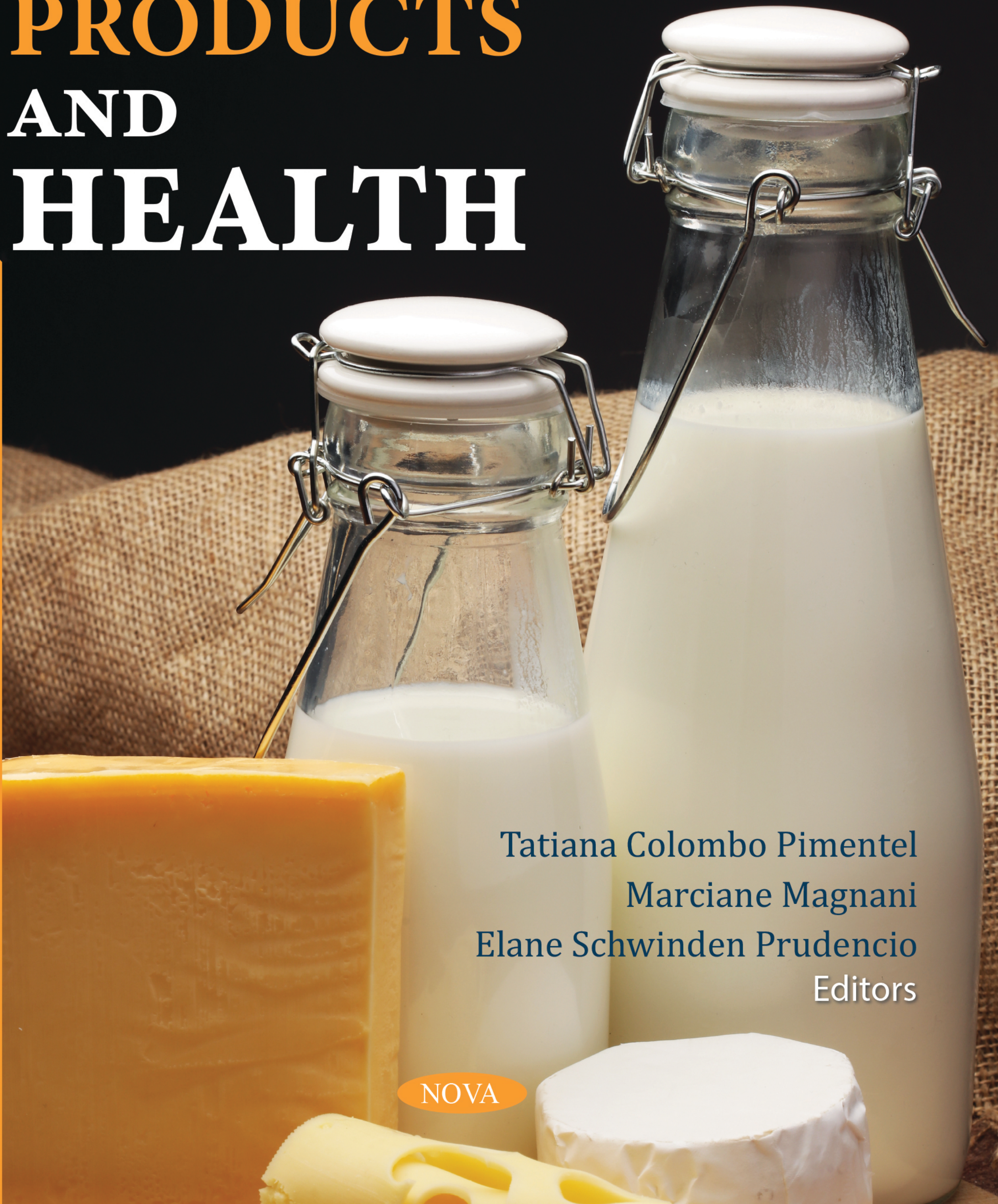
Food Science and Health

# Dairy Products and Health



Tatiana Colombo Pimentel
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# Contents

# Preface

Milk and dairy products provide many essential nutrients for consumers of all ages. Their consumption can give health benefits by decreasing fractures rate, blood pressure, and cholesterol and reducing cardiovascular diseases, diabetes, body weight, and colon cancer risk.

