Antibiotic Resistance in Lactic Acid Bacteria

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ABSTRACT

Most starter cultures belong to the lactic acid bacteria recognized as safe by the US Food and Drug Administration (FDA) and the European Food safety Authority. However, LAB may act as intrinsic reservoirs for antibiotic resistance (AR) genes. This fact may not constitute a safety concern itself, as the resistance gene transfer is vertical. Nevertheless, external genetic elements may induce changes that favour the horizontal transfer transmission of resistance from pathogens as well as from the human intestinal microbiota, which represents a severe safety issue. Some genus of AR LAB includes *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* isolated from fermented meat and milk products. Currently, the WHO recommends that LAB used in the food industry should be free of resistance.

Keywords: Lactic acid bacteria; *Lactobacillus*; antimicrobial resistance

INTRODUCTION

The antimicrobial resistance has become one of the main safety issues for humanity, and several organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization (EFSA) among others, have raised awareness on this issue. The antimicrobial resistance has become one of the main safety issues for humanity, and several organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the US Food and Drug Administration (FDA), and the European Food Safety Authority (EFSA) among others, have raised awareness on this issue. The antimicrobial resistance can take place when microorganisms (bacteria, fungi, viruses and parasites) are continuously exposed to antimicrobials (antibiotics, antivirals, antifungals, etc.), and as a result of an adaptation process, some microorganisms can survive and grow in the presence of the antimicrobial, which in normal conditions would inactivate them. In particular, antibiotics are drugs used to treat bacterial infections in humans and animals, preventing the reproduction of bacteria or inactivating them through several mechanisms, either inhibiting the synthesis of the cell wall or the cytoplasmic membrane, blocking the protein synthesis or the DNA copying processes, altering the metabolism, or acting directly against the bacterial resistance pathway. The use of antibiotics in humans (cephalosporins, broad-spectrum penicillins, and fluoroquinolones) has increased 36% from the years 2000 to 2010, mainly due to their inappropriate prescription and consumption for the treatment of viral instead of bacterial infections. This fact may be correlated with the global report on antimicrobial resistance, with raising scenery to 10 million deaths each year by 2050.

The antimicrobial resistance involves several mechanisms associated to the presence of resistant genes that allow the direct inactivation of the active antimicrobial molecule as well as the loss of susceptibility to the antimicrobial by modification of the target site or reduction of the antimicrobial uptake. Therefore, antimicrobials become ineffective, and resistant microorganisms can survive and transfer their resistant machinery to other microorganisms and become a threat to public health. The presence of antimicrobial-resistant microorganisms not only affects both the human and animal health but also increases the risk for spread and contamination of foods, crops, livestock, and aquaculture.

The growing world population results in an increased demand for food, where antimicrobials such as antibiotics and fungicides are frequently used to treat infections in food-producing animals (cattle, swine, poultry, and fish), as well as in crops, to prevent diseases and as growth promoters. This practice is frequently seen in developing countries where unauthorized high amounts

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antibiotics are used that have been associate to the occurrence of multiple antibiotic-resistant *Enterococcus* and *Lactobacillus* strains from Indian poultry. The FAO also reports that 90% of antibiotics may be excreted into the water and soil thus contaminating the environment, with the consequent exposure increment and development of AR microorganisms that can transfer their resistant genes to other microorganisms. For instance, bacterial populations from the intestine of animals exposed to antibiotics (tetracycline, penicillin, sulfonamide, and polymyxins) were five times more likely to be resistant. The resistant microorganisms can be spread to humans from contaminated foods and water or from the environment. Various practices such as adequate animal vaccination and the use of additives that promote health and efficiency of feed conversion, in combination with good hygiene and husbandry practices would reduce the need for antimicrobials and antibiotics for food production.

Lactic acid bacterial constitute one of the most important groups of microorganisms present in several habitats; they are in large numbers in the gastrointestinal tract of animals and humans and form part of microbiota in several foods [1]. Historically, LAB have been recognized as safe with a GRAS (generally recognized as safe) and QPS (qualified presumption of safety) status given by the FDA and EFSA authorities. However, the recent detection of antibiotic-resistant LAB and the continuous exposure to environmental conditions may promote that LAB became as intrinsic or extrinsic reservoirs for AR genes, which can be horizontally transmissible to pathogens through the food chain. The resistance to a specific antimicrobial may be intrinsic (when a microorganism does not possess target sites for the antimicrobial) or required. The acquired resistance is more complex and involves the presence of enzymes that inactivate the antimicrobial, posttranscriptional, or posttranslational modifications of the target site or reduction uptake and active efflux of the antimicrobial; those mechanisms derive from the gain of exogenous DNA or the mutation of indigenous DNA. In general, the AR genes can be horizontally transferred from one organism to another by transduction (bacteriophages) or by transformation between microorganisms (when released DNA is taken up by other microorganisms). However, it is claimed that the primary mechanism to acquire resistant is by direct cell contact or conjugation between different genera of bacteria, especially when the resistant genes are present on mobile genetic elements such as plasmids and transposons. LAB are highly adaptable and capable of developing resistance to antibiotics; most AR studies were focused on pathogenic microorganisms, but recently some investigators have questioned the safety commensal LAB as some strains of *Lactococcus lactis*, *Enterococci*, and *Lactobacillus* isolated from fermented foods showed genes conferring resistance to tetracycline, erythromycin, and vancomycin.

