		R	*L. fermentum*	
		S	*L. casei*	de Souza et al. (2019)
aac(6′)-Ie-aph(2)-Ia	Aminoglycosides (Gentamicin)	*R*	*L. delbrueckii* subsp. *bulgaricus*	(Yang e Yang and Yu, 2019)
		R	*L. plantarum*	(Todorov et al., 2017; Yang e Yang and Yu, 2019)
		nd	*L. curvatus*	de Castilho et al. (2019)
aph(3′)-III	Aminoglycosides (Kanamycin/Neomycin)	*R*	*L. helveticus*	Guo et al. (2017)
		R	*L. paracasei*	Ouoba et al. (2008)
		nd	*L. cuvatus*	de Castilho et al. (2019)
		R, S	*L. casei*	(de Souza et al., 2019; Ouoba et al., 2008)
aadA	Aminoglycosides (Streptomycin)	*R*	*L. paracasei*	Ouoba et al. (2008)
		R	*L. casei*	
		R	*L. rahmnosus*	Anisimova and Yarullina (2019)
aadE	Aminoglycosides (Streptomycin)	*R*	*L. plantarum*	(Anisimova and Yarullina, 2019; Ouoba et al., 2008)
		R	*L. casei*	Ouoba et al. (2008)
ant(6)	Aminoglycosides (Streptomycin)	*R*	*L. delbrueckii* subsp. *bulgaricus*	Yang and Yu (2019)

R: resistant.
S: sensitive.
ND: not determined.

Table 10
Genes of resistance to β-lactam antibiotics found in *Lactobacillus* spp.

Gene	Coded Enzyme	Species	Reference
*bla*CTX-M	ESBL[a]	*Lactobacillus* spp.	Khan et al. (2019)
*bla*SHV	ESBL[a]	*L. brevis* ssp. *gravesensis* *L. buchneri* *L. plantarum*	Anisimova and Yarullina (2019)
*bla*TEM	ESBL[a]	*L. brevis* ssp. *gravesensis* *L. brevis* *L. buchneri* *L. rhamnosus* *L. fermentum* *L. plantarum*	Anisimova and Yarullina (2019)
*bla*Z	β-lactamase	*L. plantarum*	Aquilanti et al. (2007)
*bla*OXA-1	Carbapenemase	*L. brevis* ssp. *gravesensis* *L. buchneri* *L. fermentum*	Anisimova and Yarullina (2019)
*bla*OXA-48	Carbapenemase	*L. rhamnosus*	(Hazlrolan et al., 2019)

[a] ESBL = extended spectrum β-lactamase.

Table 11
Ciprofloxacin resistance genes found in *Lactobacillus* spp.

Gene	Species	Reference
gyrA	L. curvatus	Hummel et al. (2007)
	L. acidophilus	(Hummel et al., 2007; Ouoba et al., 2008)
	L. reuteri	Ouoba et al. (2008)
	L. plantarum	(Guo et al., 2017; Hummel et al., 2007; Ouoba et al., 2008)
	L. casei L. helveticus	Guo et al. (2017)
parC	L. acidophilus L. curvatus	Hummel et al. (2007)
	L. buchneri L. brevis L. brevis subsp. gravesensis	Anisimova and Yarullina (2019)
	L. plantarum	(Anisimova and Yarullina, 2019; Hummel et al., 2007)

2.7. Chloramphenicol

Generally, members of the *Lactobacillus* genus are susceptible to the action of chloramphenicol (Anisimova and Yarullina, 2019; de Souza et al., 2019; Kastner et al., 2006; Ouoba et al., 2008), which acts binding to the ribosomal 50 S subunit preventing bacterium protein synthesis (Kapoor et al., 2017). However, some studies reported MIC values for this antibiotic above the cut-offs, such as several isolates of *L. plantarum*, with MIC ≥16 μg/mL (Arellano et al., 2020), or a strain of *L. reuteri* for which MIC concentration reached 128 μg/mL (Egervärn et al., 2009). In support of the possible resistance to this antibiotic, Campedelli et al. (2019) found resistance to chloramphenicol in 49% of the 182 strains of *Lactobacillus* spp. tested.

Resistance depends on the inactivation of chloramphenicol usually driven by the presence of a chloramphenicol transacetylase enzyme, encoded by genes of the *cat* family (Chloramphenicol Acetyl Transferase) (Kapoor et al., 2017). The presence of these genes has been observed in strains of *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* isolated from yogurt, *L. plantarum* isolated from salami and green tea, and *L. reuteri* isolated from dog feces (Table 12). However, the phenotypic resistance was not always accompanied by the presence of the *cat* gene, and vice versa. Arellano et al. (2020) lit upon the *cat* gene in two strains of *L. plantarum* susceptible to the antibiotic, an outcome in agreement with the results obtained by Todorov et al. (2017). Similarly, the gene was present, but unexpressed, in one strain of *L. acidophilus* and one of *L. delbrueckii* subsp. *bulgaricus*, resulting in susceptibility to the antibiotic (Table 12), probably due to the presence of mutations in the regulatory region that prevent the expression of the gene (Hummel et al., 2007). The same authors also reported the opposite situation, observing how in *Lactiplantibacillus pentosus* and *L. plantarum* resistant to chloramphenicol, the *cat* gene was not present. A similar result was obtained from the analysis of 43 lactobacilli isolated from dairy products, more than half of which demonstrated resistance to the antibiotic, without however highlighting the related gene (Yang and Yu, 2019). In this case, the mechanism triggering the acquired resistance *cat* is not well understood.

As regards the problem of possible horizontal transfer, the presence of the gene on plasmids was observed, consequently, the possible movement cannot be excluded (Egervärn et al., 2009).

Table 12
Chloramphenicol resistance genes found in *Lactobacillus* spp.

Gene	Species	Phenotype	Reference
cat	*L. acidophilus*	S	Hummel et al. (2007)
	L. delbrueckii subs. bulgaricus	S	
	C. alimentarius	5/7 R	Campedelli et al. (2019)
	L. brevis	7/8 R	
	L. buchneri	1/1 S	
	L. casei maniotivorans	1/1 R	
	L. collinoides	1/4 R	
	L. plantarum	3/7 R	
	L. reuteri - vaccinostercus	1/2 R	
	L. sakei	1/2 R	
	L. saivarius	1/3 R	
*cat*A	*L. plantarum*	S	Todorov et al. (2017)
		S	(Arellano et al., 2020)
cat-TC	*L. reuteri*	R	(Egervärn et al., 2009)

S: susceptible.
R: resistant.

2.8. Other antibiotics - bacitracin, rifampicin, and sulfamethoxazole

Literature counts very few studies analyzing resistance and the presence of related resistance genes to antibiotics such as bacitracin, rifampicin, and sulfamethoxazole. In the case of bacitracin, resistance was observed in strains of *L. delbrueckii* subsp. *bulgaricus* and *L. plantarum* isolated from fermented milk (Liu et al., 2009), in a strain of *L. curvatus* isolated from salami (de Castilho et al., 2019), and in strains of *L. plantarum*, *L. pentosus*, *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, *L. sakei,* and *L. curvatus* (Danielsen and Wind, 2003). Some authors, although not evaluating the phenotypic resistance, highlighted in lactobacilli the presence of genes related to antibiotic resistance such as *bcr*B, *bcr*D, and *bcr*R (Table 13). *bcr*B encodes proteins necessary for the drug efflux, *bcr*D encodes an undecaprenol kinase, and *bcr*R identifies a presumed regulatory gene upstream of the *bcr*ABD operon (Manson et al., 2004). Of the cases reported in Table 13, only Arellano et al. (2020) observed the presence of all three genes of the operon in two strains of *L. plantarum*; in other cases, it would be useful to analyze the phenotypic result to assess whether the presence of a single gene of the operon can affect the susceptibility.

Concerning rifampicin, resistance is generally related to the presence

Table 13
Bacitracin resistance genes found in *Lactobacillus* spp.

Gene	Species	Phenotype	Reference
bcrB	*L. plantarum*	nd	(Arellano et al., 2020)
	L. curvatus	nd	de Castilho et al. (2019)
	L. casei	nd	(Casarotti et al., 2017; de Souza et al., 2019)
	L. fermentum	nd	de Souza et al. (2019)
bcrD	*L. plantarum*	nd	(Arellano et al., 2020)
bcrR	*L. plantarum*	nd	(Arellano et al., 2020)
	L. casei	nd	Casarotti et al. (2017)

Nd: not determined.

of mutations in the RRDR region (RIF Resistance-Determining Region) of the *rpo*B gene encoding the β subunit of RNA polymerase. In the strains with this mutation, the antibiotic will no longer be able to inhibit the bond between DNA and RNA polymerase, and protein synthesis proceeds (Ma et al., 2006). Strains of L. *plantarum* and *L. casei* showed resistance to rifampicin (Anisimova and Yarullina, 2019; Guo et al., 2017). In the literature, there is a lack of studies that evaluate the potential presence of mutations within the *rpo*B gene that induces resistance in lactobacilli.

