Enterobacteriaceae in food safety with an emphasis on raw milk and meat

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Abstract

There has been a growing interest in traditional dairy (such as raw milk cheeses) and meat products, in recent years. However, these products are suitable, nutrient medium and may be easily contaminated by microorganisms such as *Enterobacteriaceae*. *Enterobacteriaceae* are considered to be the indicator bacteria for microbiological quality of food and hygiene status of a production process. Additionally, the food contaminated by *Enterobacteriaceae* poses a microbiological risk for consumers. In fact, the contamination of raw milk and meat by *Enterobacteriaceae* amid manufacturing may easily occur from various environmental sources and this group of bacteria is frequently detected in dairy and meat products. Therefore, monitoring microbiological quality of the used raw material and maintaining high standards of hygiene in the production process are mandatory for high quality of traditional products and the safety of the potential consumers. The goal of this review is to present the most recent survey on *Enterobacteriaceae* growth, number and distribution in raw milk cheeses and meat, as well as to discuss the sources of contamination and methods of control.

Key points

- *Enterobacteriaceae*: role and importance in milk and meat products, EU legal regulations.
- Dynamics, distribution, and survival of *Enterobacteriaceae* in milk and meat.
- Mechanisms of control of *Enterobacteriaceae* in dairy products.

Keywords: *Enterobacteriaceae*, food contamination, stress adaptation, development controlling

Introduction

Milk and meat are the two basic livestock food products. They provide a rich source of high-quality protein and a variety of important nutrients that are vital for optimal health. Milk and dairy products, as well as meat (red meat and poultry) are considered to be a valuable source of energy, highly digestible proteins of good nutritional quality, saturated and unsaturated lipids, as well as carbohydrates (lactose), vitamins A, D, B-group (mainly thiamin, vitamin B6, and pantothenic acid) and minerals (such as calcium, phosphorus, iron, zinc, copper). Based on the large role of these nutrient products, scope of this manuscript is a comprehensive overview of the role of *Enterobacteriaceae* in milk and meat with reference to their distribution, abundance and product protection measures and legal acts of microbiological safety of food.

Traditional cheeses are dairy products typical of a certain geographic region where the method of production is passed down from generation to generation. The type and quality of traditional cheeses are greatly influenced by the climatic conditions, the type of animal milk, as well as the type and content of animal feeds (grass, herbs, hay, silage) (Motahari et al., 2017). Unlike industrial cheeses, traditional ones are usually produced on a farm or in small dairies that use unpasteurized (raw milk) or low heat-treated milk (below the temperature of pasteurization) inoculated with various starter combinations. The cheeses produced in this way are recognized for their diversity and characteristic sensory properties. The lack of pasteurization step in cheese production preserves the indigenous microbial communities (Montel et al. 2014). Cheese production and development of cheese flavor is a complex process which is composed of both volatile and non-volatile fractions, which originate from milk fat, protein, and carbohydrate. The process of cheese ripening has been reviewed extensively including the difference in sensory profile between the raw milk cheeses and the pasteurized milk cheeses. It is concluded that the diverse indigenous microbiota is responsible for the specific sensory properties of raw milk cheeses, as well as for more intense flavor than the flavor of pasteurized milk cheeses (Chambers et al. 2010). Cheeses are comprised of diverse, wild microbiota which evolves in a successional process of cheese production. Microbiological monitoring has demonstrated that raw milk cheeses could be associated with several genera of *Enterobacteriaceae* family, including *Escherichia coli* species, that are recognized as indicators of the hygiene in the production process. Some species and strains are pathogens that have been clearly implicated in foodborne illness (Metz et al. 2020). Therefore, their detection, as well as permitted number is stipulated in legal regulations for the microbiological safety of food.

Meat and meat products are the first choice of animal proteins in the human diet and their consumption is continuously increasing worldwide. As one of the most perishable foods, raw meat, due to its chemical composition, favors microbial growth to unacceptable levels contributing significantly to meat deterioration or spoilage. The presence of many microorganisms in raw meat leads to changes that make it unappealing and unsuitable for human consumption (Dave and Ghaly 2011; Doulgeraki et al. 2012). Drying and fermentation, as one of the oldest methods of meat preservation, extend the shelf life of meats giving the final product distinctive properties such as microbiological safety, flavor, and palatability (Settanni and Moschetti 2014). *Enterobacteriaceae* are very common in fresh and frozen beef, pork and chicken meat (Gwida et al. 2014; Jansen et al. 2018) but could also be found in the traditional fermented meat products (Castano et al. 2002; Talon et al. 2007). The incidence and number of *Enterobacteriaceae* in raw meat and fermented meat products are effective indicators of hygiene and quality, particularly in relation to contamination of fecal origin.

The family *Enterobacteriaceae* comprises a heterogenous group of Gram - negative, facultative anaerobic, non-spore forming, rod-shaped bacteria. Some members of the *Enterobacteriaceae* possess the ability to ferment lactose producing acid and gas. These are collectively termed coliform bacteria and are frequently used as (faecal) indicator organisms by the food and water industry.

