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Review

Clarifying the Dual Role of *Staphylococcus* spp. in Cheese Production

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Simple Summary

Bacteria from the genus *Staphylococcus* can have both beneficial or harmful effects on cheese quality and safety. While some Coagulase Negative Strains enhance flavor and texture, others, particularly *Staphylococcus aureus* but not limited to it, can produce enterotoxins that cause food intoxication. Additionally, several species within the genus cause infections in dairy herds affecting the health of livestock, in addition to being resistant to many antibiotics. This study examines the behavior of different species of *Staphylococcus* during cheese production, ranging from beneficial strains to risks imposed by harmful ones due to enterotoxin production, antibiotic resistance, and virulence factors. The review also highlights the need for strict hygiene measurements, temperature control, and monitoring to ensure safety while preserving cheese quality.

Abstract

Staphylococcus spp. present a dual role during cheese production as some species may be pathogenic, while others bring beneficial characteristics to the final products. Coagulase-positive *staphylococci* (CoPS) species, particularly *Staphylococcus aureus*, are of concern due to their potential to produce enterotoxins commonly linked to foodborne outbreaks. These enterotoxins, encoded by a set of genes, can cause severe gastroenteritis, particularly vomiting. Many other members of the genus can harbor a wide range of genes encoding virulence factors and capability of forming biofilms on various surfaces. The alarming prevalence of antibiotic-resistant strains, including methicillin-resistant *S. aureus* (MRSA), further complicates their control. In contrast, some strains of coagulase-negative *Staphylococcus* species (CoNS) positively contribute to cheese ripening, influencing flavor and texture. Some strains are even considered safe for use in food production and have been studied as inhibitors of foodborne pathogens. Conversely, the expression of enterotoxin genes in some *Staphylococcus* species, particularly *S. aureus,* is regulated by different mechanisms including quorum sensing. Understanding enterotoxin gene expression in various environmental conditions, including during cheese production and ripening, can aid in developing targeted interventions. The risks posed by enterotoxin producing *Staphylococcus* in cheese are evident since numerous food poisoning outbreaks have been reported. Also concerning is the fact that several *Staphylococcus* species pose risks to animal health and livestock production. Effective control measures include adherence to microbiological criteria for CoPS and enterotoxin levels in cheeses with special attention to animal health, good manufacturing practices (GMP), temperature control, and strict hygiene protocols. This review highlights the need to balance the beneficial roles of CoNS in cheese production with the risks associated with virulent and enterotoxigenic strains of CoNS and CoPS.

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1. Introduction

The genus *Staphylococcus* is composed of Gram-positive, spherical bacteria that characteristically divide in more than one plane, forming arrangements resembling clusters of grapes. These bacteria have a diameter between 0.5 and 1.5 μm, are non-motile and non-sporogenic, catalase-positive, and can be aerobic, facultative an-aerobes or strictly anaerobic, such as *Staphylococcus aureus* subsp. *anaerobius* [1–3]. This group is susceptible to lysis by lysostaphin and resistant to lysis by lysozyme [4]. Acting either as commensals or opportunistic pathogens, they are taxonomically classified under the Kingdom Bacteria, Phylum Bacillota, Class Bacilli, Order Bacillales, and Family Staphylococcaceae [5].

The genus *Staphylococcus* is composed of 72 species and 14 subspecies, some of which are common inhabitants of the skin and respiratory tract of humans and warm-blooded animals, with few species that can be found in soil and aquatic environments [6,7]. In general, *Staphylococcus* spp. are present in most diverse environments and are considered symbionts [8]. According to Foster [9], *Staphylococcus* spp. are among the most resistant non-sporulating microorganisms: they can withstand high concentrations of salt, desiccation, heat and are more tolerant to common disinfectants than most bacteria.

Species in the *Staphylococcus* genus are classified as coagulase-positive (CoPS) or coagulase-negative (CoNS) according to their ability to produce coagulase [4]. However, different strains from some species, such as *Staphylococcus hyicus*, present variation in coagulase production, being considered coagulase-variable staphylococci (CoVS) [10]. Most CoPS species are recognized as pathogenic, although some strains may asymptomatically colonize healthy individuals, while CoNS are primarily saprophytic or associated with opportunistic infections [4]. Coagulase is the main virulence factor of CoPS, functioning as a critical mechanism of defense by inducing fibrin deposition around the cells, protecting them in the infected area. Moreover, there is a correlation between CoPS and enterotoxin production, further enhancing their pathogenicity [11,12].

The first time *Staphylococcus* was associated with foodborne illness dates back to as early as 1884 when spherical organisms in cheese caused a large food-poisoning outbreak in the United States. Other outbreaks attributed to the consumption of staphylococcal contaminated foods occurred in France in 1894, in the United States in 1907, and in the Philippines in 1914. In 1930, Gail Dack and his colleagues at the University of Chicago were able to demonstrate that the cause of a food poisoning that occurred from the consumption of a contaminated Christmas sponge cake with cream filling was due to a toxin produced by the isolated staphylococci [13].