In the case of sulfamethoxazole, resistance within the *Lactobacillus* genus is generally considered intrinsic, probably due to the structure of the cell wall and the impermeability of the membrane (Yang and Yu, 2019). This resistance was found in 27 strains of *L. reuteri*, *L. plantarum*, *L. fermentum*, *L. salivarius*, *L. acidophilus*, *L. rhamnosus*, *L. paracasei*, *L. casei,* and *L. delbrueckii* subsp. *bulgaricus* with MIC values even greater than 1024 μg/mL (Ouoba et al., 2008; Yang and Yu, 2019).

3. Virulence

Lactobacilli are generally considered non-pathogenic for humans. However, some clinical isolates belonging to this heterogenous group have been identified as responsible for health problems and diseases (especially in the case of individuals with previous medical conditions), thanks to the presence of genes capable of encoding virulence factors, which are detailed below.

3.1. Adhesion and aggregation factors

The ability to adhere to human tissues and the gastrointestinal tract is the first critical factor for the virulence of various pathogenic microorganisms. Regarding probiotic microorganisms and gut microbiota bacteria, it is usually a positive feature and defines a selection criterion in the evaluation of new probiotics (Casarotti et al., 2017; de Castilho et al., 2019; Dlamini et al., 2019). This property allows the colonization and maintenance of these microorganisms within the gastrointestinal tract. Lactobacilli produce different types of proteins able to adhere to different targets in human tissues and intestinal mucosae such as mucin, collagen, and fibronectin. Several genes coding for these proteins have been reported, as the mucin binding genes *mub* (mucus-binding-protein), *msa* (mannose-specific adhesin) (Arellano et al., 2020; de Castilho et al., 2019; Vélez et al., 2007), and genes encoding proteins able to bind fibrinogen, fibronectin, and collagen such as *fbp*B in *L. acidophilus* (Hymes et al., 2016), *fbp*A in *L. acidophilus* and *L. casei* (Muñoz-Provencio et al., 2010; Vélez et al., 2007), *cbs*A, *slp*A and *cn*BP in *L. crispatus*, *L. brevis* and *L. reuteri,* respectively (Vélez et al., 2007).

Although the ability to bind fibrinogen is generally considered positive, Collins et al. (2012) reported that *L. salivarius* CCUG_47,825 isolated from a case of septicemia was able, thanks to the presence of a specific protein, to bind fibrinogen and subsequently induce platelet aggregation to a comparable level with *Staphylococcus aureus*. The discovered protein gene was renamed CCUG_2371 (Table 14) and encodes a surface protein rich in serine with similar traits with proteins of *Clostridium perfringens* (38%), *Streptococcus infantarius* (34%), and *Corynebacterium diphtheria* (27%), whose expression is regulated by

Table 14
Virulence genes found in *Lactobacillus* spp.

Virulence factor	Gene	Species	Reference	Virulence factor	Gene	Species	Reference
Sex pheromone	*ccf*	*L.curvatus*	de Castilho et al. (2019)	Enterococcal surface protein	*esp*	*L. curvatus*	(de Castilho et al., 2019; Todorov et al., 2019)
		L. delbrueckii subsp. *bulgaricus*	Casarotti et al. (2017)			*L. casei*	Casarotti et al. (2017)
		L. casei				*L. mucosae*	de Moraes et al. (2017)
	cob	*L. plantarum*	Todorov et al. (2017)			*L. delbrueckii*	Todorov et al. (2019)
		L. delbrueckii subsp. *bulgaricus*	Casarotti et al. (2017)	Endocarditis antigen	*efaA*	*L. kefiri*	Soleymanzadeh et al. (2017)
	cpd	*L. plantarum*	Todorov et al. (2017)			*L. paracasei*	
		L. delbrueckii subsp. *bulgaricus*	Casarotti et al. (2017)			*L. gasseri*	
		L. casei	(Casarotti et al., 2017; de Souza et al., 2019)			*L. plantarum*	
Citolisine	*cylA*	*L. curvatus*	Todorov et al. (2019)			*L. mucosae*	de Moraes et al. (2017)
		L. fermentum	Casarotti et al. (2017)			*L. casei*	de Souza et al. (2019)
		L. casei				*L. fermentum*	
		L. mucosae	de Moraes et al. (2017)			*L. curvatus*	Todorov et al. (2019)
	cylB	*L. paraplantarum*	Soleymanzadeh et al. (2017)			*L. fermentum*	
		L. kefiri		Gelatinase	*gelE*	*L. gasseri*	Soleymanzadeh et al. (2017)
		L. paracasei				*L. plantarum*	(Soleymanzadeh et al., 2017; Todorov et al., 2014)
		L. gasseri				*L. casei*	Casarotti et al. (2017)
Adhesion	*ace*	*L. plantarum*	(Soleymanzadeh et al., 2017; Todorov et al., 2014, 2017)			*L. mucosae*	de Moraes et al. (2017)
		L. paracasei	Soleymanzadeh et al. (2017)			*Lb. delbrueckii*	(Casarotti et al., 2017; Todorov et al., 2019)
		L. curvatus	de Castilho et al. (2019)			*L curvatus*	Todorov et al. (2019)
	CCUG_0873	*L. salivarius 47,825*	Collins et al. (2012)		*fsrA*	*L. casei*	Casarotti et al. (2017)
	CCUG_2731				*fsrB*	*L. casei*	Casarotti et al. (2017)
	eno	*L. crispatus* ST1	Antikainen et al. (2007)		*fsrC*	*L. delbrueckii* subsp. *bulgaricus*	Casarotti et al. (2017)
		L. johnsonii F133				*L. casei*	
Aggregation	*agg*	*L. reuteri*	Dlamini et al. (2019)	Hyaluronidase	*hyl*	*L. mucosae*	de Moraes et al. (2017)
	asa1	*L. plantarum*	(Arellano et al., 2020; Todorov et al., 2014, 2017)			*L. plantarum*	Todorov et al. (2014)
		L. delbrueckii subsp. *bulgaricus*	Casarotti et al. (2017)			*L. delbrueckii* subsp. *bulgaricus*	Casarotti et al. (2017)
		L. curvatus	de Castilho et al. (2019)			*L. curvatus*	de Castilho et al. (2019)

another gene, CCUG_0873. Collagen binding is also a positive factor in probiotic microorganisms, but a factor associated with pathogenicity in pathogens. The bound is mediated by proteins, such as alpha enolases encoded by *eno* genes (Antikainen et al., 2007; Salzillo et al., 2015). These proteins, in pathogenic microorganisms such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, mediate the bound to fibronectin, collagen, and laminin and are also able to bind the plasmin activator (plasminogen), causing uncontrolled proteolysis inducing the possibility of host tissues invasion (Antikainen et al., 2007; Díaz-Ramos et al., 2012). α-enolases produced by a strain of *L. curvatus* isolated from chicken (*eno* 1, *eno* 2) an a strain of *L. johnsonii* isolated from calf feces (*eno*), shared functional similarity in the traits associated with virulence with α-enolase of *Streptococcus pyogenes*, *Streptococcus pneumoniae,* and *Staphylococcus aureus*, showing the same ability to activate plasminogen and bind laminin, a constituent of various tissues, including heart valves. Further studies are needed to better understand the potential risk connected with these findings (Antikainen et al., 2007).

In some cases, lactobacilli were associated with the presence of virulence genes related to adhesion and colonization properties of *Enterococcus* spp. (Table 14). This may be the result of gene transfer, as these factors are generally encoded by genes located in conjugative plasmids (Fisher and Phillips, 2009; Madsen et al., 2017). The *ace* gene, implicated in the pathogenesis of *E. faecalis,* was also observed in strains of *L. plantarum*, *L. paracasei,* and *L. curvatus* (Table 14). This gene encodes a surface protein with adhesive characteristics that mediates adhesion to extracellular matrix proteins, such as type I and IV collagen and laminin, (Chajęcka-Wierzchowska et al., 2017; Fisher and Phillips, 2009; Nallapareddy et al., 2000). As previously described, the ability to bind these compounds is usually not considered a negative factor for lactobacilli as they are involved in better adhesion and colonization of the gastrointestinal (GI) tract. However, a problem could be due to the presence of enterococcal genes associated with bacterial aggregation such as *agg* and *asa*1. These genes are located on the pAD1 pheromone-inducible plasmid and mediate the production of conjugative aggregates of cells to promote the exchange of genetic material (Chajęcka-Wierzchowska et al., 2017; Fisher and Phillips, 2009; Madsen et al., 2017). Consequently, the presence of these enterococcal genes in *L. plantarum*, *L. reuteri*, *L. delbrueckii* subsp. *bulgaricus* and *L. curvatus* (Table 14) can be of concern in terms of horizontal transfer of virulence or AR genes to opportunistic pathogens. Other virulence enterococcal genes found in *Lactobacillus* spp. are *efa*A, enterococcal antigen associated with endocarditis, and *esp*, encoding a surface protein, both involved in biofilm formation. *esp* is frequently observed in clinical isolates of *Enterococcus* spp. (Bittencourt De Marques and Suzart, 2004). It is involved in adhesion to human tissues, such as the GI tract and urinary tract, and can mediate the production of biofilm in *Enterococcus* spp. This property is strictly connected to adhesion and resistance to host defense mechanisms, such as phagocytosis, thus promoting pathogenicity (Fisher and Phillips, 2009; Hashem et al., 2017). It was observed in strains of *L. curvatus*, *L. casei, Limosilactobacillus mucosae,* and *L. delbrueckii* (Table 14). However, the adhesive properties conferred by this gene do not constitute a direct risk in a potential probiotic, but only in the case of transfer to other bacteria. In fact, the ability to adhere to intestinal epithelial cells and to inhibit pathogens' growth in the human gastrointestinal tract (GIT) through competitive exclusion is a desirable feature for probiotic bacteria, as it increases persistence in the GIT and the ability to effectively colonize the intestine (de Moraes et al., 2017; Casarotti et al., 2017). Similarly, the presence of the biofilm mediating *efa*A gene, which encodes a specific antigen of enterococci found in clinical isolates of endocarditis cases and urinary tract infections (Bittencourt De Marques and Suzart, 2004; Kafil and Mobarez, 2015), was found in strains of *Lentilactobacillus kefiri*, *L. paracasei*, *Lactobacillus gasseri*, *L. plantarum*, *L. mucosae*, *L. casei* and *Limosilactobacillus fermentum* (Table 14). Being specific of *Enterococcus* spp, the presence of these genes in lactobacilli is certainly the result of horizontal transfer. The problem, therefore, lies in the possibility of re-transfer of these genes to other potentially pathogenic bacteria rather than in the induced capacity to create biofilms.