Chapter One highlights the occurrence and the sources of coagulase-positive staphylococci in dairy products and the factors that influence the formation of staphylococcal enterotoxins. Moreover, some preventive measures to avoid their presence are recommended.

Chapter Two discusses the potential and beneficial health effects of fermented dairy products with probiotic starters such as *Lactobacillus* (and amended genera) and *Bifidobacteria*, as well as the possible role of probiotic fermentation metabolites in the treatment and prevention of some diseases and disorders.

Chapter Three aimed to elucidate current concepts about probiotics, prebiotics, synbiotics, and postbiotics, addressing the changes made in these terms, their actions on the health of consumers, their advantages, and their applications in dairy products, including their functional property claims.

Chapter Four includes a literature review about adding plant extracts and fruits to dairy products, highlighting the following dairy products: cheeses, fermented milks, ice creams, dairy beverages, and butter. The bioactive compounds of natural origin, including pulp, fruit juice, and plant extracts, with great sensory acceptance, improving the nutritional and functional properties of dairy products are discussed.

Chapter Five is a review of the innovative, better-for-you ice cream category. For better understanding, was approach classical issues involving the ice cream definition, stages and components used in ice cream production, the quality of ice cream, ice cream viscosity, the melting rate of the ice cream, ice cream freezing point, the microbiological quality of ice cream, and finally, the better-for-you ice cream category.

Chapter Six takes an approach to brown cheese's health and technological aspects. This chapter involves brown cheeses' definition, origin, and nutritional composition. The highlight of this chapter was the possibility to elaborate the brown cheeses from milk, cream and/or whey, either from cow or goat. Another relevant point of this chapter is the use of whey by the industry, due to the large volume generated in the manufacture of cheeses and, therefore, its low cost.

Chapter Seven focuses on recent advances on the possible benefits of milk polar lipids on different aspects of cardiometabolic health, including favorable effects on lipid digestion and absorption, reduction of lipid markers of cardiovascular risk, and potential benefits on metabolic inflammation via modulations of the gut microbiota and barrier.

Finally, Chapter Eight provides a brief overview of functional dairy products, focusing on the potential application of lactic acid bacteria in promoting health effects and as a producer of biopolymers like exopolysaccharides (EPS) and bacteriocins. Also noteworthy is the development of functional dairy products and cheese whey by lactic acid bacteria (LAB) to produce these bioactive compounds.

# Chapter 1

# *Staphylococcus Aureus* in the Dairy Production Chain

**Maria Schirone*** 
**and Pierina Visciano**
Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

## Abstract

Milk and dairy products provide many essential nutrients for consumers of all ages. They are high sources of easily digestible proteins, carbohydrates, vitamins A, D, E, C, and B group, minerals (i.e., calcium, potassium, zinc, phosphorus, and magnesium), and saturated fatty acids. In particular, the individual consumption of such foods can give health benefits by decreasing fractures rate, blood pressure, and cholesterol and contributing to reducing cardiovascular diseases, diabetes, body weight, and colon cancer risk. Further advantages are the prevention of tooth decay, arthritis, and good functioning of the digestive apparatus. Yogurt and other fermented dairy products may be vehicles of different probiotic bacteria.

Dairy products are highly nutritious for humans and a wide range of microorganisms, providing the ideal environment for their growth. The production process, type, and treatment of milk, milking practices, storage conditions, air, water, soil, and feeding management can affect the quality, shelf-life, and safety characteristics of such foods. Therefore, undesirable microorganisms belonging to different genera, such as *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Streptococcus*, and *Bacillus,* are relatively common in raw milk, sometimes reaching

* Corresponding Author's Email: mschirone@unite.it.