This review explores the dual role of *Staphylococcus* spp. in cheese production, highlighting both the beneficial contributions of CoNS to cheese ripening and the risks posed by CoNS and CoPS, particularly *S. aureus*, due to their potential to produce enterotoxins and to form biofilms. The review examines the regulatory mechanisms of enterotoxin gene expression, including quorum sensing, and discusses effective control measures to minimize the risks associated with enterotoxin-producing strains in cheese. Ultimately, this work shows that not all *Staphylococcus* spp. are detrimental in the production of dairy products.

2. Coagulase-Positive Staphylococci (CoPS)

The *coa* gene, responsible for encoding coagulase production, plays a pivotal role in the virulence of CoPS, especially in *S. aureus*, by facilitating the conversion of fibrinogen into fibrin, which aids in clot formation and immune evasion. Genetic analyses have revealed considerable variability in the *coa* gene, indicating the adaptability of CoPS in diverse environments [14]. These genetic variations can occur both chromosomally and through horizontal gene transfer via plasmids, contributing to

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the spread of virulent and antimicrobial-resistant strains. Notably, livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) strains have been shown to harbor novel recombinant staphylocoagulase types, highlighting the significant role of horizontal gene transfer in the evolution and dissemination of these strains [15]. Phylogenetic analyses of CoPS isolates demonstrate distinct clusters based on *coa* types, which are often associated with specific infection sites and geographic origins [16].

The CoPS species (n = 9) are shown in Table 1. All other species are CoNS (n = 67), except for a few species that are CoVS (n = 4) [7,10,17,18]. Five species that belonged to the genus *Staphylococcus* were reclassified to the genus *Mammaliicoccus*: *M. fleurettii*, *M. lentus*, *M. sciuri*, *M. stepanovicii* and *M. vitulinus* [19].

Table 1. Species of the coagulase-positive (CoPS), coagulase-negative (CoNS), and coagulase-variable (CoVS) staphylococci.

Coagulase-positive staphylococci (CoPS) species	
Staphylococcus argenteus	*Staphylococcus intermedius*
Staphylococcus aureus	*Staphylococcus lutrae*
Staphylococcus coagulans	*Staphylococcus pseudintermedius*
Staphylococcus cornubiensis	*Staphylococcus schweitzeri*
Staphylococcus delphini	-
Coagulase-negative staphylococci (CoNS) species	
Staphylococcus americanisciuri	*Staphylococcus leei*
Staphylococcus argensis	*Staphylococcus lloydii*
Staphylococcus arlettae	*Staphylococcus lugdunensis*
Staphylococcus auricularis	*Staphylococcus lyticans*
Staphylococcus borealis	*Staphylococcus marylandisciuri*
Staphylococcus brunensis	*Staphylococcus massiliensis*
Staphylococcus caeli	*Staphylococcus microti*
Staphylococcus caledonicus	*Staphylococcus muscae*
Staphylococcus canis	*Staphylococcus nepalensis*
Staphylococcus capitis	*Staphylococcus pasteuri*
Staphylococcus caprae	*Staphylococcus petrasii*
Staphylococcus carnosus	*Staphylococcus pettenkoferi*
Staphylococcus casei	*Staphylococcus piscifermentans*
Staphylococcus chromogenes	*Staphylococcus pragensis*
Staphylococcus cohnii	*Staphylococcus pseudolugdunensis*
Staphylococcus condimenti	*Staphylococcus pseudoxylosus*
Staphylococcus croceilyticus	*Staphylococcus ratti*
Staphylococcus debuckii	*Staphylococcus rostri*
Staphylococcus devriesei	*Staphylococcus saccharolyticus*
Staphylococcus durrellii	*Staphylococcus saprophyticus*
Staphylococcus edaphicus	*Staphylococcus schleiferi*
Staphylococcus epidermidis	*Staphylococcus shinii*
Staphylococcus equorum	*Staphylococcus simiae*
Staphylococcus felis	*Staphylococcus simulans*
Staphylococcus gallinarum	*Staphylococcus succinus*
Staphylococcus haemolyticus	*Staphylococcus taiwanensis*
Staphylococcus hominis	*Staphylococcus ureilyticus*
Staphylococcus hsinchuensis	*Staphylococcus warneri*
Staphylococcus kloosii	*Staphylococcus xylosus*
Coagulase-variable staphylococci (CoVS) species	
Staphylococcus agnetis	*Staphylococcus roterodami*
Staphylococcus hyicus	*Staphylococcus singaporensis*

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Adapted from Casanova et al. [10]; NCBI [7]; Velázquez-Guadarrama et al. [17]; Foronda-García-Hidalgo [18]; Madhaiyan et al. [19].

2.1. Staphylococcus aureus

S. aureus is a highly adaptable pathogen with a remarkable ability to thrive in diverse environments, contributing to its broad spectrum of infections. It can grow across a wide range of conditions, including temperatures from 7 to 48.5 °C (optimal 30–37 °C), pH levels from 4.2 to 9.3 (optimal 7.0–7.5), and sodium chloride concentrations up to 15% [20,21]. This adaptability makes *S. aureus* particularly significant in food safety, especially in foods requiring extensive handling during processing.