3.2. Sex pheromones

As already mentioned, generally the presence of AR or virulence genes in lactobacilli it is due to a genetic acquisition mechanism mediated by the presence of mobile genetic elements such as plasmids. The ensuing concern is the possibility of retransfer of these determinants to commensal and pathogenic microorganisms. The presence of genes acquired from *Enterococcus* spp. coding for sex pheromones (*ccf*, *cob*, *cpd*) represents a potential hazard if transferred. Sex pheromones are small peptides produced by Gram-positive microorganisms that work as signal molecules able to mediate the quorum-sensing mechanism (Ali et al., 2017). Their presence induces conjugation processes mediated by pheromone-inducible plasmids (e.g. pAD1, pPD 1, pCF10), supporting the possibility of transferring determinants that could increase pathogenicity in some bacteria (Chajęcka-Wierzchowska et al., 2017; Eaton and Gasson, 2001). For example, the pAD1 plasmid can carry genes for hemolysin and aggregating substances (Madsen et al., 2017). Sex pheromones are produced by potential recipient cells and received by donor cells, which import the exogenous pheromone, causing the expression of genes involved in the conjugation process of the related pheromone-inducible plasmid. The expression of genes encoding aggregation substances (*agg*, *asa*1) present in plasmids is induced to promote bacterial aggregation by facilitating contact between donor and recipient and, consequently, the passage of the plasmid itself (Chajęcka-Wierzchowska et al., 2017; Eaton and Gasson, 2001; Fisher and Phillips, 2009). In this way, the strains containing the genes encoding the sex pheromones can acquire the corresponding plasmids and the associated virulence or AR determinants. Several lactobacilli highlighted the presence of the genes *ccf*, *cob,* and *cpd* encoding sex pheromones. They were detected in strains of *L. curvatus*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei,* and *L. plantarum* (Table 14). In some cases, these genes were observed in lactobacilli that also carried the *asa*1 gene responsible for bacterial aggregation activity (Casarotti et al., 2017; Todorov et al., 2017). The presence of these molecules may cause concern given the possibility of inducing the horizontal transfer mechanism by promoting the spread of potentially harmful determinants. Further studies are needed to evaluate the actual role of these molecules within lactobacilli, evaluating the possibility of the latter working as recipients of virulence genes.

3.3. Cytolysin toxin

Another factor related to the virulence of pathogenic microorganisms is the ability to produce cytolysin, an exotoxin with β-hemolytic activity. This molecule can be considered an antimicrobial (lantibiotic bacteriocin) given its action against various Gram-positive microorganisms, but also a virulence factor considering the activity against eukaryotic cells such as erythrocytes and human epithelial cells, inducing the onset of infections such as endocarditis (Cotter et al., 2005; Shankar et al., 2004).

This toxin consists of two subunits and its production is delegated to the combined action of eight genes (*cyl*R1, *cyl*R2, *cyl*LL, *cyl*LS, *cyl*M, *cyl*B, *cyl*A, and *cyl*I) transcribed as a single operon. Only if the entire operon is transcribed, the lytic activity is observed. Of the eight genes, *cyl*LS and *cyl*LL are required for the ribosomal synthesis of the small and large subunits, which subsequently undergo a post-translational modification by the *cyl*M gene creating the two products CylLS * and CylLL * with modifications characteristic of lantibiotic bacteriocins. The latter will then be secreted and proteolytically processed by the membrane transporter encoded by the *cyl*B gene creating the two CylLL 'CylLS' subunits, which will be again subjected to a proteolytic event catalyzed by a serine protease encoded by the *cyl*A gene, generating the two active subunits CylLL ″ CylLS ''. The product encoded by the *cyl*I gene allows

protection of the producer strain against the lytic activity itself. *cyl*R1 and *cyl*R2 function as repressors, encoding two proteins that repress the transcription of the operon (Shankar et al., 2004). The operon can be located on the same pAD1-sensitive plasmid carrying the *asa*1 adhesive gene (Chajęcka-Wierzchowska et al., 2017; Madsen et al., 2017) or within pathogenicity islands on the associated chromosome to other genes responsible for aggregation and adhesion, such as the *esp* gene (Shankar et al., 2004). Some operon genes described above were observed in lactobacilli. (Table 14). The *cyl*A gene was detected in strains of *L. curvatus*, L. *mucosae*, *L. fermentum,* and *L. casei* isolated from smoked salmon, goat milk, and buffalo mozzarella water, respectively, while *cyl*B gene in *L. paraplantarum*, *L. kefiri*, *L. paracasei,* and *L. gasseri* strains. The reported studies generally evaluated only the presence or absence of the *cyl*A gene without considering the entire operon. However, the activity is linked to several associated genes, as reported by Casarotti et al. (2017) who observed the presence of *cyl*A gene in a strain of *L. casei*, unable to induce hemolysis, as also reported by Todorov et al. (2019) who showed a negative phenotype for a strain of *L. curvatus* *cyl*A+. Soleymanzadeh et al. (2017) showed that of the four strains found to be *cyl*B + none exhibited hemolytic activity due to the absence of associated genes such as *cyl*A and *cyl*M.

In the case reported by de Moraes et al. (2017), the presence of *cyl*A gene, the only gene evaluated, was related to the hemolytic activity for a strain of *L. mucosae.* This does not mean that the mere presence of cylA induced the positive phenotype because other associated genes were not investigated. This suggests that in-depth research should evaluate the presence of all genes present in the operon. However, Casarotti et al. (2017) found the presence of three strains belonging to the species *L. delbrueckii* subsp. *bulgaricus* and a strain of *L. casei* able to induce partial hemolysis but none containing the *cyl*A gene, probably due to other lytic genes.

3.4. Gelatinase

Gelatinase is a zinc-dependent extracellular metalloendopeptidase and is considered a virulence factor as it contributes to the degradation of host tissues such as collagen, fibrin, and elastin to supply nutrients to the cell favoring invasion, and it is also involved in biofilm formation (Chajęcka-Wierzchowska et al., 2017; Fisher and Phillips, 2009; Hashem et al., 2017). It is encoded by the *gel*E gene and generally secreted by clinical isolates belonging to *Enterococcus faecalis* and *Enterococcus faecium,* but it was also found in samples obtained from dairy products (Lopes et al., 2006). It was observed that this peptidase contributed to various disorders such as peritonitis, ulcerative colitis, endocarditis, and irritable bowel disorder in humans and mice (Ali et al., 2017). The *gel*E gene was discovered in isolates of *L. curvatus* and *L. delbrueckii* from smoked salmon (Todorov et al., 2019), *L. mucosae*, *L. gasseri,* and *L. plantarum* isolated from milk samples (de Moraes et al., 2017; Soleymanzadeh et al., 2017), *L. casei* and *L. delbrueckii* subsp. *bulgaricus* from buffalo mozzarella water (Casarotti et al., 2017) and *L. plantarum* isolated from papaya (Todorov et al., 2014) (Table 14). However, the presence of the *gel*E gene alone is not directly correlated to gelatinase activity (de Moraes et al., 2017; Soleymanzadeh et al., 2017; Todorov et al., 2019). This is because the expression of this gene is regulated by a quorum-sensing mechanism driven by the *fsr* locus. This mechanism can induce the transcription of specific genes based on the presence and relative concentration in the extracellular space of specific inducing molecules. The *fsr* locus is placed directly in contact with the virulence gene and is made up of three genes: *fsr*A, *fsr*B, *fsr*C. The first works as a regulator for the expression of the *gel*E gene, while *fsr*B encodes a transmembrane transporter able to process and produce the inducing molecule GBAP (Gelatinase Biosynthesis Activating Pheromone), which accumulates in the environment. When the inducer reaches a threshold concentration, the transmembrane histidine protein kinase FsrC, encoded by the *fsr*C gene, perceives the signal induced by GBAP and, after being subjected to phosphorylation, activates the FsrA regulator, which will then subsequently activate the transcription of gelatinase (Ali et al., 2017; Fisher and Phillips, 2009). Therefore, the presence of the entire operon is required to obtain a positive GEL + phenotype (Lopes et al., 2006). The same authors underlined how laboratory manipulation, resulting in the loss of the *fsr* operon caused the consecutive loss of gelatinase activity in several enterococcal isolates. Among the published studies, only Casarotti et al. (2017) evaluated the presence of the *gel*E-associated *fsr*A, *fsr*B, and *fsr*C genes in one strain *of L. fermentum*, six strains of *L. casei,* and three strains of *L. delbrueckii* subsp. *bulgaricus* (Table 14), but none of the strains showed the presence of the entire operon.