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amounts of $10^6$-$10^7$ cfu/g in cheeses and contributing consequently to a worsening of their sensorial attributes or constituting a health risk in case of pathogenic species occurrence. Among pathogens, *Staphylococcus aureus* is frequently isolated from the raw milk used for cheese production the dairy managing equipment and environment, and food handlers. Such microorganism produces different potent enterotoxins remaining stable even after milk pasteurization. Their ingestion can result in a staphylococcal food poisoning outbreak, the most common foodborne disease in the European Union (EU) due to the consumption of raw milk and artisanal cheeses. The onset of illness is rapid, with symptoms such as nausea, vomiting, and stomach cramps, generally enduring less than 24 hours. According to the Commission Regulation (EC), No 2073/2005 and further amendments, the coagulase-positive staphylococci (CPS) must be checked in cheeses made from raw milk or pasteurized, ripened, or unripened cheeses. If their values exceed $10^5$ cfu/g, the cheese batch should be tested for staphylococcal enterotoxins. In the year 2020, 16 outbreaks described in 8 EU Member States (Austria, Denmark, Finland, Germany, Italy, the Netherlands, and Slovakia) were linked to the consumption of contaminated milk, cheeses, and dairy products. *S. aureus* toxins were also involved in 3 outbreaks.

This chapter will highlight the occurrence and the sources of CPS in dairy products and the factors that influence the formation of staphylococcal enterotoxins. At the primary production level and in the dairy environments, the food business operators, the lack of proper hygiene measures during cheese-making, an inadequate thermal treatment, and/or post-pasteurization contamination may lead to intoxication caused by the growth of *S. aureus* and the production of staphylococcal enterotoxins in milk and cheese. Moreover, some preventive measures to avoid their presence will be recommended.

**Keywords**: *Staphylococcus aureus*, enterotoxins, outbreaks, milk, dairy products

## Introduction

Dairy production is an important industry worldwide. Its products are highly valuable foods providing several essential nutrients, such as proteins, carbohydrates, lipids, vitamins, and minerals, particularly calcium, potassium, phosphorus, zinc, etc. Moreover, the consumption of milk, cheese, yogurt, and other derivative products lasts during childhood and throughout adulthood (Meunier-Goddik and Waite-Cusic, 2019).

Due to their high content of nutritional components, low acidity, and high-water activity ($a_w$), such commodities offer a suitable medium for the growth of various microorganisms, including bacteria, yeasts, and molds. Therefore, air, soil, water, feed, feces, inadequate sanitation of milking machines and storage tanks, and incorrect cold chain maintenance during manufacturing and transport can be important sources of milk contamination (Machado et al., 2017; Iannetti et al., 2019). Further crucial factors are the size and management practices at the the farm level, the animal's health status, the processing environment's sanitary conditions, and the season and geographical location (Davis et al., 2018).

Then, the principal aspects of dairy products able to cause foodborne diseases are:

- the presence of harmful microorganisms (scarce quality of raw matter);
- environmental contamination during processing;
- accidents during the pasteurization;
- inadequate storage conditions and poor temperature control;
- cross-contamination after production.

The microbial contamination may occur at different steps throughout dairy production and involves both Gram-negative (e.g., *Pseudomonas*, *Citrobacter*, *Acinetobacter*) and Gram-positive bacteria (such as *Bacillus* and *Paenibacillus*) and a wide range of fungal genera (Martin et al., 2021). Yeasts (e.g., *Rhodotorula*, *Kluyveromyces*, *Candida*) and molds (like *Alternaria*, *Fusarium*, and *Penicillium*) are not heat resistant. Therefore, their presence in pasteurized cheese is linked to contaminated equipment, air, or brine, representing post-processing contamination (Garnier et al., 2017). Even during refrigerated temperature storage, some microorganisms can grow and produce different degradative enzymes resulting in off-odors, off-flavors, body defects, and pigment production (Reichler et al., 2018).

In addition, many pathogens comprising *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Cronobacter sakazakii*, *Clostridium botulinum*, *Clostridium perfringens,* and enteropathogenic *Escherichia coli* have been involved in severe foodborne outbreaks or consumer illnesses reported in association with milk and dairy products (Paswan and Park, 2020; Tohoyessou et al., 2020).