Members of the *Enterobacteriaceae* are widely distributed. Although strains of some species are harmless commensals, others are important human and animal pathogens. Their importance is increasing since the natural habitat of many members of *Enterobacteriaceae* family is located in the intestinal tract of animals. Thus, *Enterobacteriaceae* have been used for a long time as indicator organisms in the food industry. While testing the microbiological quality, the *Enterobacteriaceae* number and presence of coliforms/*E. coli* are used as effective parameters to assess the poor hygiene status and possible failure of a manufacturing process (Halkman and Halkman 2014).

This family includes several important foodborne pathogens such as toxin-producing *Salmonella* spp. or *Shigella* spp. In addition, despite the fact that most strains of *E. coli* are harmless commensals, several serotypes of *E. coli* produce toxins and they are considered to be pathogenic. The most significant for food is *E. coli* O157:H7, which has become one of the most important foodborne pathogens (Baylis et al. 2011). The resistance of the *Enterobacteriaceae* to various antibiotics is a major problem of current medicine (Rock and Donnenberg 2014).

Based on the above mentioned, the aim of this paper is to review the literature on importance of the *Enterobacteriaceae* for food safety associated with two basic types of food, traditional cheese product made from raw milk, and traditional meat products.

Legal acts of microbiological safety of food

The European Commission provides the microbiological criteria that represent guidance on the acceptability of foodstuffs and their manufacturing processes. Preventative actions, such as the application of Good Hygiene and Manufacturing Practices (GHP, GMP) and the Hazard Analysis Critical Control Point (HACCP) principles contribute to achieving food safety.

In the context of the European Legislation, two different types of microbiological criteria for foodstuffs are established by Regulation No. 2073/2005, namely process hygiene criteria and food safety criteria (EC 2005). As a measure of process hygiene, requirements are established for the cattle, sheep, goats, horses, and pig carcasses, in terms of aerobic colony count and levels of *Enterobacteriaceae*. Process hygiene criteria are indicators of the acceptable functioning of the HACCP system during the slaughter, dressing, and production process. For milk and dairy products, process hygiene criterion applies to *Enterobacteriaceae* in pasteurized milk, other pasteurized liquid dairy products, milk powder, whey powder, and ice-cream. Requirements are also established for cheeses made from milk or whey that has undergone heat treatment in terms of *E. coli* levels. The process hygiene criteria set indicative contamination values above which corrective actions are required to implement HACCP systems in the production place. When a food safety criterion for the absence of *Salmonella* in different categories of dairy and meat products are not met, the batch of food in question should be withdrawn from or not placed on the market (EC 2005). Regulation No. 2073/2005 harmonized the microbiological food safety and process hygiene criteria for foodstuffs in the European Union. However, many member states of the European Union have established their national legislation that oversees microbiological process hygiene criteria in stricter terms than EU 2073/2005.

Contamination of raw milk cheeses and meat by *Enterobacteriaceae*

Lack of personal hygiene amongst food handlers in retail is one of the most reported practices contributing to food-borne illness. Despite an increased awareness of safe food handling practices and a food handler receiving food hygiene training, in a study by Lues and Van Tonder (2007), *Enterobacteriaceae* were present on 44% of food handler's hands and on 16% of aprons. *Klebsiella* spp. were found to be the most abundant species found on the hand swabs of meat sellers, followed by genera *Citrobacter, Raoultella* and *Escherichia coli* (Gwida et al. 2014).

Raw milk cheeses

Many countries use *E. coli* and coliforms as indicators of sanitary quality of food and have set limits for cheeses, including raw milk cheeses (Metz et al. 2020). Milk is a suitable medium for the growth of various microorganisms since its rich in nutrients, contains high moisture, and has an initially neutral pH. Their rapid growth, particularly at high ambient temperatures, may lead to the change of the liquid composition of milk, as well as to the change in manufacturing dairy products. As Chege and Ndungu (2016) summarized, the raw milk could be a subject of contamination at different points through the whole chain process and from different sources. In general, microorganisms (bacteria, yeasts, and moulds) can contaminate raw milk in two ways. The first way is an endogenous contamination where the milk is contaminated by a direct transfer from the unhealthy animal (systemic infection, mastitis). The second way is an exogenous contamination, where the milk is contaminated while or after milking by the feces, the exterior of the udder and teats, the skin, the environment, the equipment, etc. However, the contamination of cheeses is possible during entire production process, not just during milking.

Raw milk can be contaminated in several ways. If the equipment for milking, processing and storage is not properly maintained and clean, milk contamination can occur. After the muse, everything should be well cleaned because even the smallest amount of milk can be a source of nutrients for bacteria from the fam. Enterobacteriaceae. The water used for cleaning should be of good quality, because it can also be a source of coliform bacteria that can contaminate milk. Persons involved in milk production should pay attention to the hygiene of clothing and hands, and the hygiene of milking equipment. Caution is also recommended during milking, processing, and storage, as milk contamination can occur at every step (Freitas et al. 2013). Raw milk and dairy products should be subjected to appropriate hygienic conditions, which can prevent spoilage and/or growth of foodborne pathogens.

