



# Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages



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## ABSTRACT

Dry-fermented sausages are meat products highly valued by many consumers. Manufacturing process involves fermentation driven by natural microbiota or intentionally added starter cultures and further drying. The most relevant fermentative microbiota is lactic acid bacteria (LAB) such as *Lactobacillus*, *Pediococcus* and *Enterococcus*, producing mainly lactate and contributing to product preservation. The great diversity of LAB in dry-fermented sausages is linked to manufacturing practices. Indigenous starters development is considered to be a very promising field, because it allows for high sanitary and sensorial quality of sausage production.

LAB have a long history of safe use in fermented food, however, since they are present in human gastrointestinal tract, and are also intentionally added to the diet, concerns have been raised about the antimicrobial resistance in these beneficial bacteria. In fact, the food chain has been recognized as one of the key routes of antimicrobial resistance transmission from animal to human bacterial populations. The World Health Organization 2014 report on global surveillance of antimicrobial resistance reveals that this issue is no longer a future prediction, since evidences establish a link between the antimicrobial drugs use in food-producing animals and the emergence of resistance among common pathogens. This poses a risk to the treatment of nosocomial and community-acquired infections. This review describes the possible sources and transmission routes of antibiotic resistant LAB of dry-fermented sausages, presenting LAB antibiotic resistance profile and related genetic determinants. Whenever LAB are used as starters in dry-fermented sausages processing, safety concerns regarding antimicrobial resistance should be addressed since antibiotic resistant genes could be mobilized and transferred to other bacteria.

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## 1. Introduction

There is a great diversity of dry-fermented sausage as a result of manufacturing with different raw meat types and origins, formulations, condiments, additives, meat grinding size, casing diameter, smoking and drying periods (Toldrá et al., 2007).

Meat sausages preservation is achieved by fermentation with a pH decrease due to lactate production by natural microbiota or added starter cultures. The main fermentative microbiota is lactic acid bacteria (LAB) including *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Leuconostoc*, *Lactococcus* and *Weissella* (Fontana et al., 2012). LAB constitutes a diverse group widely distributed throughout nature, being also an important component of indigenous microbiota in healthy humans and animals. LAB diversity in dry-fermented sausages' is linked to manufacturing practices, and it has been reported that indigenous starters development is very promising approach since it enables sausages' production with high sanitary and sensory characteristics

(Talon et al., 2007). However, some issues concerning consumers' safety have been expressed around this late use of LAB.

LAB have a long history of safe use in fermented food production and consumption that support their GRAS (generally recognized as safe) and QPS (qualified presumption of safety) status provided by FDA (US Food and Drug administration) and EFSA (European Food safety Authority), respectively. However the detection of antibiotic resistant (AR) strains among LAB has resulted in their recognition as reservoir of AR genes horizontally transmissible to pathogens through the food chain, this being a matter of concern (Devirgiliis et al., 2013; Marshall et al., 2009).

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This review describes the diversity and role of LAB in fermented sausages, stressing safety requirements for LAB regarding the antibiotic resistance issue. The possible sources and transmission routes of

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antibiotic resistant LAB in dry-fermented meat sausages are discussed and common LAB antibiotic resistance profile of isolates from fermented sausages and related genetic determinants are presented. Finally, some remarks are pointed out considering safety concerns for LAB selected to be used as starters in dry-fermented sausages.

## 2. Antibiotic resistance issue: facts and trends

Since the discovery of Penicillin in 1928 and the introduction of the first antimicrobial sulfonamides (1930) (Davies and Davies, 2010), antibiotics have been used, playing a decisive role in human health and life expectancy. However, concerns have been raised about pathogenic bacteria and their antibiotic resistances. In fact, infections caused by these resistant microorganisms are more difficult to treat and have higher costs, due to more intensive and time consuming care needed. According to the recent report of WHO (2014), antibiotic resistance within a wide range of infectious agents is a growing public health threat of broad concern to society and multiple sectors, since many of the available treatment options for common infections may become ineffective.

The resistance to antibiotics is an eventual characteristic in bacterial biomes and can be seen as an adaptation result that easily occurs under the effect of an environmental change (Rodríguez-Rojas et al., 2013). Antimicrobial resistance is the bacterial ability to survive and grow in the presence of a chemical molecule that would normally kill them or limit their growth. From the presence of particular resistant genes the bacteria will be able to avoid antimicrobials by major mechanisms described: direct inactivation of the active molecule; loss of bacterial susceptibility to the antimicrobial by modification of the target of action; and reduction of the drug concentration that reaches the target molecule without modification of the compound itself (efflux pump). The antibiotic defense mechanisms of intrinsic resistance are, in most of cases, related to the presence of low affinity targets, absence of targets, innate production of enzymes that inactivate the drug, inaccessibility of the drug into the bacterial cell by decreased drug uptake or extrusion by efflux of drug (Kumar and Schweizer, 2005).