Besides the bacteria mentioned above, *Staphylococcus aureus* is one of the most common agents responsible for a broad and different spectrum of complications in animals and humans, also causing toxin-mediated foodborne diseases (Li et al., 2015). Staphylococci are spherical, Gram-positive, and mesophilic bacteria belonging to the *Staphylococcus* genus, catalase-positive, oxidative negative, non-motile, non-spore-forming, facultatively anaerobic, and resistant to adverse parameters, such as low $a_w$, high salt concentrations, and osmotic stress (Baran et al., 2017). They are distinguished in coagulase-positive staphylococci (CPS), for example, *S. aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus*, and coagulase-negative staphylococci, such as *Staphylococcus epidermidis* (Becker et al., 2014). The coagulase test is used to differentiate the species belonging to the *Staphylococcus* genus into 2 groups based on the ability of the microorganism to produce the enzyme coagulase. Such enzyme-like protein converts fibrinogen into fibrin, determining blood to clot. Therefore, CPS are considered potential food enterotoxin-producing species. Moreover, when the number of CPS is over $10^5$ per gram during cheese manufacturing, staphylococcal enterotoxins production can occur, and the risk of intoxication is high.

*S. aureus* is ubiquitous in the environment and can be found on the skin (primarily on the hands, chest, and abdomen), gastrointestinal tract (Fisher et al., 2018), and mucous membranes of both animals and humans (Abril et al., 2020). This pathogen causes inflammatory infections in bovine, ovine, and caprine species consisting in milk production and quality decreases; somatic cell count increases as well as important economic losses in the dairy industry (Merz et al., 2016). The microorganism can get into milk either by direct excretion from the udders of ruminants with clinical (red and swollen mammary glands) or subclinical (no visible signs) staphylococcal mastitis or by the environmental conditions throughout the handling and manufacturing of milk for derivate products (Riva et al., 2015; Schirone and Visciano, 2018). If the udders are infected, the counts of *S. aureus* could reach values up to $10^8$ cfu/ml (Jahan et al., 2015). In humans, infective endocarditis, bacteremia, osteomyelitis, abscesses, skin lesions, and lung infections are frequently reported (Tong et al., 2015).

## Foodborne Outbreaks Caused by *S. Aureus* Enterotoxins

The most important public health problems are due to the ability of some *S. aureus* strains to synthesize a great variety of highly heat-stable and water-soluble enterotoxins and develop resistance to 3 or more classes of antibiotics (Dittmann et al., 2017). Such toxins are low molecular weight and proteolytic-resistant proteins produced by *S. aureus* during the logarithmic step of cell growth or through the passage from the exponential to stationary phase (Derzelle et al., 2009).

Table 1 shows some factors, such as an extensive range of temperature (7-48°C), pH (4-10), sodium chloride concentration (0-20%), and $a_w$ affecting the growth and production of staphylococcal enterotoxins by the pathogen. Such growth parameters can vary among the different *S. aureus* strains, and the amounts of staphylococcal enterotoxins are strain dependent. However, in the absence of milk thermal treatment, the initial concentrations of *S. aureus* can reach unsafe levels of enterotoxins due to its multiplication potential. To date, a total of 24 staphylococcal enterotoxins, designated with alphabetical letters and distinguished into 2 groups, the classical (SEA to SEE) and the newer enterotoxin-like proteins (SEG to SElY and counting), have been identified (Oliveira et al., 2022). Staphylococcal enterotoxins A and D are mainly correlated with foodstuffs; therefore, are the most clinically relevant. Then, if a food contains preformed toxins in sufficient quantities, one or more, they can cause staphylococcal food poisoning.

The symptoms (Figure 1) appear a few hours (3-9) after the consumption of contaminated food and are usually self-limited, completely resolving within 12-24 hours or without specific treatment depending on the individual health status (Tong et al., 2015). Therefore, the true incidence of this intoxication may be underestimated or misclassified due to similar symptoms to other illnesses. However, a few micrograms of staphylococcal enterotoxins can cause more severe or fatal disease, even if occasionally, in some people, such as children, elderly, and immunocompromised (Wu et al., 2016).