Meat and meat products

The three main mechanisms for meat and meat products spoilage (after slaughtering, amid processing, and storage) imply the growth of microorganisms, lipid oxidation, and autolytic enzymatic spoilage. According to Dave and Ghaly (2011), the change of pH, formation of slime, structural components degradation, off odors, and appearance changes in meat is induced by microbial growth. When conditions favor their growth, some members of the fam. *Enterobacteriaceae* establish an important spoilage group in meat (Doulgeraki et al. 2011). They cause green discoloration of meat products as well as the production of putrescine and cadaverine, diamines of foul odor (Doulgeraki et al. 2011; Gwida et al. 2014).

According to Reiche et al. (2019), the quality of raw meat is seriously affected by conditions during the process of slaughtering animals. Bacteria from the fam. *Enterobacteriaceae* represent normal and healthy parts of the intestinal microbiota of animals. Therefore, they are present and can be spread by cross-contamination. Meat processing conditions, such as poor operating techniques and low levels of hygiene in facilities, can lead to meat loss, reduced meat quality and meat spoilage. Therefore, prevention of contamination after slaughter, during cutting and processing of meat, is a key measure that can contaminate meat.

Dry fermented sausages are a good substrate for the survival and even growth of certain pathogens, such as *E. coli. S. tiphimurium* and *L. monocytogenes*. *Clostridium botulinum* and *Toxoplasma gondii* have also been identified as potential microbial risks for consumers of this type of product. Pathogenic microorganisms can be entered by cross-contamination from meat processing equipment or personnel involved in meat processing or retail. Conditions during meat processing and pathogen characteristics determine the ability of pathogens to grow and survive and to determine possible pathogen removal strategies to ensure the microbiological quality of the food (Holck et al., 2017).

Schwaiger et al. (2012) in their study showed a lower prevalence of *Salmonella* spp. in chicken drumsticks from the slaughterhouse (14%) than in retail samples (21%) which can be explained by the ability of salmonella to reproduce at low temperatures. Furthermore, the high frequency of *E. coli*-positive chicken and pork samples. *E.coli*, indicates that fecal contamination in the middle of the slaughter process. *Enterobacter*, *Citrobacter*, and *Klebsiella* were the most detected Enterobacteriaceae from slaughterhouse samples (Schvaiger et al. 2012). The study by Carney et al. (2006) performed in a beef slaughterhouse in Ireland confirmed the presence of *E. coli* O157 in 2.4% of beef samples, 3.0% of carcasses and 3.0% of head meat samples, indicating the need for stricter control measures to reduce the spread of pathogens in slaughterhouses.

Growth and distribution of *Enterobacteriaceae*

Bacterial growth in foods follows the normal pattern for bacterial growth. The lag phase may have a variable duration in a food. This depends on the properties of the contaminating bacterial species and the food. The mandatory time needs to the population density reach a significant level in each food product depends on the amount of the initial inoculum and the rate of growth during the exponential phase. The rate of bacterial growth during the exponential phase depends on the temperature, the nutrient value of the food, and other growth conditions.

Raw milk cheeses

The growth and survival of *Enterobacteriaceae* in raw milk cheeses have been studied. In general, the highest number of *Enterobacteriaceae* was found throughout the first week of ripening. Afterwards the number decreased with time of ripening at a variable rate depending on the strain, growth conditions and on the physico-chemical characteristics of cheese. Generally, the growth of bacteria depended on external factors (environmental parameters) and the internal characteristics of the food products (pH, a_w, temperature, etc.).

According to Mladenović et al. (2018a; 2018b), *K. oxytoca*, *K. pneumoniae*, *Klebsiella ornithinolytica* and *E. coli* were the most dominant species in raw milk cheese, at first stage of ripening. Trnčić et al. (2016) indicated that the milk type used in cheese production was also significantly associated with detection of coliforms. Metz et al. (2020) indicated that *E. coli* and coliforms are detected in different types of raw milk but usually at <100 CFU/ml or not found at all. They also noticed that indicators which were present in raw milk, during cheese-making, would frequently increase in numbers, but their levels declined with decreasing of pH. A quick initial acidification is the most important factor in reducing coliform loads and preventing defects such as early blowing in cheese (Sheehan, 2011). Trnčić et al. (2016) indicated between the 47 tested cheese samples with pH <5.0, only two were positive for coliforms. They also indicated that water activity was significantly associated with detection of coliforms. None of the 20 cheese samples with water activity <0.932 were positive for coliforms. However, among all mentioned factors, water activity seems to be the only factor that determines the concentration at which coliforms are present. Except for fresh cheeses, indicator levels are further reduced by 2–3 log10 CFU/g or more, amid the ripening process. As a result of ripening and pH decreasing, indicator levels in final cheese products are often low and within the limits of <10 or <100 CFU/g (Metz et al. 2020). De Pasquale et al. (2014) demonstrated that raw milk cheeses made using good quality raw milk, under hygienic conditions and properly aged, should have not contained high levels of indicator bacteria in the final product.