The virulence of *S. aureus* is driven by a variety of mechanisms, including the production of toxins such as alpha-hemolysin and Panton-Valentine leukocidin (PVL), along with superantigens like toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEs). These virulence factors can lead to severe conditions such as tissue necrosis, vascular thrombosis, and bacteremia [22]. In the context of foodborne illness, staphylococcal food poisoning (SFP) is specifically attributed to the production of SEs, which are the primary virulence factor responsible for the gastrointestinal symptoms [23].

S. aureus is also noted for its role in hematogenous metastasis, biofilm formation, and the persistence of chronic infections, contributing to its ability to evade the immune system and resist treatment with antibiotics [24]. The combination of toxin production, invasiveness, and antibiotic resistance enables *S. aureus* to cause a wide range of symptoms, from superficial skin infections to more severe illnesses like toxic shock syndrome (TSS-like).

Strains of *S. aureus* can be classified into two groups based on their resistance to oxacillin/methicillin: methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). MRSA strains emerged soon after the introduction of semisynthetic penicillins [25] and these strains are resistant to nearly all beta-lactam antibiotics, with the exception of ceftaroline and ceftobiprole [26]. Its clinical relevance lies in its association with poor prognoses and increased healthcare demands, as patients with MRSA infections often face longer hospitalizations, more extensive diagnostics, and higher mortality rates [27]. While recent data suggests a decline in the proportion of MRSA isolates, it remains a critical pathogen in the European Union/European Economic Area (EU/EEA), especially in Southern and Eastern European countries, where resistance levels remain high [28–30].

The presence of *S. aureus* in many environments is a significant concern due to its potential to cause severe infections and its increasing resistance to antibiotics. Thus, effective monitoring and control strategies are essential to reduce the risk posed by this pathogen in both clinical and food safety contexts.

2.2. Others Coagulase-Positive Staphylococci (CoPS)

Other CoPS, such as *S. intermedius* and *S. coagulans*, as well as *S. hyicus*, a CoVS, also pose significant health risks. These species, alongside *S. aureus*, can produce enterotoxins and coagulase, contributing to foodborne illnesses and animal infections.

S. aureus subsp. *aureus* is the most extensively studied subspecies and is a common cause of foodborne diseases through the production of heat-stable enterotoxins, leading to SFP [16,20].

In veterinary medicine, *S. hyicus* is a significant pathogen in swine, causing exudative epidermitis, also known as “greasy pig disease”. While primarily of concern in veterinary contexts, human infections, though rare, have been documented [31].

S. intermedius is commonly associated with animals such as dogs, and rarely as the cause of SFP in humans. However, it was implicated in an outbreak in 1991, when more than 265 people in the western United States became ill after consuming food contaminated with *S. intermedius* [32,33]. Its close relatives, *S. pseudintermedius* and *S. delphini*, are known to colonize various animal species, with increasing antibiotic resistance adding to their public health concern [34,35]. *S. pseudintermedius*,

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primarily associated with canine and feline infections like pyoderma and otitis externa, has gained attention due to the emergence of methicillin-resistant strains (MRSP), presenting a challenge similar to that of MRSA in humans. The rise of MRSP underscores the need for improved infection control measures in veterinary healthcare [35,36].

Other CoPS, such as *S. lutrae*, have been predominantly isolated from wildlife, like otters [37], with no evidence of human infection. Meanwhile, *S. coagulans* has been linked to infections in dogs and occasional human cases, particularly in immunocompromised individuals [38]. While more commonly associated with skin infections in companion animals, *S. coagulans* can also pose a food safety risk due to its presence in animal hosts.

S. delphini and *S. argenteus* have been isolated from both animals and humans. *S. delphini* is mainly found in dolphins and horses, while *S. argenteus*, closely related to *S. aureus*, has emerged as a significant human pathogen [39]. Finally, *S. schweitzeri*, primarily isolated from primates, shares genetic similarities with *S. aureus* [40], raising concerns about its zoonotic potential.

3. Coagulase-Negative Staphylococci (CoNS)

Most research on antibiotic resistance of staphylococci isolated from food has focused on the species *S. aureus*, while less attention has been paid to the CoNS group [41]. For many years, CoNS were considered non-pathogenic and were usually identified only at the genus level. Their role in food can be considered dual in nature, as some species bring beneficial characteristics to food, while others may be pathogenic.

CoNS belong to the saprophytic microbiota of the skin and mucous membranes of warm-blooded animals and humans, but are also found in foods such as meat, cheese and milk, and have been considered emerging pathogens with high incidence [42]. Their incidence in food is much higher than that of CoPS and, generally, most species are commensals; however, in other circumstances, some can act as pathogens [42,43]. In dairy products, especially on the surface of various types of cheese, CoNS are frequently found, either as contaminating species or as useful species to determine flavor or develop organoleptic properties. Their presence may not be an immediate hazard to public health, but they can become a risk factor [44]. In processed foods, CoNS may be indicative of hygiene failures in handling [45], and in foods derived from raw milk, in particular, they are of great importance, since *Staphylococcus* spp. are the most common causes of mastitis [46].