3.5. Hyaluronidase

Hyaluronidase is a degradative enzyme, able to depolymerize the mucopolysaccharides that constitute the human connective tissue, such as hyaluronic acid and cartilage, to provide nourishment to pathogens and promote its diffusion inside the host (Chajęcka-Wierzchowska et al., 2017; Fisher and Phillips, 2009). These enzymes are produced by species belonging to the genera *Staphylococcus* spp, *Streptococcus* spp, *Clostridium* spp. and *Enterococcus* spp. The corresponding *hyl* gene encoding the hyaluronidase enzyme was found mainly in clinical isolates of *E. faecium*, and food isolates of *Enterococcus casseliflavus*, *Enterococcus mundtii,* and *Enterococcus durans* (Chajęcka-Wierzchowska et al., 2017; Van Reenen and Dicks, 2011). Although there is no evidence in the literature that lactobacilli produces this enzyme, the *hyl* gene was found in strains of *L. mucosae*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus* and *L. curvatus* isolated from food (Table 14). The gene presence didn't always result in virulence induction, so the expression evaluation is fundamental. However, the major concern is the possible re-transfer of the gene to pathogenic microorganisms since, in clinical isolates of *E. faecium*, the *hyl* gene is positioned on a conjugative plasmid (Van Reenen and Dicks, 2011).

3.6. Glycosidase and arylamidase

As previously reported, lactobacilli were responsible for infectious endocarditis (Aaron et al., 2017; Martinez et al., 2014; Patnaik et al., 2015; Stroupe et al., 2017; Zeba et al., 2018). This disorder involves bacterial infection of the endocardial surface through initial bacteremia induced by bacteria that can subsequently adhere to and colonize the tissue of the heart valve (fibrinogen, laminin, collagen). Consequently, the formation of the so-called vegetations, infected masses formed by the deposition of successive cycles of platelet aggregations, which create a protective matrix for the microorganisms capable of proliferating, occurs (Holland et al., 2017).

It was observed that some species of lactobacilli (*L. rhamnosus*, *L. paracasei*, *L. casei*, *L. salivarius*) had factors favoring the potential colonization of cardiac tissue by adhering to collagen, fibronectin, and fibrinogen, and mediating platelet aggregation (Collins et al., 2012; Harty et al., 1993, 1994; Kirjavainen et al., 1999). Colonization depends also on other factors, such as the ability to obtain nutrients and to evade the host's defense mechanisms. In this regard, Oakey et al. (1995) observed the ability to produce characteristic enzymes, as glycosidase and arylamidase in lactobacilli isolates from cases of endocarditis (Table 15). They highlighted that the combination of *N*-acetyl-β-D-glucosaminidase and α-D-galactosidase production was a characteristic feature of clinical isolates. Their action is delegated to the release of carbohydrates from glycoproteins allowing the inflow of nutrients to the bacterium. They also observed the presence of arylamidase with fibrinolytic and pro-and anticoagulant activity, with its main activities in the evasion of host defense mechanisms. Among these, the presence of (Ca)-like C protein, a human physiological anticoagulant with fibrinolytic activity able to convert plasminogen into plasmin causing the invasion of vegetation (Esmon, 1983) was found. The same activity was obtained by the presence of a protein, produced by

Table 15
Enzymes found in *Lactobacillus* spp. associated with infectious endocarditis.

Enzyme		Role	Species	Reference
Glycosidase	*N-acetyl-β-D-glucosaminidase*	Release carbohydrates from glycoproteins favoring the inflow of nutrients to the bacterium, an important factor for the microbial colonization of thrombotic vegetation	*L. rhamnosus* L. *paracasei* *L. acidophilus*	Oakey et al. (1995)
	α-D-galattosidase			
Protease (Arylamidase)	*Activated factor X*	Pro-coagulant activity		
	(Xa)-like	Aids evasion from the host's defense mechanism		
	Activated protein C	Fibrinolithic activity		
	(Ca)-like	Aids evasion from the host's defense mechanism		
	Hageman factor- like	Pro-coagulant activity		
	Kallikrein-like	Fibrinolithic and indirect ro-coagulant activity		

clinically isolated strains of *L. rhamnosus* and *L. paracasei,* similar to the proteolytic enzyme of plasma kallikrein origin, which is also able to activate the Hageman factor (XII), the zymogenic form of a serine protease with action in the early stages of coagulation (Tans et al., 1983), thus inducing pro-coagulant activity. This ability determines the expansion of the vegetation around the bacterium giving more resistance from the host's defense mechanisms. The pro-coagulant activity has also been associated with the presence of an Xa-like activated factor, a plasma glycoprotein involved in blood clotting responsible for the conversion of prothrombin into thrombin, which subsequently catalyzes the conversion of fibrinogen into fibrin, a component of the clot (Mertens and Bertina, 1980).

4. Conclusions

Antibiotic resistence and virulence factors are important aspects to be considerend in lactic acid bacteria due to their important role in food production. After a critical reading, it emerged that several studies highlighted the lack of standards in the field of susceptibility testing and the relative definition of the cut-offs for different species of lactobacilli (Anisimova and Yarullina, 2019; Aquilanti et al., 2007; Hummel et al., 2007; Połka et al., 2016; Sanders et al., 2010; Yang and Yu, 2019). As regards the tests for the assessment of susceptibility, for example, possible interferences in the determination of the MIC value related to the medium used were underlined (Huys et al., 2002). Klare et al. (2005) developed and tested a specific medium (LSM) for lactic acid bacteria (LAB) able to provide optimal results in terms of growth support and a correct indication of the MIC value. However, the latter has not been included in a standardized method, consequently, in many studies, the De Man, Rogosa, and Sharpe (MRS) medium is still used, of which components can cause potential interference with specific antibiotics such as trimethoprim and sulfonamides (Klare et al., 2005). Furthermore, the low pH of the MRS medium (pH 6.2 ± 0.2) may be responsible for the reduction of the activity of some antibiotics such as aminoglycosides (optimal pH 7.8), while the pH of the LMS medium, adjusted to pH 6.8, appears not to interfere (Klare et al., 2005, 2007). A further problem in the sensitivity analysis is the determination of the MIC cut-off values of different lactobacilli, which is important since it can affect the decision to consider a bacterium susceptible or resistant. Hummel et al. (2007) underline this problem by presenting discordant results regarding the antibiotic resistance of various lactic bacteria considering the cut-offs dictated by FEEDAP (2005), and European Commission (2002), and Danielsen and Wind (2003).

Lactobacilli are generally considered to be more resistant to aminoglycosides and vancomycin and susceptible to erythromycin, β-lactam antibiotics, chloramphenicol, and tetracycline. However, strains resistant to the latter antibiotics have also been identified with the presence of the related genes. Resistance to tetracycline, erythromycin, and aminoglycosides are the most discussed, and the *tet*(M) and *erm*(B) genes the most observed, studied and evaluated also considering their possible transfer. From the analyzed data, it emerged that *L. plantarum* is one of the most documented species characterized by different resistance traits and by the presence of the corresponding potentially transmissible genes. It is also important to remember the possible presence, within the same strain, of resistance to multiple antibiotics, as highlighted by Campedelli et al. (2019) who noted multiple resistance in 152 of the 182 strains analyzed (84%). Finally, for a complete evaluation of the safety of lactobacilli, it would be necessary to take into consideration all the factors reported above, both virulence traits and AR.

Considering AR, it is important to investigate on all the possible determinants to avoid confusing an extrinsic resistance, with the relative concern of the possibility of transfer, with an intrinsic resistance, much less problematic.

Regarding virulence factors, although the known genetic determinants of *lactobacilli* are limited, in this review it is exposed that there is the possibility of acquiring virulence genes from pathogenic microorganisms, with the possibility of consequent clinical problems, in particular in immonocompromised patients.

As reported by EFSA, whole genome sequencing can be a valid technique to screen for bacteria intentionally used in the food chain (EFSA, 2021) but to date, there is no specific genetic database for lactobacilli associated with a safety assessment, and existing databases tend to focus primarily on pathogens. For example, in some studies, the VFDB database (Virulence Factors of Pathogenic Bacteria) (Liu et al., 2019) built to analyze virulence factors in genomes of 32 well-established human pathogen genera of which genomes are not part of *Lactobacillus* spp, is mistakenly used. This leads to misleading results, as factors that can induce virulence in certain pathogens are not necessarily given the same result about lactobacilli. For example, Zhang et al. (2012) conducted the safety assessment of L. plantarum JDM1 using this database, resulting in the presence of 126 virulence genes, subsequently not considered problematic as they do not encode toxins or proteins of invasion. This underlines the usefulness of the presence of potential virulence and AR-related gene lists for lactobacilli.