Yoon et al. (2016) described that the native microbiota of raw milk was made from many bacterial genera, but the most dominant were lactic acid bacteria (LAB) and members from *Enterobacteriaceae* family. As a result of the presence of native microbiota, raw milk cheeses exhibit higher amounts of volatile compounds such as carboxylic acids, alcohols and esters compared to pasteurized milk cheeses, which also affect the dynamic of growth of *Enterobacteriaceae* (Ocak et al. 2015). In addition, milk pasteurization, low pH and low water activity significantly contribute to lower prevalence of coliforms in cheese.

Meat and meat products

Members of the Enterobacteriaceae family are often found on freshly cut meat (Doulgeraki et al. 2011; Jansen et al. 2018). Various treatments for raw meat, such as preservative addition, storage temperature, vacuum, and modified atmosphere packaging (MAP), can affect the survival and growth of microorganisms that can cause food spoilage (Doulgeraki et al. 2012). Several authors have reported the appearance of many members of the Enterobacteriaceae family on raw poultry, beef, and pork (Kozačinski et al. 2006; Gvida et al. 2014; Jansen et al. 2018).

Globalization, international trade, and an increasing flow of goods and people enable foodborne zoonotic and multi-resistant bacteria to spread worldwide. An interesting study on the safety and quality of fresh poultry meat and fresh pork filets imported on the European market at border inspection post Hamburg harbor showed that *E. coli* was the most frequent microbial contamination detected on poultry in 67% and on pork in 50% of all samples. The 33 isolates were confirmed as extended-spectrum *β*-lactamase producing *E. coli*. The most likely source of these zoonotic pathogens in imported food is improper personal hygiene amid meat handling and processing accompanied by poor storage conditions during transport (Jansen et al. 2018). The order *Enterobacterales* contains species such as *Serratia* spp. (Yersiniaceae) and *Proteus* spp. (Morganellaceae) that can be found together with members of the Enterobacteriaceae family. While *Serratia proteamaculans* and *Serratia liquefaciens* frequently constitute the community of fresh meat, *Citrobacter freundii* (Enterobacteriaceae) and *Proteus vulgaris* were recovered from minced beef stored aerobically and under MAP, respectively (Doulgeraki et al. 2011). *S. liquefaciens* has been found as predominant *Enterobacterales* in raw meat stored in different atmospheres at the retail level. A psychrotolerant *H. alvei* is very often detected in minced beef stored in MAP at 5-10 °C (Doulgeraki et al. 2011; Kilonzo-Nthenge et al. 2012).

The hygienic condition of fermented meat products that do not receive any thermal treatment is regulated only by the fermentation and drying process they undergo. The *Enterobacteriaceae* found in minced meat, as a raw material for the preparation of sausage mass, mostly derives from the animal tissue, working environment, the tools used for the cutting up and mincing the meat. During the mincing of the meat potential microbial contamination is more widely distributed by the liberated meat juices, as an ideal substrate for microbial growth (Castano et al. 2002; Talon et al. 2007). It has been established that the *Enterobacteriaceae* count in the artisanal Spanish sausages has not been completely reduced after fermentation and ripening process, unlike in the industrial sausages. Moreover, in the artisanal sausage, only three species of *Enterobacteriaceae* were isolated during ripening, while their variety in the industrial sausage was much higher, with *E. coli* and *H. alvei* isolated in higher proportion, followed by *S. liquefaciens* and *Salmonella choleraesuis* (Castano et al. 2002). Factors that favor the growth of *Enterobacteriaceae* during meat fermentation include a high initial water activity and high pH values, low concentration of fermentable carbohydrates, low number of lactic acid bacteria in fresh sausage mixture, low levels of nitrite as the curing agent and high ripening temperatures. Therefore, in order to control the population of *Enterobacteriaceae* during the production of traditional fermented meat products, the addition of curing agents, sugars and starter cultures is recommended accompanied by better control of the conditions during processing (Talon et al. 2007; Settanni and Moschetti 2014).