The CoNS group has 67 species, some of which have Generally Recognized As Safe (GRAS) status and are considered positive microbiota as they are responsible for the organoleptic characteristics of the final products. Some CoNS can even be used as starter culture in the production of cheeses, sausages, and fermented meats, due to their aromatic and pigmenting capacity [47].

Some CoNS species may play a beneficial role in producing certain fermented foods. However, safety concerns arise due to identified risk factors associated with some strains, as well as reports of nosocomial and urinary tract infections linked to *S. epidermidis* and *S. saprophyticus*, which are CoNS species commonly found in fermented foods [48,49]. Risk factors also identified correspond to virulence, in particular the production of enterotoxins, antibiotic resistance, and the ability to adhere and form biofilms [48,50–52].

Food poisoning of staphylococcal (SFP) origin is among the most common foodborne diseases and, contrary to what was previously thought, can be associated with both CoPS and CoNS strains [53]. This generates increasing interest in CoNS strains, since they have been associated with infections in humans, and in the induction of SFP, due to their ability to produce enterotoxins [54,55]. Food processing does not eliminate these toxins, which, unlike bacteria, have greater resistance to high temperatures, a wide pH range and proteolytic enzymes [56]. The toxins are also resistant to drying or freezing, and are insensitive to enzymatic digestion in the human gastrointestinal tract [57].

For a long time, the production of cytolytic toxins was attributed exclusively to *S. aureus*, but toxigenic factors or corresponding genes have been detected also in *S. epidermidis* and other CoNS species [58]. Exfoliative toxins (ETs), including ExhA, ExhB, ExhC, and ExhD, have been identified in some strains of *S. hyicus*. These toxins likely cause exudative epidermitis in pigs, a skin lesion that

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has several features in common with staphylococcal scalded skin syndrome (SSSS) in humans and share sequence similarities with the ETs of *S. aureus*: ETA, ETB, and ETD. Furthermore, TSST-1-associated enterotoxins and ETs were identified in a CoNS collection, following detection of hemolytic activities during a comprehensive immunoblot analysis, where a significant proportion of the tested strains produced the toxins [59].

SEs are toxins that cause vomiting after reaching the gastrointestinal tract, but other toxins, called staphylococcal enterotoxin-like proteins (SEls) and that lack the ability to induce vomiting, can also be produced [60]. More than 24 different serological types of SE have been identified in strains from different foodborne outbreaks, clinical cases or isolated from animals. The first five SE genes identified, which code for SEA, SEB, SEC, SED and SEE, known as classical enterotoxins, are frequently linked to foodborne outbreaks due to their ability to induce vomiting in humans. SEls are also considered a threat to humans, since they have been identified in cases of SFP outbreaks even without the presence of SEs. In addition, the new SE genes and the TSST-1, which belongs to a family of SE-associated toxins, are capable of stimulating large populations of T cells [61] (Table 2).

Table 2. Characterization of genetic element, activities, and source of staphylococcal enterotoxins (SEs), SEs-like (SEls), and toxic shock syndrome toxin-1 (TSST-1).

SEs/SEls/ TSST-1	Gene	Genetic element	Superantigenic activity	Emetic activity	Source
SEA	*sea*	Prophage	Yes	Yes	Food poisoning, dairy products, human, bovine, caprine, ovine
SEA	*sea*	Prophage	Yes	Yes	Food poisoning, dairy products, human, bovine, caprine, ovine
SEB	*seb*	SaPI3, chromosome, plasmid	Yes	Yes	Food poisoning, dairy products, human, bovine, caprine, ovine
SEC	*sec*	SaPI*	Yes	Yes	Food poisoning, dairy products, human, bovine, caprine, ovine
SEC-1	*sec*	SaPI	Yes	Yes	Human
SEC-2	*sec*	SaPI	Yes	Not evaluated	Human
SEC-3	*sec*	SaPI	Yes	Yes	Human
SED	*sed*	Plasmid	Yes	Yes	Food poisoning, bovine
SEE	*see*	Prophage (hypothetical location)	Yes	Yes	Food poisoning, unpasteurized milk soft cheese
SEG	*seg*	*egc1*, *egc2*, *egc3*, *egc4*	Yes	Yes	Bovine
SEH	*seh*	Transposon	Yes	Yes	Empyema human
SEI	*sei*	*egc1*, *egc2*, *egc3*	Yes	Yes	Mastitis cows, humans
SElJ	*selj*	Plasmid	Yes	Not evaluated	Epidemiologically implicated in food poisoning
SElK	*selk*	SaPI1, SaPI3, SaPI5, SaPIbov1, prophages	Yes	Not evaluated	Human
SElL	*sell*	SaPIn1, SaPIm1, SaPImw2, SaPIbov1	Yes	Yes	Human
SElM	*selm*	*egc1*, *egc2*	Yes	Yes	Bovine
SElN	*seln*	*egc1*, *egc2*, *egc3*, *egc4*	Yes	Yes	Human