Declaration of competing interest

The authors have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

Aaron, J.G., Sobieszczyk, M.E., Weiner, S.D., Whittier, S., Lowy, F.D., 2017. Lactobacillus rhamnosus endocarditis after upper endoscopy. Open Forum Infect. Dis. 4, 4–6. https://doi.org/10.1093/ofid/ofx085.

Ali, L., Goraya, M.U., Arafat, Y., Ajmal, M., Chen, J.L., Yu, D., 2017. Molecular mechanism of quorum-sensing in Enterococcus faecalis: its role in virulence and therapeutic approaches. Int. J. Mol. Sci. 18 https://doi.org/10.3390/ijms18050960.

Ammor, M.S., Flórez, A.B., Van Hoek, A.H.A.M., De Los Reyes-Gavilán, C.G., Aarts, H.J. M., Margolles, A., Mayo, B., 2007. Molecular characterization of intrinsic and acquired antibiotic resistance in lactic acid bacteria and bifidobacteria. J. Mol. Microbiol. Biotechnol. 14, 6–15. https://doi.org/10.1159/000106077.

Ammor, M.S., Gueimonde, M., Danielsen, M., Zagorec, M., Van Hoek, A.H.A.M., De Los Reyes-Gavilán, C.G., Mayo, B., Margolles, A., 2008. Two different tetracycline resistance mechanisms, plasmid-carried tet(L) and chromosomally located transposon-associated tet(M), coexist in Lactobacillus sakei rits 9. Appl. Environ. Microbiol. 74, 1394–1401. https://doi.org/10.1128/AEM.01463-07.

Anisimova, E.A., Yarullina, D.R., 2019. Antibiotic resistance of LACTOBACILLUS strains. Curr. Microbiol. 76, 1407–1416. https://doi.org/10.1007/s00284-019-01769-7.

Antikainen, J., Kuparinen, V., Lähteenmäki, K., Korhonen, T.K., 2007. Enolases from Gram-positive bacterial pathogens and commensal lactobacilli share functional similarity in virulence-associated traits. FEMS Immunol. Med. Microbiol. 51, 526–534. https://doi.org/10.1111/j.1574-695X.2007.00330.x.

Aquilanti, L., Garofalo, C., Osimani, A., Silvestri, G., Vignaroli, C., Clementi, F., 2007. Isolation and molecular characterization of antibiotic-resistant lactic acid bacteria from poultry and swine meat products. J. Food Protect. 70, 557–565. https://doi.org/10.4315/0362-028X-70.3.557.

Arellano, K., Vazquez, J., Park, H., Lim, J., Ji, Y., Kang, H.J., Cho, D., Jeong, H.W., Holzapfel, W.H., 2020. Safety evaluation and whole-genome annotation of lactobacillus plantarum strains from different sources with special focus on isolates from green tea. Probiotics antimicrob. Proteins 12, 1057–1070. https://doi.org/10.1007/s12602-019-09620-y.

Bittencourt De Marques, E.B., Suzart, S., 2004. Occurrence of virulence-associated genes in clinical Enterococcus faecalis strains isolated in Londrina, Brazil. J. Med. Microbiol. 53, 1069–1073. https://doi.org/10.1099/jmm.0.45654-0.

Broaders, E., Gahan, C.G.M., Marchesi, J.R., 2013. Mobile genetic elements of the human gastrointestinal tract: potential for spread of antibiotic resistance genes. Gut Microb. 4, 271–280. https://doi.org/10.4161/gmic.24627.

Campagne, J., Guichard, J.F., Moulhade, M.C., Kawski, H., Maurier, F., 2020. Lactobacillus endocarditis: a case report in France and literature review. IDCases 21, e00811. https://doi.org/10.1016/j.idcr.2020.e00811.

Campedelli, I., Mathur, H., Salvetti, E., Rea, M.C., Torriani, S., Ross, R.P., Hill, C., O'Toole, P.W., 2019. Genus-wide assessment of antibiotic resistance in lactobacillus spp. Appl. Enveron. Microbiol. 85, 1–21. https://doi.org/10.1128/AEM.01738-18.

Cannon, J.P., Lee, T.A., Bolanos, J.T., Danziger, L.H., 2005. Pathogenic relevance of Lactobacillus: a retrospective review of over 200 cases. Eur. J. Clin. Microbiol. Infect. Dis. 24, 31–40. https://doi.org/10.1007/s10096-004-1253-y.

Casarotti, S.N., Carneiro, B.M., Todorov, S.D., Nero, L.A., Rahal, P., Penna, A.L.B., 2017. In vitro assessment of safety and probiotic potential characteristics of Lactobacillus strains isolated from water buffalo mozzarella cheese. Ann. Microbiol. 67, 289–301. https://doi.org/10.1007/s13213-017-1258-2.

Cauwerts, K., Pasmans, F., Devriese, L.A., Haesebrouck, F., Decostere, A., 2006a. Cloacal Lactobacillus isolates from broilers often display resistance toward tetracycline antibiotics. Microb. Drug Resist. 12, 284–288. https://doi.org/10.1089/mdr.2006.12.284.

Cauwerts, K., Pasmans, F., Devriese, L.A., Martel, A., Haesebrouck, F., Decostere, A., 2006b. Cloacal Lactobacillus isolates from broilers show high prevalence of resistance towards macrolide and lincosamide antibiotics. Avian Pathol. 35, 160–164. https://doi.org/10.1080/03079450600598137.

Chajęcka-Wierzchowska, W., Zadernowska, A., Łaniewska-Trokenheim, Ł., 2017. Virulence factors of Enterococcus spp. presented in food. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 75, 670–676. https://doi.org/10.1016/j.lwt.2016.10.026.

Chang, Y.C., Tsai, C.Y., Lin, C.F., Wang, Y.C., Wang, I.K., Chung, T.C., 2011. Characterization of tetracycline resistance lactobacilli isolated from swine intestines at western area of Taiwan. Anaerobe 17, 239–245. https://doi.org/10.1016/j.anaerobe.2011.08.001.

Chery, J., Dvoskin, D., Morato, F.P., Fahoum, B., 2013. Lactobacillus fermentum, a pathogen in documented cholecystitis. Int. J. Surg. Case Rep. 4, 662–664. https://doi.org/10.1016/j.ijscr.2013.04.034.

Collins, J., van Pijkeren, J.P., Svensson, L., Claesson, M.J., Sturme, M., Li, Y., Cooney, J. C., van Sinderen, D., Walker, A.W., Parkhill, J., Shannon, O., O'Toole, P.W., 2012. Fibrinogen-binding and platelet-aggregation activities of a Lactobacillus salivarius septicaemia isolate are mediated by a novel fibrinogen-binding protein. Mol. Microbiol. 85, 862–877. https://doi.org/10.1111/j.1365-2958.2012.08148.x.

Cotter, P.D., Hill, C., Ross, R.P., 2005. Food microbiology: bacteriocins: Developing innate immunity for food. Nat. Rev. Microbiol. 3, 777–788. https://doi.org/10.1038/nrmicro1273.

Danielsen, M., Wind, A., 2003. Susceptibility of Lactobacillus spp. to antimicrobial agents. Int. J. Food Microbiol. 82, 1–11. https://doi.org/10.1016/S0168-1605(02)00254-4.

Darbro, B.W., Petroelje, B.K., Doern, G.V., 2009. Lactobacillus delbrueckii as the cause of urinary tract infection. J. Clin. Microbiol. 47, 275–277. https://doi.org/10.1128/JCM.01630-08.

de Castilho, N.P.A., Nero, L.A., Todorov, S.D., 2019. Molecular screening of beneficial and safety determinants from bacteriocinogenic lactic acid bacteria isolated from Brazilian artisanal calabresa. Lett. Appl. Microbiol. 69, 204–211. https://doi.org/10.1111/lam.13194.

de Moraes, G.M.D., de Abreu, L.R., do Egito, A.S., Salles, H.O., da Silva, L.M.F., Nero, L. A., Todorov, S.D., dos Santos, K.M.O., 2017. Functional properties of lactobacillus mucosae strains isolated from Brazilian goat milk. Probiotics Antimicrob. Proteins 9, 235–245. https://doi.org/10.1007/s12602-016-9244-8.

de Souza, B.M.S., Borgonovi, T.F., Casarotti, S.N., Todorov, S.D., Penna, A.L.B., 2019. Lactobacillus casei and lactobacillus fermentum strains isolated from mozzarella cheese: probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. Probiotics Antimicrob. Proteins 11, 382–396. https://doi.org/10.1007/s12602-018-9406-y.

Dec, M., Nowaczek, A., Stępień-Pyśniak, D., Wawrzykowski, J., Urban-Chmiel, R., 2018. Identification and antibiotic susceptibility of lactobacilli isolated from turkeys. BMC Microbiol. 18, 1–14. https://doi.org/10.1186/s12866-018-1269-6.