***Enterobacteriaceae* in dairy and meat products as reservoirs of virulence and antimicrobial resistance genes**

In addition to the role of indicators of unsanitary conditions in food production, some members of the fam. *Enterobacteriaceae* have emerged as potential opportunistic pathogens (Ntuli et al. 2016). Several important virulence genes have been found in fam. Enterobacteriaceae including thermolabile toxin (LT), thermostable toxin (STa and STb), shiga-like toxin (Stk1 and Stk2), binding and deletion (Eae) and also rmpA (mucoid phenotype regulator), vabG (lipopolysaccharides), kfu (iron intake), magA (mucus viscosity), fimH (fimbriae) and uge (lipopolysaccharides), which were detected in *Klebsiella pneumoniae* (Jian-li et al. 2017). In addition, antibiotic resistance genes encoding AmpC enzymes in Enterobacteriaceae are both chromosomally and plasmid-mediated which increases its potential for lateral transfer. Antimicrobial resistance has recently been discovered (Khari et al. 2016) in bacteria isolated from dairy products such as *E. coli* and *Salmonella* spp. (Bread et al. 2015).

Resistance to antibiotics

Antibiotics are often used in animals with the aim of preventing and treating diseases, as well as increasing growth and development (Murphy et al. 2016). The administration of antibiotics can affect the food industry because antibiotic-resistant microorganisms from animals can be transferred to food products (Rolain 2013). There is also an indirect risk of horizontal transmission of resistance genes to pathogenic microorganisms at various points along the food chain (Capita et al. 2013; 2020).

Multidrug resistance is often associated with *E. coli* and *S. enterica*, which are considered the most common foodborne pathogens. According to Chauhan et al. (2013) and Fakruddin et al. (2014) described multiresistant *K. pneumoniae* from raw milk samples. Multiresistant such as *Enterobacter, Citrobacter* and *Klebsiella* have also been described by Fakruddin et al. (2014) in various food samples, including milk powder. Schwaiger et al. (2012) and Uzeh et al. (2021) described the occurrence of multiresistant strains in members of the genera *Enterobacter, Serratia, Klebsiella* and *Citrobacter*, with more frequent resistance in chicken meat. Capita et al. (2020) described the emergence of multiresistant enterobacteria isolated from red meat and poultry.

The ability to produce extended-spectrum β-lactamase (ESBL) by fam. Enterobacteriaceae is the factor of most concern to the scientific community (Tekiner and Ozpinar 2016). ESBL inhibitors are widely used to treat bacterial infections, especially gram-negative bacteria. *E coli* and *K. pneumoniae* are the most common ESBL (Saito et al. 2010). ESBL production can confer resistance to many classes of antibiotics. Foods with certain characteristics may facilitate the spread of ESBL bacteria. For example, Calbo et al. (2011) described the transmission of an ESBL-producing K. pneumoniae strain by food consumption at a health facility.

Biofilm production

Biofilms present on equipment surfaces in the food industry have been identified as the cause foodborne diseases (Capita et al. 2020). Bacteria in biofilms show resistance to various environmental stresses, thus encouraging their longevity and increasing the risk of food contamination (Mladenović et al. (2018a; 2018b). Biofilms are the dominant mode of community of microorganisms in nature (Diez-Garcia et al. 2012) .

The formation of biofilms on milk processing equipment occurs very quickly. Milk is rich in nutrients, especially calcium, which favors the formation of biofilm (Flint et al. 2015). A study conducted by Cherif-Antar et al. (2016) indicated the presence of biofilm producers *Enterobacteriaceae* (*K. pneumoniae, Serratia marcescens* and *Enterobacter* spp.) on stainless steel pipe surfaces in milk processing plants. According to Malek et al. (2012), enterobacteria may be resistant to cleaning products which must be taken into account. Malek et al. (2012) proved the presence of *Enterobacter* sp. after disinfection. Mladenović et al. (2018a; 2018b) indicated the ability of members of the genera *Serratia* and *Klebsiella* isolated from raw milk cheese to produce biofilm.

Resistance to disinfectants and the great potential for transmission of bacteria from biofilms to meat products by direct contact, is one of the biggest concerns in the industries (Wang 2019). Currently, in the meat industry, pathogens on animal skins are considered to be the main source of carcass contamination during processing. Strains *E coli* O157: H7 and STEC O157: H7, as well as *Salmonella* spp. and *L. monocytogenes* isolated from various meat products, have the ability to create a biofilm (Wang 2019). In most cases, antibiotic resistance is higher among biofilm-producing strains than among non-biofilm-producing strains (Saha et al. 2018; Capita et al. 2020).

Adaptive ability of *Enterobacteriaceae* to environmental conditions

Members of the *Enterobacteriaceae* family are widely distributed and adapted to various environmental conditions. This means that it is imminent that some members of the *Enterobacteriaceae* family will enter the food chain (Baylis et al. 2011). They possess the extreme ability to adapt. They grow in the pH range between 3.8 and 9. To control acid tolerance of microorganisms (enterobacteria) in foods, the prevention of organisms from becoming acid-adapted is crucial. If the acidification process of a food product is performed slowly, enterobacteria present in the food product will become adapted to the gradual reduction in pH. Therefore, they will be unaffected by the final pH value of the food product and survive longer in the acidic foods (Alvarez-Ordóñez et al. 2015).