In the United States, *S. aureus* affected approximately 241,000 cases of foodborne illness between 2000 and 2008, identifying the pathogen fifth among the most reported microorganisms (Dai et al., 2019; Zeaki et al., 2019). In Australia, *S. aureus* intoxication was described in 1% of all foodborne outbreaks in the years 2000-2012, while in China it corresponded to 12.5% in 2013 (Pillsbury et al., 2013).

**Table 1.** Factors influencing *S. aureus* growth and enterotoxin production.

| **Parameters** | **Bacterial growth** | | | **Staphylococcal enterotoxin production** | |
|---|---|---|---|---|---|
| | **Optimum** | **Range** | | **Optimum** | **Range** |
| Temperature (°C) | 37 | 7-48 | | 37-45 | 10-45 |
| pH | 6-7 | 4-10 | | 7-8 | 4-9.6 |
| $a_w$ | 0.98 | 0.83-0.99 | | 0.98 | 0.85-0.99 |
| NaCl (%) | 0 | 0-20 | | 0 | 0-10 |
| Atmosphere | Aerobic | Aerobic-Anaerobic | | Aerobic | Aerobic-Anaerobic |
| Redox potential ($E_h$) | > +200 mV | < -200 mV – +200 mV | | > +200 mV | < -100 mV – > +200 mV |

Modified from Tatini, 1973; ICMSF, 1996.


**Figure 1**. Symptoms of staphylococcal food poisoning were analyzed with a word cloud generator.

In the EU, 8% of foodborne outbreaks reported in the year 2016 were caused by staphylococcal enterotoxins, and the main vehicle was mixed food (31%) followed by milk and milk products with a percentage of 22 (EFSA and ECDC, 2017). In 2020, 43 outbreaks, 402 cases, and 32 hospitalizations were reported by 6 EU Member States (Belgium, Finland, France, Italy, Spain, and Sweden), and staphylococcal enterotoxins were found in samples of pasteurized cheeses, according to the recent European Food Safety Authority Report (EFSA and ECDC, 2021). Moreover, an important increase in antimicrobial resistance among zoonotic pathogens was observed.

Most infections are caused by methicillin-resistant *S. aureus* (MRSA) strains. Such resistance is given by the *mec*A gene encoding the production of a different penicillin-binding protein with a low affinity for all beta-lactam antimicrobials. The increasing resistance is caused by the use and, in some cases, the overuse of antibiotics in various sectors, like human communities and hospitals, as well as at the farm level and in pharmacological treatments of pets (Kwon et al., 2006). In the year 2019, 6 pathogens, including *S. aureus*, resulted responsible for more than 250,000 deaths associated with antimicrobial resistance (Antimicrobial Resistance Collaborators, 2022).

According to Directive 2003/99/EC, data on animals, food, and feed must be stated on a compulsory basis for some zoonotic agents, such as *Campylobacter* spp., *Salmonella* spp., and *Listeria monocytogenes*. The EU Member States also provide data on staphylococcal enterotoxins. Then, *S. aureus* toxins are one of the most frequently described causes of food poisoning outbreaks.

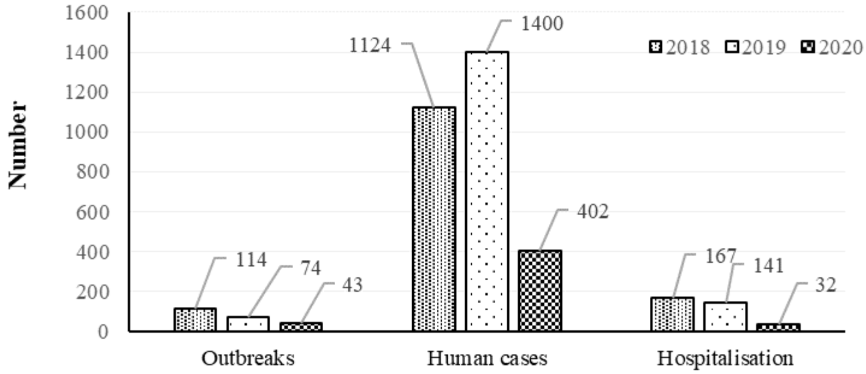


**Figure 2**. Number of outbreaks and cases of illness (human cases and hospitalization) caused by *S. aureus* toxins in EU Member States in the years 2018-2020.