Deghorain, M., Goffin, P., Fontaine, L., Mainardi, J.L., Daniel, R., Errington, J., Hallet, B., Hols, P., 2007. Selectivity for D-lactate incorporation into the peptidoglycan precursors of Lactobacillus plantarum: role of Aad, a VanX-like D-alanyl-D-alanine dipeptidase. J. Bacteriol. 189, 4332–4337. https://doi.org/10.1128/JB.01829-06.

Devirgiliis, C., Coppola, D., Barile, S., Colonna, B., Perozzi, G., 2009. Characterization of the Tn916 conjugative transposon in a food-borne strain of Lactobacillus paracasei. Appl. Environ. Microbiol. 75, 3866–3871. https://doi.org/10.1128/AEM.00589-09.

Díaz-Ramos, À., Roig-Borrellas, A., García-Melero, A., López-Alemany, R., 2012. α-enolase, a multifunctional protein: its role on pathophysiological situations. J. Biomed. Biotechnol. https://doi.org/10.1155/2012/156795, 2012.

Dlamini, Z.C., Langa, R.L.S., Aiyegoro, O.A., Okoh, A.I., 2019. Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. Probiotics Antimicrob. Proteins 11, 397–402. https://doi.org/10.1007/s12602-018-9430-y.

Drago, L., Rodighiero, V., Mattina, R., Toscano, M., De Vecchi, E., 2011. In vitro selection and transferability of antibiotic resistance in the probiotic strain lactobacillus reuteri DSM 17938. J. Chemother. 23, 371–373. https://doi.org/10.1179/joc.2011.23.6.371.

Eaton, T.J., Gasson, M.J., 2001. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. 67, 1628–1635. https://doi.org/10.1128/AEM.67.4.1628-1635.2001.

EFSA, 2021. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. EFSA J 19. https://doi.org/10.2903/j.efsa.2021.6506.

EFSA, 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 10, 1–10. https://doi.org/10.2903/j.efsa.2012.2740.

EFSA, 2007. Introduction of a qualified presumption of safety (QPS) approach for assessment of selected microorganisms referred to EFSA - opinion of the scientific committee. EFSA J 5, 1–16. https://doi.org/10.2903/j.efsa.2007.587.

Egervärn, M., Lindmark, H., Olsson, J., Roos, S., 2010. Transferability of a tetracycline resistance gene from probiotic Lactobacillus reuteri to bacteria in the gastrointestinal tract of humans. Antonie van Leeuwenhoek. Int. J. Gen. Mol. Microbiol. 97, 189–200. https://doi.org/10.1007/s10482-009-9401-0.

Egervärn, M., Roos, S., Lindmark, H., 2009. Identification and characterization of antibiotic resistance genes in Lactobacillus reuteri and Lactobacillus plantarum. J. Appl. Microbiol. 107, 1658–1668. https://doi.org/10.1111/j.1365-2672.2009.04352.x.

Esmon, C.T., 1983. Protein-C: biochemistry, physiology, and clinical implications. Blood 62, 1155–1158.

European Commission, 2002. Opinion of the Scientific Committee on Ani- Mal Nutrition on the Criteria for Assessing the Safety of Micro-organisms Resistant to Antibiotics of Human Clinical and Veterinary Importance. Euro- pean Commission, Health and Consumer Protection Directorate G.

Feedap, 2005. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance. EFSA J 3, 1–12. https://doi.org/10.2903/j.efsa.2005.223.

Feld, L., Bielak, E., Hammer, K., Wilcks, A., 2009. Characterization of a small erythromycin resistance plasmid pLFE1 from the food-isolate Lactobacillus plantarum M345. Plasmid 61, 159–170. https://doi.org/10.1016/j.plasmid.2009.01.002.

Fisher, K., Phillips, C., 2009. The ecology, epidemiology and virulence of Enterococcus. Microbiology 155, 1749–1757. https://doi.org/10.1099/mic.0.026385-0.

Founou, L.L., Founou, R.C., Essack, S.Y., 2016. Antibiotic resistance in the food chain: a developing country-perspective. Front. Microbiol. 7, 1–19. https://doi.org/10.3389/fmicb.2016.01881.

Gevers, D., Danielsen, M., Huys, G., Swings, J., 2003a. Molecular characterization of tet (M) genes in Lactobacillus isolates from different types of fermented dry sausage. Appl. Environ. Microbiol. 69, 1270–1275. https://doi.org/10.1128/AEM.69.2.1270-1275.2003.

Gevers, D., Huys, G., Swings, J., 2003b. In vitro conjugal transfer of tetracycline resistance from Lactobacillus isolates to other Gram-positive bacteria. FEMS Microbiol. Lett. 225, 125–130. https://doi.org/10.1016/S0378-1097(03)00505-6.

Gfeller, K.Y., Roth, M., Meile, L., Teuber, M., 2003. Sequence and genetic organization of the 19.3-kb erythromycin- and dalfopristin-resistance plasmid pLME300 from Lactobacillus fermentum ROT1. Plasmid 50, 190–201. https://doi.org/10.1016/j.plasmid.2003.08.001.

Groga-Bada, P., Mueller, I.I., Foschi, F., Gawaz, M., Eick, C., 2018. Mitral valve endocarditis due to lactobacillus. Case Rep. Med. 38–40. https://doi.org/10.1155/2018/8613948, 2018.

Guo, H., Pan, L., Li, L., Lu, J., Kwok, L., Menghe, B., Zhang, H., Zhang, W., 2017. Characterization of antibiotic resistance genes from lactobacillus isolated from traditional dairy products. J. Food Sci. 82, 724–730. https://doi.org/10.1111/1750-3841.13645.

Hammad, A.M., Shimamoto, T., 2010. Towards a compatible probiotic-antibiotic combination therapy: assessment of antimicrobial resistance in the Japanese probiotics. J. Appl. Microbiol. 109, 1349–1360. https://doi.org/10.1111/j.1365-2672.2010.04762.x.

Harty, D.W.S., Oakey, H.J., Patrikakis, M., Hume, E.B.H., Knox, K.W., 1994. Pathogenic potential of lactobacilli. Int. J. Food Microbiol. 24, 179–189. https://doi.org/10.1016/0168-1605(94)90117-1.

Harty, D.W.S., Patrikakis, M., Hume, E.B.H., Oakey, H.J., Knox, K.W., 1993. The aggregation of human platelets by Lactobacillus species. J. Gen. Microbiol. 139, 2945–2951. https://doi.org/10.1099/00221287-139-12-2945.

Hashem, Y.A., Amin, H.M., Essam, T.M., Yassin, A.S., Aziz, R.K., 2017. Biofilm formation in enterococci: genotype-phenotype correlations and inhibition by vancomycin. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-05901-0.

Hazlrolan, G., Gündoğdu, A., Nigiz, S., Altun, B., Gür, D., 2019. Presence of OXA-48 gene in a clinical isolate of lactobacillus rhamnosus. Foodb. Pathog. Dis. 16, 840–843. https://doi.org/10.1089/fpd.2019.2651.

Holland, T.L., Baddour, L.M., Bayer, A.S., Hoen, B., Miro, J.M., Fowler, V.G., 2017. Infective endocarditis HHS public access. Nat. Rev. Dis. Prim. 2, 1–49. https://doi.org/10.1038/nrdp.2016.59.Infective.

Hummel, A.S., Hertel, C., Holzapfel, W.H., Franz, C.M.A.P., 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl. Environ. Microbiol. 73, 730–739. https://doi.org/10.1128/AEM.02105-06.

Huys, G., D'Haene, K., Swings, J., 2002. Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. Lett. Appl. Microbiol. 34, 402–406. https://doi.org/10.1046/j.1472-765X.2002.01109.x.

Hymes, J.P., Johnson, B.R., Barrangou, R., Klaenhammer, T.R., 2016. Functional analysis of an S-layer-associated fibronectin-binding protein in Lactobacillus acidophilus NCFM. Appl. Environ. Microbiol. 82, 2676–2685. https://doi.org/10.1128/AEM.00024-16.

Imperial, I.C.V.J., Ibana, J.A., 2016. Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. Front. Microbiol. 7, 1–10. https://doi.org/10.3389/fmicb.2016.01983.

Kafil, H.S., Mobarez, A.M., 2015. Assessment of biofilm formation by enterococci isolates from urinary tract infections with different virulence profiles. J. King Saud Univ. Sci. 27, 312–317. https://doi.org/10.1016/j.jksus.2014.12.007.

Kapoor, G., Saigal, S., Elongavan, A., 2017. Action and resistance mechanisms of antibiotics: a guide for clinicians. J. Anaesthesiol. Clin. Pharmacol. 33, 46–50. https://doi.org/10.4103/joacp.JOACP.

Kastner, S., Perreten, V., Bleuler, H., Hugenschmidt, G., Lacroix, C., Meile, L., 2006. Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food. Syst. Appl. Microbiol. 29, 145–155. https://doi.org/10.1016/j.syapm.2005.07.009.

Khan, U., Afsana, S., Kibtia, M., Hossain, M., Choudhury, N., Ahsan, C.R., 2019. Presence of blactx-m antibiotic resistance gene in lactobacillus spp. Isolated from hirschsprung diseased infants with stoma. J. Infect. Dev. Ctries. 13, 426–433. https://doi.org/10.3855/jidc.10968.