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SElO	*selo*	*egc1*, *egc2*, *egc3*, *egc4*, transposon	Yes	Yes	Human
SElP	*selp*	Prophage	Yes	Yes	Human, ulcer
SElQ	*selq*	SaPI1, SaPI3, SaPI5, prophage	Yes	Yes	Human
SElR	*selr*	Plasmid	Yes	Yes	Human
SElS	*sels*	Plasmid	Yes	Yes	Not found
SElT	*selt*	Plasmid	Yes	Yes	Not found
SElU	*selu*	*egc2*, *egc3*	Yes	Not evaluated	Human
SElV	*selv*	*egc4*	Yes	Not evaluated	Not found
SElW	*selw*	*egc4*	Yes	Not evaluated	Human
SElX	*selx*	Chromosome	Yes	Not evaluated	Milk, raw meat, human
SElY	*sely*	Chromosome	Yes	Not evaluated	Human
SElZ	*selz*	Chromosome	Not evaluated	Not evaluated	Bovine
TSST-1	*tst*/*TssT*	Chromosome	Yes	No	Human

Adapted from Cieza et al. [61]. The structures of some enteroxins can be found in: http://www.ebi.ac.uk/ebisearch/search.ebi?db=macromolecularStructures&t=%22staphylococcal+enterotoxin%22&requestFrom=navigateYouResults. * SaPI - *Staphylococcus aureus* Pathogenicity Island.

Studies using PCR and/or DNA microarrays revealed that the occurrence of SE genes in CoNS isolated from foods is very rare [62]. However, Nunes *et al.* [63] isolated CoNS from Minas Frescal cheese marketed in southeastern Brazil, and all strains carried multiple enterotoxin genes. The most frequently detected genes using an enzyme-linked immunosorbent assay (ELISA) were *sea* and *seb* (90% and 70%, respectively), followed by *sec*/*see*, *seh*/*sei*, and *sed* with intermediate incidence (60%, 50%, and 40%, respectively). The lowest incidence was observed for *seg*/*selk*/*selq*/*selr* and *selu* (20% and 10%, respectively). Notably, the most frequent species were *S. saprophyticus* (40%), *S. xylosus* (30%), *M. sciuri* (20%, former name *S. sciuri*), and *S. piscifermentans* (10%). Additionally, Andrade *et al.* [64] observed that among the CoNS and CoPS species with enterotoxigenic potential, the *seg* and *seh* genes occurred in the species *S. cohnii* subsp. *cohnii*, *S. chromogenes*, *S. epidermidis*, *S. hominis*, *S. hyicus*, *S. lugdunensis*, *S. saprophyticus*, *S. ureilyticus*, and *S. xylosus*, with *seg* gene being the most predominant.

Chajecka-Wierzchowska *et al.* [65] evaluated 118 CoNS isolates from food and observed that 72% were positive for at least one gene encoding for enterotoxin, while 28% were negative for the genes tested. The study also examined the presence of exfoliative genes (*eta*, *etd*), as well as the *tsst-1* gene. The presence of the *tsst-1* gene encoding TSST-1 was confirmed in 31.4% of CoNS strains belonging to the following species: *S. simulans* (n = 8), *S. carnosus* (n = 6), *S. epidermidis* (n = 3), *S. warneri* (n = 3), *S. xylosus* (n = 3), *S. saprophyticus* (n = 2), *S. pasteuri* (n = 1), *S. petrasii* (n = 1), and *S. piscifermentas* (n = 1). Although positive for the genes, the strains were unable to produce these toxins in the tested conditions.

Other species of the *Staphylococcus*, especially the CoNS, have significant roles as infectious agents for human or animal hosts, revealing a more restricted repertoire of virulence factors when compared to *S. aureus*. They act as infectious agents, with moderately pathogenic species typically causing subtler infections characterized by a subacute or chronic clinical course. These infections rarely present with fulminant signs and are seldom fatal [66]. The most notable representative of this group is *S. epidermidis*.

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Found widely on human skin, wounds or surgeries, which may be the factors for the entry of this microorganism into the host's bloodstream, *S. epidermidis* has been highly related to hospital infections in recent years. Like *S. aureus, S. epidermidis* strains are highly resistant to antibiotics. The *S. epidermidis* species comprises a group of pathogens characterized by pronounced genomic diversity and when detected in clinical samples, clinicians face the challenge of determining whether they represent a true infection or just colonization/contamination [67]. With great clinical impact, this species has become the most important model microorganism for the study of healthcare-associated infections linked to inserted or implanted medical devices [68].

In addition to *S. epidermidis*, species such as *S. saprophyticus*, *S. haemolyticus*, and *S. lugdunensis* are occasionally observed as infectious agents of humans and animals, especially in patients with compromised immune systems [67,69].

