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Review article

Antibiotic resistance of lactic acid bacteria

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ABSTRACT

About 50 years ago, antibiotics were introduced for the treatment of microbial diseases. Since then, the greatest threat to the use of antimicrobial agents for therapy of bacterial infections has been the development of antimicrobial resistance in pathogenic bacteria. Lactic acid bacteria (LAB) are a heterogeneous group of bacteria found widely in nature. They colonize the gastrointestinal and urogenital tracts of humans and animals, and are present in foods such as dairy products, fermented meats, fruits and vegetables. Recently many investigators have speculated that commensal bacteria including LAB may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens. The aim of this review is to consider the current evidence on antibiotic resistance of lactic acid bacteria.

1. Introduction

Lactic acid bacteria (LAB) are a heterogeneous group of bacteria found widely in nature. They colonize the gastrointestinal and urogenital tracts of humans and animals, and are present in foods such as dairy products, fermented meats, fruits and vegetables. The LAB forms a taxonomically diverse group of microorganisms, which can convert fermentable carbohydrates into lactic acid. The most typical LAB members are Gram-positive, catalase-negative organisms of the low C+G branch, belonging to the genera *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc* (Carr et al., 2002; Leroy and Vuyst, 2004). Infections have been the major cause of disease throughout the history of human population. With the introduction of antibiotics, it was thought that this problem should disappear. However, bacteria have been able to evolve to become resistant to antibiotics (Tenover, 2001). Antibiotic resistance or drug resistance can be defined as the ability of bacteria and other microorganisms to withstand an antibiotic to which they were once sensitive (and were once stalled or killed outright) (Patel et al., 2012). According to the FAO/WHO, the development of commercial probiotics requires their unequivocal taxonomic identification, as well as their in vitro and in vivo functional characterization and safety assessment. In Europe, the European Food Safety Agency (EFSA) proposed a system for a pre-market safety assessment of selected groups of microorganisms used in food/feed and the production of food/feed additives leading to a Qualified Presumption of Safety (QPS) status. According to the EFSA approach, most LAB species are included in the QPS list and, therefore, demonstration of their safety only requires confirmation of the absence of determinants of resistance to antibiotics of human and veterinary clinical significance. However, in the case of enterococci, a more thorough, strain-specific evaluation is required to assess the risk associated to their intentional use in the food chain (Munoz-Atienza et al; 2013; FAO/WHO, 2006; EFSA, 2007). In this review, we summarize the current knowledge on antibiotic resistance of lactic acid bacteria.

2**. Lactic acid bacteria**

LAB grows anaerobically, but unlike most anaerobes, they grow in the presence of O_2 as aerotolerant anaerobes. Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term Lactic Acid Bacteria is conventionally reserved for genera in the order *Lactobacillales*, which includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, in addition to *Carnobacterium*, *Enterococcus*, Oenococcus, *Tetragenococcus*, *Vagococcus*, and *Weisella*. Other genera are: *Aerococcus*, *Microbacterium*, *Propionibacterium* and *Bifidobacterium*. Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. There has been much recent interest in the use of various strains of LAB as probiotics, since because these bacteria, mainly *lactobacilli* and *bifidobacteria*, may have several therapeutic functions. Probiotics are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. *Lactobacillus* and *Bifidobacterium* spp. are prominent members of the commensal intestinal flora. They cause reduced lactose intolerance alleviation of some diarrhoeas, lowered blood cholesterol, increased immune response and prevention of cancer. Lactic acid bacteria used as probiotics are given in Table 1 (Lavanya et al., 2012; Suvarna and Boby, 2005; Kazemi Darsanaki et al., 2012).

Table 1
Lactic acid bacteria used as probiotics.