Kirjavainen, P.V., Tuomola, E.M., Crittenden, R.G., Ouwehand, A.C., Harty, D.W.S., Morris, L.F., Rautelin, H., Playne, M.J., Donohue, D.C., Salminen, S.J., 1999. In vitro adhesion and platelet aggregation properties of bacteremia- associated lactobacilli. Infect. Immun. 67, 2653–2655. https://doi.org/10.1128/iai.67.5.2653-2655.1999.

Klare, I., Konstabel, C., Müller-Bertling, S., Reissbrodt, R., Huys, G., Vancanneyt, M., Swings, J., Goossens, H., Witte, W., 2005. Evaluation of new broth media for microdilution antibiotic susceptibility testing of lactobacilli, pediococci, lactococci, and bifidobacteria. Appl. Environ. Microbiol. 71, 8982–8986. https://doi.org/10.1128/AEM.71.12.8982-8986.2005.

Klare, I., Konstabel, C., Werner, G., Huys, G., Vankerckhoven, V., Kahlmeter, G., Hildebrandt, B., Müller-Bertling, S., Witte, W., Goossens, H., 2007. Antimicrobial susceptibilities of Lactobacillus, Pediococcus and Lactococcus human isolates and cultures intended for probiotic or nutritional use. J. Antimicrob. Chemother. 59, 900–912. https://doi.org/10.1093/jac/dkm035.

Klein, G., 2011. Antibiotic resistance and molecular characterization of probiotic and clinical Lactobacillus strains in relation to safety aspects of probiotics. Foodb. Pathog. Dis. 8, 267–281. https://doi.org/10.1089/fpd.2010.0672.

Leclercq, R., 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. 34, 482–492. https://doi.org/10.1086/324626.

Lee, C., Wongjarupong, N., Narayan, M., Melzer, A.C., 2020. Acute cholecystitis complicated with gallbladder perforation and peritonitis caused by vancomycin-resistant lactobacillus paracasei. Cureus 12. https://doi.org/10.7759/cureus.7476.

Lerner, A., Matthias, T., Aminov, R., 2017. Potential effects of horizontal gene exchange in the human gut. Front. Immunol. 8, 1–14. https://doi.org/10.3389/fimmu.2017.01630.

Lerner, A., Shoenfeld, Y., Matthias, T., 2019. Probiotics: if it does not help it does not do any harm. really? Microorganisms 7, 1–20. https://doi.org/10.3390/microorganisms7040104.

Li, Shaoying, Li, Z., Wei, W., Ma, C., Song, X., Li, Shufen, He, W., Tian, J., Huo, X., 2015. Association of mutation patterns in GyrA and ParC genes with quinolone resistance levels in lactic acid bacteria. J. Antibiot. (Tokyo) 68, 81–87. https://doi.org/10.1038/ja.2014.113.

Liu, B., Zheng, D., Jin, Q., Chen, L., Yang, J., 2019. Vfdb 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res. 47, D687–D692. https://doi.org/10.1093/nar/gky1080.

Liu, C., Zhang, Z.Y., Dong, K., Yuan, J.P., Guo, X.K., 2009. Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomed. Environ. Sci. 22, 401–412. https://doi.org/10.1016/S0895-3988(10)60018-9.

Lopes, M. de F.S., Simões, A.P., Tenreiro, R., Marques, J.J.F., Crespo, M.T.B., 2006. Activity and expression of a virulence factor, gelatinase, in dairy enterococci. Int. J. Food Microbiol. 112, 208–214. https://doi.org/10.1016/j.ijfoodmicro.2006.09.004.

Ma, Q., Fu, Y., Sun, H., Huang, Y., Li, L., Yu, Q., Dinnyes, A., Sun, Q., 2017. Antimicrobial resistance of Lactobacillus spp. from fermented foods and human gut. LWT (Lebensm.-Wiss. & Technol.) 86, 201. https://doi.org/10.1016/j.lwt.2017.07.059.

Ma, X., Wang, H., Deng, Y., Liu, Z., Xu, Y., Pan, X., Musser, J.M., Graviss, E.A., 2006. rpoB gene mutations and molecular characterization of rifampin-resistant Mycobacterium tuberculosis isolates from Shandong Province, China. J. Clin. Microbiol. 44, 3409–3412. https://doi.org/10.1128/JCM.00515-06.

Madsen, K.T., Skov, M.N., Gill, S., Kemp, M., 2017. Virulence factors associated with Enterococcus faecalis infective endocarditis: a mini review. Open Microbiol. J. 11, 1–11. https://doi.org/10.2174/1874285801711010001.

Magalhães, M.L., Blanchard, J.S., 2019. Aminoglycosides: mechanisms of action and resistance. Amin. Antibiot. A Guid. To Ther. 23–44 https://doi.org/10.1201/9780429280184-2.

Manson, J.M., Keis, S., Smith, J.M.B., Cook, G.M., 2004. Acquired bacitracin resistance in Enterococcus faecalis is mediated by an ABC transporter and a novel regulatory protein. BcrR. Antimicrob. Agents Chemother. 48, 3743–3748. https://doi.org/10.1128/AAC.48.10.3743-3748.2004.

Marosevic, D., Kaevska, M., Jaglic, Z., 2017. Resistance to the tetracyclines and macrolide-lincosamide-streptogramin group of antibiotics and its genetic linkage – a review. Ann. Agric. Environ. Med. 24, 338–344. https://doi.org/10.26444/aaem/74718.

Martinez, R.M., Hulten, K.G., Bui, U., Clarridge, J.E., 2014. Molecular analysis and clinical significance of lactobacillus spp. Recovered from clinical specimens presumptively associated with disease. J. Clin. Microbiol. 52, 30–36. https://doi.org/10.1128/JCM.02072-13.

Mater, D.D.G., Langella, P., Corthier, G., Flores, M.J., 2007. A probiotic Lactobacillus strain can acquire vancomycin resistance during digestive transit in mice. J. Mol. Microbiol. Biotechnol. 14, 123–127. https://doi.org/10.1159/000106091.

McDermott, P.F., Zhao, S., Wagner, D.D., Simjee, S., Walker, R.D., White, D.G., 2002. The food safety perspective of antibiotic resistance. Anim. Biotechnol. 13, 71–84. https://doi.org/10.1081/ABIO-120005771.

Mertens, K., Bertina, R.M., 1980. Pathways in the activation of human coagulation factor X. Biochem. J. 185, 647–658. https://doi.org/10.1042/bj1850647.

Muñoz-Provencio, D., Pérez-Martínez, G., Monedero, V., 2010. Characterization of a fibronectin-binding protein from Lactobacillus casei BL23. J. Appl. Microbiol. 108, 1050–1059. https://doi.org/10.1111/j.1365-2672.2009.04508.x.

Naas, T., Cuzon, G., Bogaerts, P., Glupczynski, Y., Nordmann, P., 2011. Evaluation of a DNA microarray (check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum β-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. J. Clin. Microbiol. 49, 1608–1613. https://doi.org/10.1128/JCM.02607-10.

Nallapareddy, S.R., Qin, X., Weinstock, G.M., Hook, M., Murray, B.E., 2000. Enterococcus faecalis adhesin, Ace, mediates attachment to extracellular matrix proteins collagen type IV and laminin as well as collagen type I. Infect. Immun. 68, 5218–5224. https://doi.org/10.1128/IAI.68.9.5218-5224.2000.

Naqvi, S.S.B., Nagendra, V., Hofmeyr, A., 2018. Probiotic related Lactobacillus rhamnosus endocarditis in a patient with liver cirrhosis. IDCases 13, e00439. https://doi.org/10.1016/j.idcr.2018.e00439.

Oakey, H.J., Harty, D.W.S., Knox, K.W., 1995. Enzyme production by lactobacilli and the potential link with infective endocarditis. J. Appl. Bacteriol. 78, 142–148. https://doi.org/10.1111/j.1365-2672.1995.tb02834.x.

Ouoba, L.I.I., Lei, V., Jensen, L.B., 2008. Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: determination and transferability of the resistance genes to other bacteria. Int. J. Food Microbiol. 121, 217–224. https://doi.org/10.1016/j.ijfoodmicro.2007.11.018.

Patnaik, S., Davila, C.D., Chennupati, A., Rubin, A., 2015. Endocarditis of the native aortic valve caused by Lactobacillus jensenii. BMJ Case Rep. 3–6. https://doi.org/10.1136/bcr-2014-206288, 2015.

Petersen, A., Jensen, L.B., 2004. Analysis of gyrA and parC mutations in enterococci from environmental samples with reduced susceptibility to ciprofloxacin. FEMS Microbiol. Lett. 231, 73–76. https://doi.org/10.1016/S0378-1097(03)00929-7.

Połka, J., Morelli, L., Patrone, V., 2016. Microbiological cutoff values: a critical issue in phenotypic antibiotic resistance assessment of lactobacilli and bifidobacteria. Microb. Drug Resist. 22, 696–699. https://doi.org/10.1089/mdr.2015.0328.