Figure 2 shows the notification rates for such intoxication reported by the EU Member States in the years 2018-2020. A decreasing trend and no death were observed. In 2018, the foodborne outbreaks caused by *S. aureus* enterotoxins were related to 15 Member States (Bulgaria, Croatia, Czechia, Denmark, France, Germany, Greece, Hungary, Italy, Latvia, Poland, Portugal, Romania, Slovakia, Spain), while 13 (Bulgaria, Croatia, Cyprus, Finland, France, Germany, Hungary, Italy, Poland, Portugal, Romania, Spain, Sweden) and 8 countries (Belgium, Finland, France, Germany, Hungary, the Netherlands, Poland, Spain) described the such event in the years 2019 and 2020, respectively. Regarding the food vehicle, milk, cheese, and dairy products (other than cheeses) were responsible for 38, 17, and 16 foodborne outbreaks in the investigated period (data not shown). The lower values registered in 2020 could be connected to the closure of restaurants, pubs, or similar activities due to the COVID-19 pandemic.

In 2020, 99 out of 3,862 original notifications in milk and dairy products were communicated through the Rapid Alert System for Food and Feed (RASFF). However, for the same food category, an important decrease in original notification data (50 out of 3,699) compared to 2018 was observed. Table 2 shows some non-compliances in milk and derivate products reported by RASFF. Microbial contaminants and foreign bodies constituted the major hazards in all dairy products from 2018-2020, even if the number decreased slightly in 2020.

**Table 2.** Notifications of some non-compliances in milk and milk products in the years 2018-2020.

| **Hazard category** | **Non-compliance** | | |
|---|---|---|---|
| | **2018** | **2019** | **2020** |
| Microbial contaminants | 55 | 55 | 41 |
| Pathogenic microorganisms | - | 11 | 7 |
| Foreign bodies | 14 | 8 | 12 |
| Allergens | 2 | 3 | 12 |
| Absent/incomplete/incorrect labeling | 2 | 1 | 1 |
| Poor or insufficient controls | - | 1 | 2 |
| Residues of veterinary medicinal products | - | 1 | 3 |
| Mycotoxins | - | 1 | 1 |

Legend: - = not reported.

Based on Commission Regulation (EC) No 2073/2005 (EC, 2005) and further amendments, CPS should be detected in various categories of cheese, milk, and whey powder as process hygiene criteria (Table 3). Such criteria must be applied at the end of the manufacturing process or when the number of staphylococci is expected to be the highest, and only when such values exceed $10^5$ cfu/g the batch must be analyzed for staphylococcal enterotoxins.

Most staphylococcal food poisoning episodes are reported in those countries (i.e., France and Italy) where the consumption of unpasteurized cheese is common. Iannetti et al. (2019) analyzed 404 samples of milk and dairy products from different dairy industries in Central Italy within the framework of own checks and official controls aiming at the enumeration of CPS. Only some dairy samples (*provola* and *mozzarella*) collected by the competent authority during official controls exceeded the maximum limits fixed by the Commission Regulation (EC) No 2073/2005. Therefore, the presence of staphylococcal enterotoxins was observed, resulting in their withdrawal from the market. Di Giannatale et al. (2011) found that 4 out of 30 fresh cheeses were contaminated with *S. aureus* ranging between 750 and 2 800 cfu/g. One out of 4 strains was enterotoxigenic, and the toxins detected were C and A enterotoxins. At the same time, Normanno et al. (2007) examined 641 dairy products finding that 9.2% of samples were positive for *S. aureus*. Dittmann et al. (2017) determined the presence of *S. aureus* in 5 dairies in Brazil. A total of 31 out of 421 samples were positive for the

**Table 3.** Process hygiene criteria for the detection of coagulase-positive staphylococci in dairy products (EC, 2005).