Lactobacillus sp.	Bifidobacteriim sp.	Enterococcus sp.	Others
L. acidophilus	*B. bifidum*	*E. faecium*	*Lactococcus lactis* ssp. *lactis*
L.plantarum	*B. infantis*	*E. faecalis*	*Lactococcus lactis* ssp. *cremoris*
L. casei	*B. adolescentis*	-	*Leuconostoc mesenteroides*
L. rhamnosus	*B. lactis*	-	*Pediococcus acidilactici*
L. delbrueckii ssp. *bulgaricus*	*B. breve*	-	*Propionibacterium freudenreichii*
L. fermentum	*B. longum*	-	*Streptococcus thermophilus*
L. johnsonii	-	-	-
L. gasseri	-	-	-
L. salivarius	-	-	-

3. Types of antibiotic resistance

About 50 years ago, antibiotics were introduced for the treatment of microbial diseases. Since then, the greatest threat to the use of antimicrobial agents for therapy of bacterial infections has been the development of antimicrobial resistance in pathogenic bacteria (Mathur and Singh, 2005). According to World Health Organization (WHO) global strategy for the containment of antimicrobial resistance, the rate of emergence of antimicrobial resistance is expected to be increased by misuse of antibacterial substances. There are three types of resistance: natural (intrinsic or innate), acquired and mutational (Ashraf and Shah, 2011). Three conditions must be met for an antibiotic to be effective against bacteria: I) a susceptible antibiotic target must exist in the cell, II) the antibiotic must reach the target in sufficient quantity, and III) the antibiotic must not be inactivated or modified. There are five major modes of antibiotic mechanisms of activity:

1. Interference with cell wall synthesis
2. Inhibition of protein synthesis
3. Interference with nucleic acid synthesis.
4. Inhibition of a metabolic pathway
5. Disorganizing of the cell membrane (Table 2) (Didic et al., 2008).

Table 2
Major antibiotic families and their target of action.

Sites of inhibition	Group	Antibiotic
Cell wall synthesis	β-lactams	Amoxicillin Ampicillin Penicillin
	Glycopeptides	Vancomycin
Protein synthesis	Aminoglycosides	Gentamicin Kanamycin Neomycin Streptomycin
	Chloramphenicols	Chloramphenicol
	Tetracyclines	Tetracycline
	Macrolides	Erythromycin Tylosin
	Lincosamides	Clindamycin Lincomycin
DNA replication/transcription	Quinolones	Enrofloxacin
Folate synthesis	Sulphonamides	Trimethoprim

4. Antibiotic resistance in lactic acid bacteria

Lactic acid bacteria widely used as probiotics or in starter cultures have the potential to serve as a host of antibiotic resistance genes with the risk of transferring the genes in many lactic acid bacteria and other pathogenic bacteria (Mathur and Singh, 2005). Several approaches to the evaluation of the safety of probiotics have been applied. Assessment of the safety of a probiotic begins with the correct identification of the strain. Laboratory tests applied in the safety assessment of probiotics include in vitro assays assessing different intrinsic properties of the strains such as resistance to antibiotics or production of toxic metabolites, and different animal models, which can be used to evaluate the potential of probiotics to translocate from the hosts gut into the hosts bloodstream and tissues, or assess the infectivity of the probiotics in different disease models. In addition, the safety of probiotics may be evaluated in clinical trials. One of the main targets of the in vitro safety assessments of existing and potential probiotic strains is the determination of antibiotic resistance properties. Resistance of a probiotic strain to a certain antibiotic is clinically relevant only in the case of infections, and infections related to probiotics are extremely rare. The presence of antibiotic resistance genes in the probiotic genomic content is not a safety concern in itself, as long as the genes are not mobilized and transferred to other bacteria. Theoretically, probiotics possessing antibiotic resistance genes could serve as a reservoir of resistance for potential pathogens. Therefore, microorganisms intended for use as probiotics have to be systematically screened for antibiotic resistance susceptibility in order to avoid the transfer of antibiotic resistance genes, since the ability of these determinants to transfer in the food and gut environment has been demonstrated (Dimitris et al., 2009).

Table 3
Genes responsible for antibiotic resistance in lactic acid bacteria.