Generally Recognized as Safe (GRAS) Status

In order for a microorganism to be used in the preparation and composition of a food, it must have GRAS status. Many non-pathogenic *Staphylococcus* species are used in the food industry because they confer unique characteristics to products. Among them, some CoNS species stand out, such as *S. carnosus, S. condimenti, S. equorum, S. piscifermentans*, *S. succinus*, and *S. xylosus.* Among these, *S. carnosus*, *S. equorum*, *S. succinus*, and *S. xylosus* are used as starter cultures for the production of cheeses and fermented meat products products [49].

CoNS play a significant role in defining color and developing organoleptic characteristics, which vary according to their proteolytic and lipolytic abilities [70,71]. This bacterial group can also contribute to the sensory qualities by producing diverse aroma profiles through carbohydrates and amino acids catabolism, esters formation, interactions with fatty acids, and their protease and lipase activities. Based on these distinctive properties, CoNS can be selected for use as starter cultures for fermentation [72].

S. carnosus has been used as a starter culture in the food industry since the 1950s. Its genome sequence has been determined and has provided the means for comparative studies of pathogenic and nonpathogenic staphylococci, and it has also been used as a cloning host to study the function of specific staphylococcal genes, given its food-grade status [73,74].

S. xylosus, belonging to the novobiocin-resistant CoNS species group, is commonly isolated from human and animal skin. The type strain C2a is commonly used as a starter culture in sausage and cheese production, contributing to the orange color on the surface of certain cheeses [75]

When evaluating strains of *S. equorum* isolated from cured cheese, the strain WS 2733 demonstrated the secretion of the macrocyclic peptide antibiotic micrococcin P(1), which exhibits antilisterial activity. This property was explored in cheese fermentation as a means to control the contamination by *Listeria monocytogenes* [76]. Deetae *et al.* [77] evaluated the production of volatile aromatic compounds by CoNS bacterial strains, isolated from different French cheeses, observing that *S. equorum* produced volatile compounds such as 3-methyl-3-buten-1-ol and 4-methyl-2-pentanone, responsible for conferring the fruity and sweet characteristics to the cheese.

Other CoNS species also produce compounds of interest such as diacetyl and acetoin, observed in *S. succinus* and *S. xylosus*, when isolated from fermented sausage [78]. Regarding the enzymatic activity of CoNS, they should have amino acid converting enzymes and specific peptide uptake mechanisms to produce volatile aroma compounds [79]. Thus, the addition of CoNS as starter cultures in the fermentation of cheeses and meats proves to be a safe alternative capable of conferring desired sensory characteristics.

Lee *et al.* [80] evaluated the genetic potential of *S. equorum* KS1039 as a starter culture in the fermentation of high-salt foods and observed that this strain contains genes for the biosynthesis of all amino acids except asparagine and for the production of branched-chain fatty acids. They also found that the species carries genes necessary for the production of butane-2,3-diol, diacetyl, and acetoin via glycolysis, and ester compounds via protein degradation.

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Irlinger *et al.* [81] evaluated the genome sequence of *S. equorum* Mu2 from a French ripened cheese and observed that the strain did not possess any of the virulence factors found in *S. aureus.* Genomic evaluation of *S. succinu*s 14BME20, isolated from fermented soybeans, confirmed that it did not contain any of the known *S. aureus* virulence factor-encoding genes, but it did contain strain-specific genes for lipid degradation, which may contribute to the production of volatile compounds [82].

4. Virulence Factors

Among the various *Staphylococcus* species, *S. aureus* stands out as both a tolerated commensal and a potent pathogen, widely colonizing several animals, the human skin and mucous membranes, as well as being present in food. In addition to toxins production, its pathogenicity is attributed to a diverse arsenal of virulence factors that facilitate adhesion to host tissues, biofilm formation, immune system evasion, and survival under nutrient-limited conditions. Additionally, the ability to acquire antibiotic resistance further enhances its clinical relevance. However, due to its genomic plasticity, not all *S. aureus* strains share the same genetic composition, leading to significant variability in virulence and pathogenic potential among subpopulations. The expression of these factors is influenced by environmental conditions and the host's immune response, determining the strain's capacity to cause infections ranging from mild skin lesions to severe systemic diseases, as well as its ability to form biofilms and produce enterotoxins in food. Given its broad impact on human and animal health, *S. aureus* is considered the primary reference for comparing pathogenic *Staphylococcus* species [75,83].

Just as *Staphylococcus* spp. play a dual role in cheese production, biofilm formation by CoPS and CoNS also exhibits this duality, depending on the characteristics of the producing strain. Strains carrying antibiotic resistance genes and enterotoxin expression genes are particularly concerning in cheese.