LACTIC ACID BACTERIA

Lactic acid bacteria are a group of nonmotile, non-sporulating, acid-tolerant, gram-positive bacteria, which are detected in various habitats such as foods, many environmental niches, and gut and mucous membrane of humans and animals [2]. They may exist as coccus or rod-shaped in single cells, forming couples, tetrads, or short to long chains, and is capable to convert fermenting sugars primarily into lactic acid [3]. LAB represent two phyla, *Firmicutes* and the *Actinobacteria*; *Firmicutes phylum* consists species of the low G+C (31-49%) genera *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* that are grouped in the *Bacilli* class and reserved in the *Lactobacillus*'s order, while *Actinobacteria phylum* consists of high G+C (58%-61%) *Bifidobacterium genus* [4].

Based on sugar fermentation patterns, two broad metabolic categories of LAB exist: homofermentative and heterofermentative. They are divided according to the end-products derived from the glucose metabolism. The homofermentative LAB converts glucose to lactic acid by the Embden-Meyerhof pathway and this includes most species of *Enterococci*, *Lactococci*, *Pediococci*, *Streptococci*, *Tetragenococci*, *Vagococci* and some species of *Lactobacilli*, while the heterofermentative LAB undergo 6-phosphogluconate pathway and ferment glucose with lactic acid, ethanol or acetic acid, and carbon dioxide as by-product [5]. LAB are capable to improve food safety through bio preservation by producing antimicrobial metabolites such as organic acid (mainly lactic acid and acetic acids), hydrogen peroxide, bacteriocins, peptidoglycan hydrolyses, diacetyl, ethanol, and antifungal peptides short-chain fatty acids derived from lipolysis reactions.

Earlier, LAB was mainly used for dairy products, with time; they have been safely applied in food fermentations. They not only prevent formation of spoilage and pathogenic microorganisms, but also improve flavour, texture, and taste of vegetables such as sauerkraut and kimchi, meat products, chocolate, coffee, wine, and sourdough bread by various reactions, including proteolysis, lipolysis, and conversion of lactose into citrate (dairy products) and pyruvate intermediates that can alternatively be converted to diacetyl, acetoin, acetaldehyde, or acetic acid (yogurt flavour). The organoleptic characteristics and flavour profile of a food product can be altered by proteolysis through degradation of protein into small peptides and free amino acids, and further transformed into alcohol, aldehydes, acids and esters. Moreover, LAB strains can secrete extracellular polysaccharide or exopolysaccharides that are responsible for increasing firmness and viscosity and better texture and mouth feel of yogurt [6].

MECHANISMS OF RESISTANCE IN LAB

In order to allow interaction between antibiotic and its target, the antibiotic must identify the target and the antibiotic concentration in the target must be sufficient within the bacterial cell to have effective outcome [7]. There are two concepts that are significant when concerning the problem of antimicrobial resistance. First, antimicrobial resistance existed in a very distant past, and thus, it can occur in many organisms [8]. The vast majority of antimicrobial metabolites generate naturally, as such, co-resident bacteria overcome actions to survive by modification of their target site, without altering the structure of antibiotics; this is known as natural resistance, or intrinsic resistance. It is also defined as 'a trait that is shared universally within a bacterial species, is independent of previous

antibiotic exposure, and not related to horizontal gene transfer' [9]. In contrast, acquired resistance or extrinsic resistance occurs when the degradation of antibiotics structure or efflux pump causes the reduction of intracellular antibiotics concentration, which may lead to mutations in chromosomal genes [10]. Second, the concept of antimicrobial resistance in clinical practice is important as it could be considered as a relative phenomenon with different strata [11]. The initiation of clinical resistance breakpoints mostly depends on the *in vitro* activity of an antibiotic against a sizeable bacterial sample, together with several pharmacokinetic parameters [12]. So, the interpretation of vulnerability patterns may differ based on the clinical settings and the treatment options availability [11].

Natural resistance

Natural resistance or intrinsic resistance is the innate ability of a bacterial species that involves the absence of the target or the presence of low-affinity targets, antibiotics inactivation, efflux of antibiotics, and reduced permeability of the outer membrane [13]. Multi-drug resistant phenotype exhibited by effective Gram-negative bacteria is one of the convention examples, which the gram-negative bacteria possess an innate insensitivity towards many classes of effective Gram-positive antibiotics [14]. A few studies reported that several species of LAB have intrinsic resistance to beta-lactams, vancomycin, teicoplanin, and bacitracin [15]. When an antibiotic-probiotic combination therapy is applied, intrinsic resistance in lactic acid bacteria to certain antibiotics may provide beneficial effect. A study showed antibiotics-resistant probiotic strains from 40 samples of Japanese probiotics may interact antagonistically with antibiotics during probiotic-antibiotic combination therapy, but their isolates found absence of antibiotic resistance genes [16].