Gene	Antibiotic	Mechanism of action	References
Lactobacillus			
cat; chloramphenicol acetyl transferases	Chloramphenicol	Antibiotic acetylation	Hummel et al. (2009) Mayrhofer et al. (2010)
erm; erythromycin resistance genes	macrolides, lincosamides, streptrogramins	Ribosomal methylation	Hummel et al. (2009) Mayrhofer et al. (2010) Ammor et al. (2008) Cauwerts et al. (2006)
msrC	macrolides, lincosamides, streptrogramins	Efflux	Thumu and Halami (2010)
mef(A); macrolide efflux pumps	Macrolide	Efflux	Cauwerts et al. (2006)
tet(W), tet(M), tet(S), tet(O), tet(Q), tet(36), tet(Z), tet(W/O); tet(O/W/32/O/W/O) tetracycline resistance genes	Tetracycline	Ribosomal protection	Ammor et al. (2008) Aquilanti et al. (2012) Van Hoek et al. (2008)
tet(K) and tet(L); tetracycline efflux pumps	Tetracycline	Efflux	Ammor et al. (2008) Aquilanti et al. (2102) Thumu and Halami (2010)
aac(6')-aph(2"), ant(6) aph(3')-IIIa, aph(E) or sat(3) ; aminoglycoside resistance genes	aminoglycoside	Enzymatic modification	Rojo-Bezares et al. (2006)
(blaZ); β-lactam resistance genes	β-lactam	Antibiotic hydrolysis	Aquilanti et al. (2012)
vat(E)	Quinupristin–dalfopristin	Antibiotic acetylation	Mayrhofer et al. (2010)
Bifidobacterium			
tet(L)	Tetracycline	Efflux	Van Hoek et al. (2008)
erm(X)	macrolides, lincosamides, streptrogramins	Ribosomal methylation	Van Hoek et al. (2008)
tet(W), tet(M), tet (O), tet(W/32/O), tet(O/W)	Tetracycline	Ribosomal protection	Van Hoek et al. (2008) Florez et al. (2006) Kazimierczak et al. (2006)
Bacillus			
erm(34)	macrolides, lincosamides, streptrogramins	Ribosomal protection	Bozdogan et al. (2004)
cat(Bcl)	Chloramphenicol	Antibiotic acetylation	Galopin et al. (2009)

In regard to antibiotics acting on cell wall, *lactobacilli* are usually sensitive to penicillin and β-lactamase inhibitors, but more resistant to cephalosporins. Many *Lactobacillus* species show a high level of resistance to vancomycin. Most *Bifidobacterium* species are resistant to aminoglycosides, metronidazole and Gram-negative spectrum antibiotics. They are also intrinsically resistant to mupirocin, an antibiotic that is being used in the selective isolation of this genus. In contrast, *bifidobacteria* are very susceptible to macrolides/lincosamides, vancomycin, rifampicin, spectinomycin, chloramphenicol, and β-lactams. Different species of bifidobacteria and *lactobacilli* are the most commonly used probiotics, but other bacteria as well as the probiotic yeast *Saccharomyces boulardii* are also used as probiotics. *Saccharomyces* is a member of the domain Eukaryota, and hence naturally resistant to all antibiotics. In the case of *Saccharomyces*, resistance to fungicides is more relevant. *Lactococcus* and *Pediococcus* are LAB present in the commensal intestinal flora of humans and animals. Resistances for chloramphenicol, tetracycline, erythromycin and streptomycin have been found in different *Pediococcus* species. Relating to *Lactococcus* strains, a *Lactococcus lactis* strain resistant to streptomycin, tetracycline and chloramphenicol isolated from a raw milk cheese. (Table 3) (Dimitris et al., 2009; Hummel et al., 2007; Zhou et al., 2005; Klare et al., 2007; Perreten et al., 1997). Most probiotics are common members of the human intestinal tract, and they are ingested in large amounts in functional foods, and the presence of antibiotic resistance determinants in their genome must be systematically screened.

5. Conclusion

Lactic acid bacteria used as starter cultures or probiotic bacteria, enter into human intestines in large number where they interact with the intestinal microflora. The ability to transfer antibiotic resistance genes must be considered as an important parameter for the selection of the probiotic strains. Continuous attention should be paid to the selection of probiotic strains free of transferable antibiotic-resistance determinants. Without doubt, the uncontrolled use of antimicrobial agents in farming practice has assisted the spread of resistant organisms. Therefore a much stricter control over the use of these drugs is essential.

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