4.1. Biofilm Formation

Biofilm formation by CoPS and CoNS plays a significant role in the persistence of these bacteria on both biological materials and inert surfaces, such as cheese, equipment, and utensils used in production. Biofilm-associated cells are highly adherent and exhibit reduced susceptibility to desiccation, heat, detergents, biocides, and other antimicrobial agents [84–86]. In dairy processing environments, these biofilms are difficult to eliminate through conventional sanitation procedures, representing persistent sources of cross-contamination and posing challenges to microbiological control. When formed by enterotoxigenic strains and/or those carrying antibiotic resistance genes, such biofilms represent a significant threat to food safety, as the microorganisms can withstand adverse processing conditions and remain viable in the final product. Conversely, biofilm formation by GRAS-status CoPS and CoNS may have beneficial effects, particularly when these strains contribute to cheese ripening, promote the development of characteristic flavor and texture, and exhibit antagonistic activity against foodborne pathogens. Thus, biofilm formation by CoPS and CoNS can be seen as both a threat and an ally in cheese production, depending on the microbiological characteristics of the strains involved.

Several studies highlight the ability of different *Staphylococcus* spp. isolates from cheese to form biofilms. Friedriczewski *et al.* [87] tested twenty *S. aureus* isolates from buffalo mozzarella cheese, and observed that 10% were strong biofilm formers, 35% moderate formers, 50% weak formers, and 5% were non-biofilm formers. Souza *et al.* (2024) reported that 55% of the *S. aureus* isolates obtained from Minas Frescal and Porungo cheeses were strong biofilm formers. Meanwhile, Pineda *et al.* [88] evaluated the capability of *S. aureus* isolates from raw milk artisanal cheeses from Canastra (Brazil), observing that none of them was strong biofilm former, while 24% were moderate and 16.7% did not form biofilm. Moreover, Carvalho *et al.* [89] demonstrated that *S. aureus* ATCC 25923 is capable of growing and forming biofilms on an 18-micron low-density polyethylene (LDPE) package when

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stored at 5 °C in the presence of Minas Frescal cheese whey. Additionally, bacterial cells were able to detach from the packaging, increasing the microbial load on the product.

Fontes *et al.* [86] showed that 29.5% of the 227 CoNS isolated from soft cheeses in Brazil were able to form biofilm. Gajewska and Chajęcka-Wierzchowska [90] isolated 54 staphylococcal strains from cow's milk samples, of which 42 were classified as CoNS, belonging to the following species: *S. capitis*, *S. chromogenes*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. sciuri* (reclassified as *M. sciuri*), *S. simulans*, *S. warneri,* and *S. xylosus*, while 12 were classified as *S. aureus*. All tested isolates exhibited the capacity for biofilm formation. Of these, 85.7 and 58.3% of the CoNS and *S. aureus* isolates were capable of forming strong biofilms, while 4.8 and 8.3% formed moderate biofilms, and 9.5 and 33.3% formed weak biofilms, respectively. Interestingly, Goetz *et al.* [91] evaluated the effect of CoNS isolates with a weak-biofilm phenotype on the biofilm formation of other CoNS and CoPS isolates from the mastitis pathogen culture collection. Four of the CoNS isolates with a weak-biofilm phenotype (*S. chromogenes* C and E, and *S. simulans* F and H) significantly reduced biofilm formation in approximately 80% of the staphylococcal species tested, including *S. aureus*. These four *S. chromogenes* and *S. simulans* isolates were also able to disperse pre-established biofilms, but did not inhibit the growth of isolates with a strong-biofilm phenotype. These results suggest that some CoNS isolates can negatively affect the ability of other staphylococcal isolates and species to form biofilms via a mechanism that does not involve growth inhibition.

Formation of bacterial biofilms is a complex process comprising four main stages: adhesion, aggregation, maturation, and dispersion [92,93]. During the initial adhesion stage, *S. aureus* planktonic cells utilize various factors and regulatory mechanisms, such as the expression of cell wall-anchored (CWA) proteins, adhesins, and extracellular DNA (eDNA), to attach to biotic and abiotic surfaces [94,95]. One of the primary mechanisms involved is the organization of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), including protein A (SpA), fibronectin-binding proteins (FnBPs, such as FnbA and FnbB), fibrinogen-binding proteins (Fib), clumping factors (ClfA and ClfB), serine-aspartate repeat family proteins (SdrC, SdrD, and SdrE), biofilm-associated protein (Bap), and *S. aureus* surface proteins (SasC and SasG) [96–101]. Souza *et al.* [102] reported that some *S. aureus* isolates from Minas Frescal and Porungo cheeses exhibited expression of the fnbA and clfB genes. Pineda *et al.* [88] evaluated the virulence potential of *S. aureus* isolates from raw milk artisanal cheeses and found that most isolates possessed MSCRAMM genes, including fnbA, fnbB, fib, clfA, clfB, and eno.