The members of the *Enterobacteriaceae* family are psychotropic, mesophilic, and thermotolerant bacteria. Most of them, including foodborne pathogens, are mesophilic. An optimum growth temperature of 37 °C is common for *Enterobacteriaceae* of fecal origin (Baylis et al. 2011; Mladenović et al. 2018c; Mladenović et al. 2019d).

The best water activity (a_w) for the growth of *Enterobacteriaceae* is above 0.95, with a minimum a_w to 0.94 as to the amount of water required. Thus, foods with a higher water activity provide optimal conditions for growth. This applies particularly to food such as fresh meat and fish, fresh fruits, vegetables, and milk, with *aw* values of 0.98 or above. According to Abdulkarim et al. (2009), higher salinity has an inhibitory effect on the growth and development of enterobacteria.

One of the ways in which bacteria adapt to environmental conditions is to form a biofilm. Milk possesses characteristics that may promote biofilm production on surfaces. Its composition is rich in lipids, proteins, and certain divalent cations, e.g., calcium, which favors the formation of biofilm (Teh et al. 2014). Biofilm formation is influenced by environmental factors. They can stimulate or inhibit biofilm formation. For example, the higher salt concentration in cheese inhibits the biofilm formation of *Enterobacteriaceae* (Mladenović et al. 2018c; Mladenović et al. 2019d).

Maintaining the quality of fermented sausages consists of several strategies: lowering the pH by fermenting sugar into mostly lactic acid, reducing water activity by salting, drying by evaporating water, inhibiting the growth of aerobic bacteria by creating an anaerobic environment, inhibiting microbial growth by adding nitrates or nitrites and inhibiting surface growth by smoking or adding specific molds (Holck et al., 2017). Exposure to stress can cause varying degrees of cell damage or injury depending on the intensity of stress and the physiological state of individual cells (Alvarez-Ordonez et al. 2015). Some members of the Enterobacteriaceae family may develop resistance to extreme processing conditions. Bacteria can also respond to adverse conditions through so-called stress tolerance responses. These responses include both structural and physiological modifications in the bacterial cell.

They are mediated by complex genetic regulatory mechanisms (Alvarez-Ordonez et al. 2015). The main aspects of the adaptive response are given in Table 1.

Mechanisms for controlling the number of *Enterobacteriaceae* in dairy and meat products

Raw milk cheeses

Pasteurization is one of the methods used to standardize microbial composition and improve the microbial safety of milk (Montel et al. 2014). Proponents of pasteurization argue that it is precisely the warming of milk that leads to less frequent occurrence of pathogenic species in milk (Montel et al. 2014), and afterward in the cheese (Brooks et al. 2012). On the other hand, proponents of traditional cheeses made from fresh unpasteurized milk, discuss about preserving microbial indigenous cheese communities. Their arguments include the great diversity of microbial species combined with a certain method of production. Cheese diversity, sensory properties, as well as the low risk of developing pathogens are based on this (Montel et al. 2014).

Microfiltration is an alternative to pasteurization. Microfiltration involves filtering milk through membranes that contain pores of certain dimensions that retain microorganisms, and a sterile filtrate leads to obtaining sterile milk (Fox et al. 2000). In this way, unwanted microorganisms are removed, and the physico-chemical composition of the milk is preserved. By eliminating the autochthonous microbiota, the original taste of the cheese is changed.

In cheese manufacturing, the activity of lactic acid bacteria (LAB) may be used as food preservative and contribute to the quality of cheese. Frequently established interaction between the *Enterobacteriaceae* and LAB is antagonistic. LAB prevent the multiplication of enterobacteria, by applying several control mechanisms. First, the antagonistic activity of LAB could be related to the fact that they compete for nutrients (Gopal et al. 2001). Also, the ability of LAB to produce antimicrobial compounds is well known, e.g., lowland production (*Lactococcus lactis*) (Biscola et al. 2013). Due to the large fermentative capacity of LAB, metabolic products, especially produced acids such as acetic, formic, and lactic acids may be considered as potential inhibitors. Indirectly, decreasing the pH value, the produced acids inhibit the growth of undesirable bacteria (Nuraida 2015).

Meat and meat products

Nowadays meat preservation methods became necessary due to the long-distance transportation and meat storage in retail and supermarkets. The basic aim of preservation methods is to extend the shelf life of meat by inhibiting the microbial spoilage, including the growth of *Enterobacteriaceae*. Low temperature methods of storage help to inhibit or completely stop bacterial growth in the meat chill chain. However, freezing rate can significantly affect the quality of frozen meat, with only 60% of the viable microbial population that dies at low temperatures, while the remaining population gradually increases during frozen storage (Dave and Ghaly 2011). Foodborne pathogens from the *Enterobacteriaceae* group, *Salmonella* spp. and *E. coli* are frequently associated with chilled and frozen raw meats, poultry, and their products (Kozačinski et al. 2006; Jansen et al. 2018). Antimicrobial preservatives (chlorides, nitrates, sulfites, and organic acids) are often used in reducing microbial proliferation amid slaughtering, transportation, processing, and storage (Dave and Ghaly 2011). Sallam and Samejima (2004) found that the use of sodium chloride in combination with sodium lactate reduced the *Enterobacteriaceae* count, maintained the chemical quality, and extended the shelf life of ground beef during refrigerated storage. Organic acids have demonstrated antimicrobial activities against many pathogenic organisms such as *Salmonella* spp. and *E. coli* O157:H7, due to their abilities to reduce pH level of meat (Smulders et al. 2013). However, they should not be used as a substitute for poor processing conditions or to cover up an already spoiled meat product (Dave and Ghaly 2011).