Preethi, C., Thumu, S.C.R., Halami, P.M., 2017. Occurrence and distribution of multiple antibiotic-resistant Enterococcus and Lactobacillus spp. from Indian poultry: in vivo transferability of their erythromycin, tetracycline and vancomycin resistance. Ann. Microbiol. 67, 395–404. https://doi.org/10.1007/s13213-017-1270-6.

Rao Thumu, S.C., Halami, P.M., 2012. Presence of erythromycin and tetracycline resistance genes in lactic acid bacteria from fermented foods of Indian origin. Antonie van Leeuwenhoek. Int. J. Gen. Mol. Microbiol. 102, 541–551. https://doi.org/10.1007/s10482-012-9749-4.

Recio, R., Chaves, F., Reyes, C.A., Orellana, M.Á., 2017. Infective endocarditis due to Lactobacillus rhamnosus : risks of probiotic consumption in a patient with structural heart disease. Enferm. Infecc. Microbiol. Clín. 35, 609–610. https://doi.org/10.1016/j.eimce.2017.10.008.

Roberts, M.C., Schwarz, S., 2009. Tetracycline and chloramphenicol resistance mechanisms. Antimicrob. Drug Resist. 183–193. https://doi.org/10.1007/978-1-59745-180-2_15.

Rossi, F., Amadoro, C., Colavita, G., 2019. Members of the lactobacillus genus complex (LGC) as opportunistic pathogens: a review. Microorganisms 7. https://doi.org/10.3390/microorganisms7050126.

Saini, K., Tomar, S.K., 2017. In vitro evaluation of probiotic potential of Lactobacillus cultures of human origin capable of selenium bioaccumulation. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 84, 497–504. https://doi.org/10.1016/j.lwt.2017.05.034.

Salzillo, M., Vastano, V., Capri, U., Muscariello, L., Sacco, M., Marasco, R., 2015. Identification and characterization of enolase as a collagen-binding protein in

Lactobacillus plantarum. J. Basic Microbiol. 55, 890–897. https://doi.org/10.1002/jobm.201400942.
Sanders, M.E., Akkermans, L.M.A., Haller, D., Hammerman, C., Heimbach, J., Hörmannsperger, G., Huys, G., Levy, D.D., Lutgendorff, F., Mack, D., Phothirath, P., Solano-Aguilar, G., Vaughan, E., 2010. Safety assessment of probiotics for human use. Gut Microb. 1, 1–22. https://doi.org/10.4161/gmic.1.3.12127.
Shankar, N., Coburn, P., Pilla, C., Haas, W., Gilmore, M., 2004. Enterococcal cytolysin: activities and association with other virulence traits in a pathogenicity island. Int. J. Med. Microbiol. 293, 609–618. https://doi.org/10.1078/1438-4221-00301.
Soleymanzadeh, N., Mirdamadi, S., Kianirad, M., 2017. Incidence of virulence determinants and antibiotic resistance in lactic acid bacteria isolated from Iranian traditional fermented camel milk (chal). J. Food Biosci. Technol. 7, 1–8.
Stroupe, C., Pendley, J., Isang, E., Helms, B., 2017. Persistent bacteremia secondary to delayed identification of Lactobacillus in the setting of mitral valve endocarditis. IDCases 10, 132–134. https://doi.org/10.1016/j.idcr.2017.10.002.
Tans, G., Rosing, J., Griffin, J.H., 1983. Sulfatide-dependent autoactivation of human blood coagulation factor XII (Hageman factor). J. Biol. Chem. 258, 8215–8222.
Tavernese, A., Stelitano, M., Mauceri, A., Mollace, R., Uccello, G., Romeo, F., Cammalleri, V., 2020. Progression of Lactobacillus plantarum prosthetic valve endocarditis followed by transesophageal echocardiogram. Int. J. Infect. Dis. 97, 160–161. https://doi.org/10.1016/j.ijid.2020.05.067.
Tena, D., Martínez, N.M., Losa, C., Fernández, C., Medina, M.J., Sáez-Nieto, J.A., 2013. Acute acalculous cholecystitis complicated with peritonitis caused by Lactobacillus plantarum. Diagn. Microbiol. Infect. Dis. 76, 510–512. https://doi.org/10.1016/j.diagmicrobio.2013.03.018.
Thumu, S.C.R., Halami, P.M., 2019. Conjugal transfer of erm(B) and multiple tet genes from Lactobacillus spp. to bacterial pathogens in animal gut, in vitro and during food fermentation. Food Res. Int. 116, 1066–1075. https://doi.org/10.1016/j.foodres.2018.09.046.
Todorov, S., Wachsman, M., Ignatova-Ivanova, T., Ivanova, I., 2014. Lactobacillus plantarum st16pa–are we ready to use it as bio-protective culture? Bulg. J. Agric. Sci. 20, 55–58.
Todorov, S.D., Perin, L.M., Carneiro, B.M., Rahal, P., Holzapfel, W., Nero, L.A., 2017. Safety of lactobacillus plantarum ST8Sh and its bacteriocin. Probiotics antimicrob. Proteins 9, 334–344. https://doi.org/10.1007/s12602-017-9260-3.
Todorov, S.D., Tome, E., Nero, L.A., 2019. Not everything is a question of reputation: safety of bacteriocinogenic lab isolated from smoked salmon. Chem. Eng. Trans. 75, 451–456. https://doi.org/10.3303/CET1975076.
Van Hoek, A.H.A.M., Mayrhofer, S., Domig, K.J., Flórez, A.B., Ammor, M.S., Mayo, B., Aarts, H.J.M., 2008. Mosaic tetracycline resistance genes and their flanking regions in Bifidobacterium thermophilum and Lactobacillus johnsonii. Antimicrob. Agents Chemother. 52, 248–252. https://doi.org/10.1128/AAC.00714-07.
Van Reenen, C.A., Dicks, L.M.T., 2011. Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. Arch. Microbiol. 193, 157–168. https://doi.org/10.1007/s00203-010-0668-3.
Vélez, M.P., De Keersmaecker, S.C.J., Vanderleyden, J., 2007. Adherence factors of Lactobacillus in the human gastrointestinal tract. FEMS Microbiol. Lett. 276, 140–148. https://doi.org/10.1111/j.1574-6968.2007.00908.x.
Wallet, F., Dessein, R., Armand, S., Courcol, R.J., 2002. Molecular diagnosis of endocarditis due to Lactobacillus casei subsp. rhamnosus. Clin. Infect. Dis. 35, 117–119. https://doi.org/10.1086/344181.
Wang, H.H., Manuzon, M., Lehman, M., Wan, K., Luo, H., Wittum, T.E., Yousef, A., Bakaletz, L.O., 2006. Food commensal microbes as a potentially important avenue intransmitting antibiotic resistance genes. FEMS Microbiol. Lett. 254, 226–231. https://doi.org/10.1111/j.1574-6968.2005.00030.x.
Willis, C., 2000. Antibiotics in the food chain: their impact on the consumer. Rev. Med. Microbiol. https://doi.org/10.1097/00013542-200011030-00005.
Yang, C., Yu, T., 2019. Characterization and transfer of antimicrobial resistance in lactic acid bacteria from fermented dairy products in China. J. Infect. Dev. Ctries. 13, 137–148. https://doi.org/10.3855/jidc.10765.
Yelin, I., Flett, K.B., Merakou, C., Mehrotra, P., Stam, J., Snesrud, E., Hinkle, M., Lesho, E., McGann, P., McAdam, A.J., Sandora, T.J., Kishony, R., Priebe, G.P., 2019. Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in ICU patients. Nat. Med. 25, 1728–1732. https://doi.org/10.1038/s41591-019-0626-9.
Zeba, F., Yirerong, J., Assali, M., Tewary, G., Noska, A., 2018. A double whammy: lactobacillus acidophilus bacteremia and subsequent lactobacillus rhamnosus prosthetic valve infective endocarditis in an elderly diabetic patient. R. I. Med. J. 101, 32–35 (2013).
Zhang, S., Oh, J.-H., Alexander, L.M., Özçam, M., Van Pijkeren, J.P., 2018. D-alanyl-d-alanine ligase as a broad-host-range counterselection marker in vancomycin-resistant. Lactic Acid Bacteria 200, 1–15. https://doi.org/10.1128/JB.00607-17.
Zhang, Y., Zhang, Q., 2019. Relationship between tetracycline antibiotic susceptibility and genotype in oral cavity Lactobacilli clinical isolates. Antimicrob. Resist. Infect. Control 8, 1–8. https://doi.org/10.1186/s13756-019-0483-9.
Zhang, Z.Y., Liu, C., Zhu, Y.Z., Wei, Y.X., Tian, F., Zhao, G.P., Guo, X.K., 2012. Safety assessment of Lactobacillus plantarum JDM1 based on the complete genome. Int. J. Food Microbiol. 153, 166–170. https://doi.org/10.1016/j.ijfoodmicro.2011.11.003.
Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol. 70, 2782–2858. https://doi.org/10.1099/ijsem.0.004107.
Zonenschain, D., Rebecchi, A., Morelli, L., 2009. Erythromycin- and tetracycline-resistant lactobacilli in Italian fermented dry sausages. J. Appl. Microbiol. 107, 1559–1568. https://doi.org/10.1111/j.1365-2672.2009.04338.x.