The resistance to a specific antimicrobial drug should be considered 'intrinsic' or 'natural' when it is inherent to a bacterial species, being present in all its strains. On the other hand, resistance is named "acquired", if a susceptible species' strain becomes resistant to a specific antimicrobial drug. This can be due either to added genes (genes acquired by the bacteria via gain of exogenous DNA, gene exchange between bacteria) or to the mutation of indigenous genes (Ammor and Mayo, 2007; van Reenen and Dicks, 2011).

Antibiotic resistance genes can be spread from one bacterium to another (Horizontal transfer of genetic material) through several mechanisms. The transfers of DNA by transduction (via bacteriophages) or by transformation (when DNA is released from a bacterium and taken up by another) are not believed to be relevant mechanisms of antibiotic resistance transfer (Ammor and Mayo, 2007). By contrast, conjugation, i.e., the direct cell-to-cell contact, can potentially achieve horizontal gene transfer, as it has been shown to be a genetic information transfer mechanism with a broad host range (Courvalin, 1994).

Intrinsic resistance is estimated to present a minimal potential for horizontal spread (between different bacterial species), as was demonstrated for example with the chromosomal vancomycin resistance determinant of the *Lactobacillus rhamnosus* strain GG (Tynkkynen et al., 1998). Similar to intrinsic resistance, acquired resistance usually possesses a low risk of horizontal dissemination, when the resistance is a result of a chromosomal mutation. By contrast, acquired resistance is considered to have a higher potential for antibiotic resistance horizontal dissemination, when the resistance genes are present on mobile genetic elements (plasmids and transposons) (Devirgiliis et al., 2013; van Reenen and Dicks, 2011).

The genetic origin of antibiotic resistance is a controversial topic; usually the antibiotic resistance in bacteria from the food chain is

attributed to the contact with the antimicrobial agent in concern, and consequent development of resistance against the antibiotic (Levy and Marshall, 2004). However, recent studies have demonstrated that antibiotic resistance genes were around long before the human use of antibiotics (Bhullar et al., 2012; D'Costa et al., 2011). More than the use of antibiotics, the major problem for antibiotic resistance developing links to its misuse.

The large scale antibiotic application in human and veterinary medicine, agriculture and aquaculture is pointed out as one of the main causes of the increasing rate of resistant bacteria.

Over the last years, human antibiotic use has grown substantially (increasing 36% between 2000 and 2010), mainly in developing countries (Van Boeckel et al., 2014). The largest absolute increases in use were observed for cephalosporins, broad-spectrum penicillins and fluoroquinolones.

The frequent use of antibiotics in veterinary medicine, to treat or prevent diseases raised concerns about the potential for emerging new antibiotic resistant strains. Bacterial populations isolated from the intestine of animals exposed to antibiotics were found to be five times more likely to be resistant to any given antibiotic than other resistant microbial populations (Sarmah et al., 2006). In fact, the last European Surveillance of Veterinary Antimicrobial Consumption (ESVAC, 2013) report, regarding the overall sales in 2011 for 25 countries, states that the largest proportions, expressed as mg/PCU, were accounted for tetracyclines (37%), penicillins (23%), sulfonamides (11%) and polymyxins (7%).

The excessive use of antimicrobial agents in animal husbandry contributes for the selection of resistant bacteria in the food chain (Teale, 2002). The overuse and the misuses of antibiotics promote the selection and dissemination of antibiotic resistant bacteria and resistance genes, as well as the emergence of new resistant bacteria through genetic mutations and gene movements.

The several human activities had a predominant role in the promotion and growth of antibiotic resistance genes environmental reservoirs. In fact, the increased antibiotic pollution promoted by human activities had a role in selecting antibiotic resistant mutants and favoring the acquisition of antibiotic resistance determinants by gene-transfer elements that can be spread among the environmental microbiota (Martinez, 2009). Antibiotic abuse can enrich the population of resistant microorganisms and reduce the number of susceptible ones.

Bacteria containing resistance genes in mobile genetic elements are a threat to public health, since they can act as reservoirs, allowing for resistance genes dissemination especially in dense microbial community environments (Haug et al., 2011; Marty et al., 2012; Salyers et al., 2004). Consequently, foods colonized by bacteria that harbor such transferable antibiotic resistance genes are a major concern. Antibioresistance of foodborne bacteria has aroused great interest because they may act as reservoirs for antibiotic resistance genes (Talon and Leroy, 2011).

## 3. Common lactic acid bacteria in fermented meat sausages: their role and diversity

LAB include a diverse group of Gram-positive non-spore forming cocci, coccobacilli or rods, with common morphological, metabolic and physiological characteristics (Batt, 2000). They are facultative anaerobic with variable oxygen tolerance in different species.

LAB growth depends on the presence of fermentable carbohydrates. They are classified as homofermentative or heterofermentative based on end products of glucose metabolism. While homofermentative LAB convert glucose mainly to lactic acid using the glycolysis (Embden-Meyerhof-Parnas or Embden-Meyerhof) pathway, the heterofermentative LAB use the phosphoketolase (6-phosphogluconate) pathway and convert glucose to lactic acid, carbon dioxide and ethanol or acetic acid. LAB are a diverse group of organisms with diverse metabolic capacity. This diversity makes them easily adaptable to a wide range

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