Since the procedures throughout meat cutting and processing cannot guarantee the absence of microbial contamination in the raw materials, the addition of acidifying starter cultures to the minced meat, curing salts and decrease of water activity can improve the sanitary condition of traditional fermented meat products (Castano et al. 2002; Settanni and Moschetti 2014). In meat products, the most widely used starter cultures are lactic acid bacteria that produce several compounds with antimicrobial action. Casquete et al. (2012) demonstrated the inhibitory effect of starter culture made up of autochthonous strains of *Pediococcus acidilactici* and *Staphylococcus vitulinus* on enterobacteria in a traditional Iberian dry sausage. In the traditional Romanian dry sausage, the decrease of *Enterobacteriaceae* throughout the ripening period is explained by the low pH value, due to the inclusion of *Lactobacillus acidophilus* on the starter culture (Ciuciu Simion et al. 2014).

New techniques for controlling microorganisms in foods

Cold plasma technology

The science and technology of cold plasma are being researched and introduced as one of the methods for preserving foodstuffs in the food sector. Plasma technology is considered as a modern non-conventional technique (Bourke et al. 2018). As a novel technology, cold plasma is a technique used for sterilization of sensitive materials like food. For years cold plasma processing has been viewed as useful for microbial inactivation while maintaining quality of fresh produce. Overall application of cold plasma for microbial destruction on different food substrates like fruits, meat products, cheese etc. was considered (Thirumdas et al. 2014).

High pressure

High pressure processing is a food pasteurization technique that leads to the inactivation of microorganisms at room temperature, followed by minimizing the loss of sensory and nutritional components of the food. This process preserves the original color, flavor and nutritional content of food since smaller molecules (pigments, vitamins, volatile compounds, etc.) are less affected by high pressure (Huang et al. 2014). However, Vanlint et al. (2012) examined the potential for high hydrostatic pressure (HHP) resistance development among strains of *E. coli*, *Shigella flexneri*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Yersinia enterocolitica*. They reported that extreme HHP resistance was observed only in some *E. coli* strains, which is probably due to specific genetic predisposition. Therefore, it is important to combine two or more techniques for controlling microorganisms in foods, due to their high adaptive ability.

Natural preservatives

Some chemical preservatives such as sodium benzoate, potassium sorbate and nitrites, have been used commercially to conserve food (fruit juices, dairy products, meat, and meat products, etc.) from contamination by spoilage microorganisms. However, the usage of chemical preservatives has initiated some health issues. Therefore, the recent trend is towards the use of natural antimicrobials, such as plant antimicrobials, in food preservation. Spices and herbs are used in food since the ancient time, not only for flavoring but also for the preservation. The antimicrobial properties of plants are associated to their secondary metabolites such as phenylpropanoids, terpenes, flavonoids, and anthocyanins. Nowadays, it is proved the efficacy of plant products and various compounds isolated from the plants are used (Dhiman and Aggarwal 2019).

Bacteriocins

Bacteriocins are naturally synthesized peptides that produce bacteriostatic or bactericidal effects on other bacteria. The use of bacteriocins in natural preservation is an essential strategy to increase food safety due to its minimal impact on the nutritional and sensory properties of food products. Low pH and bacteriocins from Gram-positive bacteria (mainly lactic acid bacteria) that are presented in food may play an important role in natural food preservations. Furthermore, bacteriocins combined with natural food preservatives containing plant essential oils, with high pressure processing (HPP), temperature etc. represent a future perspective in preservations of food (Prudêncio et al. 2015).

Conclusion

Bacteria from the *Enterobacteriaceae* family are present in our environment, and we inevitably encounter them. They are an effective indicator of meat and dairy product quality and hygiene throughout the production process. Hygienic and sanitary preventive measures include protecting the food from direct or indirect contamination, applying personal hygiene practices, preserving the food in appropriate places and temperatures, proper storage. However, *Enterobacteriaceae* represents a part of frequently detected microbiota in cheeses made from fresh, unpasteurized milk which defines the final organoleptic characteristics of the resulting product. Therefore, it is recommended that fermented food made from raw milk or meat is manufactured with the addition of autochthonous microorganisms in order to preserve the original texture, taste and aroma of traditional products, as well as to avoid risks to consumers' health and their exposure to potentially pathogenic bacteria and their toxins.