| Food Category | Sampling plane | | Limits (cfu/g) | | Stage where the criterion applies | Action in case of unsatisfactory results |
|---|---|---|---|---|---|---|
| | n* | c** | M | M | | |
| Cheeses made from raw milk | 5 | 2 | $10^4$ | $10^5$ | During the manufacturing process, when the number of staphylococci is expected to be highest | • Improvements in production hygiene<br>• Selection of raw materials<br>• If values > $10^5$ cfu/g, the cheese batch is tested for staphylococcal enterotoxins |
| Cheeses made from raw milk that has undergone a lower heat treatment than pasteurization and ripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment | 5 | 2 | $10^2$ | $10^3$ | | |
| Unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurization or a stronger heat treatment | 5 | 2 | 10 | $10^2$ | End of the manufacturing process | • Improvements in production hygiene<br>• If values > $10^5$ cfu/g, the batch is tested for staphylococcal enterotoxins |
| Milk powder and whey powder | 5 | 2 | 10 | $10^2$ | | |

Legend: * = number of units comprising the sample; ** = number of sample units giving values between m and M.

pathogen, and 66 isolates were typed, assessed for 11 antibiotics compounds, and detected for the presence of enterotoxin encoding genes. No MRSA strains were found, and only 3 strains harbored enterotoxin G, and 2 of these also enterotoxin A.

Rahimi (2013) analyzed a total of 347 samples from various dairy products, traditional and commercial, collected from retail stores in Iran. A total of 20 samples were contaminated with *S. aureus,* and 7 out of 20 strains could synthesize classical enterotoxins. Enterotoxin C was the most common, followed by A. Moreover, 95 and 55% of isolates were resistant to one or more, and 3 or more antimicrobial agents, respectively.

A combination of management and control measures along the entire dairy supply chain influences the safety of raw milk. Milking hygiene is essential to hinder contamination. Practices, such as teat washing, may reduce the number of pathogenic and spoilage microorganisms in milk. The udder washing with clean water and its following drying with towels reduces the presence of transient bacteria located on the exterior surfaces of the udder, while post-milking teat disinfection diminishes the resident teat skin bacterial population is the main source of infection for the mammary gland. The rapid cooling and holding of the collected milk are particularly important, as an inappropriate temperature can lead to bacterial growth. Even the time and temperature conditions through storage and distribution influence the concentration of any contaminating pathogens. Correct sanitizing procedures for packaging and effective cold chain management practices for raw milk are important steps for minimizing cross-contamination and the growth of any microorganism present in the raw milk.

Human carriers, especially food handlers transferring the pathogen from their noses or hands, are considered the principal reservoir of staphylococcal food poisoning by direct contact or respiratory secretions (Argudín et al., 2010). Therefore, raw materials, food handlers, poor hygiene during manufacture and processing and post-production are the main sources of *S. aureus* in dairy products (Castro et al., 2016).

## Conclusion

The surveillance of raw milk is essential for understanding the risk factors associated with the milk supply chain. Good milk quality and general hygiene environmental parameters should be evaluated to guarantee public health safety. Pasteurization is suitable for killing pathogens and also, able to eliminate toxins. For example, staphylococci can directly contaminate milk due to mastitis occurrence, but inadequate environmental conditions can also be a major contamination source. Therefore, the improvement of good manufacturing and hygiene practices, milking operations, and a deep sanitary status of the herd are strongly recommended.

The potential for enterotoxin to be produced in dairy products depends on the initial count of *S. aureus*, the property of genes encoding staphylococcal enterotoxin production. Still, some factors, such as pH and temperature, can favor the growth and production of enterotoxin.

In recent years, another important problem has been the emergence of antimicrobial-resistant strains, particularly MRSA colonization in food-producing animals and the links with human health, especially personnel working with animals. The transmission of MRSA through milk has been well documented, but it is not likely to occur when animals show clinical signs of udder disease because their milk cannot be used for human consumption. Contrastingly, when mastitis is a subclinical or latent infection, the possible resistant microorganisms can be transmitted to humans via raw milk products (Visciano et al., 2014). More restrictive policies on the use of antibiotics for both humans and animals could reduce MRSA occurrence in milk and derivative products.

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