In addition to the intrinsic resistance mediated by the bacterial outer membrane and active efflux, studies have shown that a surprising number of additional genes and genetic loci also contribute to this phenotype. Antibiotic resistance is rife in both the clinic and the environment; novel therapeutic strategies need to be developed in order to prevent a major global clinical threat. The possibility of inhibiting elements comprising the intrinsic resistome in bacterial pathogens offers the promise for repurposing existing antibiotics against intrinsically resistant bacteria. Intrinsic resistance may defined as a trait that is shared universally within a bacterial species, is independent of previous antibiotic exposure, and not related to horizontal gene transfer. The most common bacterial mechanisms involved in intrinsic resistance are reduced permeability of the outer membrane and the natural activity of efflux pumps. Multidrug-efflux pumps are also a common mechanism of induced resistance.

The clinical use of antibiotics, and therefore the effective treatment of bacterial infections, in under considerable threat due to the emergence of bacteria that are resistant to many classes of commonly used antibiotics. In the traditional sense, antibiotic resistance is often considered to be a trait acquired by previously susceptible bacteria, the basis of which can be attributed to the horizontal acquisition of new genes, or the occurrence of spontaneous mutations within chromosomally located genes that are subsequently transmitted vertically as the

bacteria replicates. In addition to the ability of bacteria to 'acquire' resistance, they are also intrinsically resistant to different classes of antibiotics; a trait that is universally found within the genome of a bacterial species, is independent of antibiotic selective pressure and is not accredited to horizontal gene transfer. Indeed, the conventional example of intrinsic antibiotic resistance is the Multi-Drug Resistant (MDR) phenotype exhibited by Gram-negative bacteria, which are insensitive to many classes of clinically effective Gram-positive antibiotics. The molecular basis of this phenomenon is the presence of the Gram-negative outer membrane, which is impermeable to many molecules, and expression of numerous MDR efflux pumps that effectively reduce the intracellular concentration of the given drug.

The natural or innate ability of a bacterium to survive the effect of antibiotics, as result of mutations antibiotics. Intrinsic resistance has a minimum propagation potential between bacterial genera, as resistance genes are located into the chromosome with a limited transference to other genus, which represents low risk within non-pathogenic bacteria. Any gene responsible for intrinsic resistance could be disseminated and transferred to other bacteria if it is flanked by insertion sequences that may promote its mobilization. For instance, *Bifidobacterium* strains are commonly used as starter cultures and/or prebiotics in tradition and industrialized fermented foods although they have intrinsic resistance to quinolone (ciprofloxacin and nalidixic acid), mupirocin, tetracyclines, and aminoglycosides such as streptomycin; however, all the gens are located in the chromosome with a limited transference to other genus. It has been reported that some LAB genera have intrinsic resistance to bacitracin, vancomycin, kanamycin, teicoplanin, quinolones. This intrinsic resistance mechanisms presented by LAB include:

- Modification of the cell wall, commonly observed in the resistance to glycopeptides (vancomycin and teicoplanin) and non-ribosomal antibiotics (bacitracin). In particular, and present innate resistance to vancomycin, due to the substitution of D-alanine residues of the muramyl pentapeptide cell wall by D-lactate (high-level resistance) or D-serine (low-level resistance) in chemical structure of the peptidoglycan, thus avoiding the antibiotic interaction
- Enzymatic inactivation such as for aminoglycosides (neomycin, kanamycin, streptomycin) or quinolones (ciprofloxacin, norfloxacin, nalidixic acid) prevent the binding of these antibiotics with their specific targets, as observed for and for the 16 S rRNA of the 30 S ribosomal bacterial subunit and DNA gyrase, respectively, that explains the intrinsic resistance to both groups of antibiotics.

Acquired resistance

The acquisition of antibiotic resistance occurs via the mutation of pre-existing genes or by horizontal transmission. With some exceptions, intrinsic resistance and resistance by mutation are unlikely to be disseminated; horizontally transferred genes, particularly those carried on mobile genetic elements are those most likely to be transmitted.

Extrinsic resistance groups

It involves the absence of the target or the presence of low-affinity targets, low cell permeability, antibiotic inactivation of the antibiotics and the presence of efflux mechanisms.

CONCLUSION

Bacterial resistance to antibiotics is an emerging public concern that may compromise the efficacy of agents used for the treatment of infectious diseases. Therefore, the objective of this chapter is to present an overview of the LAB antibiotic resistance and some methods to determine this characteristic, as per the FAO/OMS guideline for testing food-related bacteria and probiotics for resistance patterns.

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