Considering the pathogenic properties of many members of the *Enterobacteriaceae* family and their high prevalence in dairy and meat products, strict observance of hygiene policies and systematical monitoring of *Enterobacteriaceae* at all stages of food production plays a crucial role in ensuring food safety and controlling the transmission of pathogenic foodborne bacteria to humans.

Declarations

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Author contributions

K. G. Mladenović, M. Ž. Grujović have idea for the article and represented literature of enterobacteria and raw milk chesses; M. Kiš, S. Furmeg, V. Jaki Tkalec were represented literature of enterobacteria in meat products; O. D. Stefanović and S. Kocić-Tanackov were represented a legal act and mechanisam of control the enterobacteria and critically revised the manuscript. All authors read and approved the final manuscript.

Consent for Publication

All the authors have read the manuscript and have approved this submission.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors declare that they have no conflicts of interest with the current work or its publication.

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Table 1. The major aspects in the adaptive response of some members from *Enterobacteriaceae* family

Adaptation	Mechanisms	Mode of action	Model microorganisms	References
The acid stress response	Homeostatic systems	Keep intracellular pH relatively constant at pH 7.6 to 7.8, even as external pH changes during growth, restoring the internal pH to neutrality	*S. Typhimurium*, *E. coli*	Foster (2000); Kieboom and Abee (2006); Alvarez-Ordóñez et al. (2010b)
	Acid shock proteins (e.g., RpoS)	Cellular regulation, molecular chaperoning, energy metabolism, transcription, translation, synthesis of fimbriae, regulation of the cellular envelopes, colonization, and virulence	*S. Typhimurium*, *E. coli*	Audia et al. (2001); Bearson et al. (2006)
	Membrane fluidity (membrane adaptation)	Effect on membrane fatty acid composition (decrease in the unsaturated to saturated fatty acid ratio and in the relative concentration of octadecenoic (oleic or vaccenic) acidc, with a concomitant increase in the content in cyclic fatty acids	*S. Typhimurium*, *E. coli*, *Yersinia enterocolitica*	de Jonge et al. (2003); Alvarez-Ordóñez et al. (2008, 2010a); Beales (2004)
	Other mechanisms	Chilled temperatures for *S. enteritidis* in low pH conditions	*S. enteritidis*	Phillips et al. (1998); Beales (2004)
High salt stress response (increasing the osmotic pressure by lowering *aw* by drying, salting, or sugaring)	Osmoregulation - The bacteria raise their internal solute levels (compatible solutes), resulting in an increase in internal osmotic pressure and restoration of turgor pressure	As the bacterium loses water, cytoplasmic level of K^+ increases. This triggers enzymes, such as glutamate dehydrogenase to form glutamate from a ketoglutamate. As the glutamate levels increase, water starts to re-enter the cell and growth resumes	*E. coli*, *Salmonella* spp.	Galinski (1995); Beales (2004)
	Gene expression	Expression of the *kdp* gene (codes for the high-affinity potassium uptake system Kdp) - This results in an uptake of potassium, which can last until the turgor is restored	*E. coli*	Gutierrez et al. (1995); Beales (2004)
Low temperature stress response	Cell membranes response - changes in fatty acid composition	An increase in the amount and/or kind of branched fatty acids, a reduction in the proportion of cyclic fatty acids and thus an increase in mono-unsaturated straight chain fatty acids	*Salmonella* spp.	Russell (1984); Russell et al. (1995); Beales (2004)

		The effect of $C_{15:0}$ on physical properties and on maintaining a fluid, liquid-crystalline state of the membrane lipids	*E. coli*, *Y. enterocolitica*	Annous et al. (1997); Beales (2004)
	Cell membranes response - synthesize elevated levels of enzymes	Production of cold adapted enzymes such as β-galactosidase	Some psychrophiles	Nakagawa et al. (2003); Beales (2004)
	Gene expression: the cold shock response	Protein RecA - Role in recombination and the induction of the SOS response	*E. coli*	Berry and Foegeding (1997); Beales (2004)
		Hsc66 (70-kDa heat shock protein) – The response thought act as a molecular chaperone in the cold shock response. Ensure the conformation of proteins and refolding of denatured proteins occurs correctly		Lelivelt and Kawula (1995); Beales (2004)
		CspA (70 amino acid protein encoded by the *cspA* gene) - It has a high induction level, increasing 200-fold after a reduction from 37 °C to 10 °C. CspA is a transcriptional regulator, which recognizes gene promoters and switches them on, thus producing cold shock proteins		Jones et al. (1987), Jones and Inouye (1994); Beales (2004)
		Cold shock inducible CspB and CspG -The temperature dependence of CspB and CspG induction is restricted to low temperature ranges		Etchegaray and Inouye (1999); Beales (2004)
		Cold shock inducible CspI – may bind to RNA and single-stranded (ss)DNA.		Wang et al. (1999); Beales (2004)