SasC promotes the formation of large cell aggregates, increases adhesion to polystyrene, and enhances biofilm formation in *S. aureus* and *S. carnosus* [103]. SasG and plasmin-sensitive protein (Pls) from *S. aureus* are homologous to accumulation-associated protein (Aap) in *S. epidermidis* and other CoNS. Aap and SasG appear as long fibrils on the bacterial cell surface [104,105] and play roles in host cell binding and biofilm formation [105–107], while Pls surface expression reduces *S. aureus* adhesion to fibronectin [108–110]. Aap has also been shown to mediate intercellular adhesion in polysaccharide intercellular adhesion (PIA)-negative *S. epidermidis* strains, leading to a proteinaceous extracellular biofilm matrix [106]. Artini *et al.* [111] demonstrated that CoNS biofilm-producing species possess the aap gene, whereas the atlE gene is absent in these strains. A class of bifunctional proteins known as adhesins facilitate biofilm attachment to host tissues and surfaces. These include AtlA and Aaa in *S. aureus* [112] and AtlE and Aae in *S. epidermidis* [113]. Adhesins play essential roles at multiple stages of biofilm formation and adhesion [114]. Although numerous surface adhesins have been identified, *S. aureus* possesses a much larger repertoire of these proteins than *S. epidermidis,* which is limited to a few adhesive proteins. The ability to attach to host tissues or surfaces is a prerequisite for the subsequent formation of multilayered biofilms, stabilized by exopolysaccharides or proteinaceous intercellular material [75].

Gajewska and Chajęcka-Wierzchowska [90] isolated 42 CoNS and 12 *S. aureus* from cow's milk. They then identified genetic determinants responsible for biofilm formation, such as the bap and eno genes. Additionally, among CoNS, they detected the aap, bhp, fbe, embP, and atlE genes. Most of the tested staphylococcal strains (90.7%) had at least one of the tested genes. Nearly half (47.6%) of the

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CoNS had the eno gene, while for *S. aureus*, the eno gene was found in 58.3% of isolates. The frequency of the bap gene occurrence was 23.8% in CoNS strains and 25% in *S. aureus*, respectively. The fbe gene was demonstrated in only three CoNS isolates. Among the CoNS, the presence of the embP (16.7%), aap (28.6%), and atlE (23.8%) genes was also demonstrated. Following the adhesion step, bacterial cells begin to divide and aggregate [115]. During the aggregation stage, bacteria regulate biofilm formation by sensing environmental signals that activate regulatory networks and intracellular signaling molecules, promoting bacterial proliferation and biofilm thickening [116]. The biofilm provides resistance against the human immune system and antibiotics [117], while bacterial cells lose direct contact with the host surface and rely on cell–cell and cell–extracellular polymeric substance (EPS) adhesion [118].

Among the EPS components in *S. aureus* biofilms, PIA is biosynthesized as a poly-N-acetylglucosamine (PNAG) polymer and is a key factor [119]. PIA has cationic properties and plays a crucial role in adhesion and aggregation [120]. In *S. aureus*, biofilm formation is controlled by PIA production through proteins encoded by the icaADBC operon. Mutant strains lacking PIA exhibit significantly reduced bacterial cell adhesion [121]. PIA-dependent biofilms are predominantly observed in MSSA strains [121,122]. PIA interacts with other small proteins, such as Bap (biofilm-associated protein) that promotes cell-to-cell aggregation during biofilm formation [123] and Aap (accumulation-associated protein) that facilitates biofilm maturation [124]. Souza *et al.* [102] reported that 90% of the *S. aureus* isolates obtained from Minas Frescal and Porungo cheese expressed the icaD gene. Also, Pineda *et al.* [88] found that icaA and icaD genes were present in 70.3% and 46.2% of *S. aureus* isolates from raw milk artisanal cheeses, respectively.

Although the ica operon is considered essential as the genetic basis for PIA production in biofilm formation, biofilm development through ica-independent mechanisms has also been observed. Specifically, ica-negative mutants of *S. epidermidis* can still form biofilms, though these exhibit a proteinaceous rather than polysaccharide composition, as evidenced by their resistance to metaperiodate and their susceptibility to protease disruption [125]. However, ica-negative strains such as *S. epidermidis* ATCC 12228 have been reported to lack biofilm formation capabilities [126]. Gajewska and Chajęcka-Wierzchowska [90] showed that, among the 42 CoNS and 12 *S. aureus* isolates from cow's milk, the icaA was detected only in CoNS strains (24.1%), while icaD was found in both CoNS strains (21.4%) and *S. aureus* (100%).

During the maturation stage, biofilms become highly organized, forming compact, three-dimensional mushroom- or tower-like structures [127]. Channels develop around microcolonies, facilitating nutrient transport to deeper biofilm layers [128]. Mature biofilms exhibit metabolic diversity, which enhances their ability to withstand environmental stressors [129]. The production of EPS promotes bacterial aggregation into microcolonies, which serve as the structural foundation of the biofilm [130]. As these microcolonies thicken, genetic or environmental cues may trigger biofilm dispersion [131].

Biofilm dispersion is a complex, multi-step process that includes the production of exoenzymes and surfactants capable of degrading the EPS matrix [131], as well as physiological adaptations that prepare cells for survival outside the biofilm [132]. Once dispersed, cells revert to the planktonic state, allowing them to colonize new sites and initiate a new biofilm formation cycle [133]. As the final stage of the biofilm life cycle, dispersion plays a crucial role in infection spread. During biofilm growth and development, surfactant-like phenol-soluble modulins (PSMs) contribute significantly to biofilm dispersion and transmission in *S. aureus*. These molecules disrupt non-covalent interactions within the biofilm matrix and facilitate the formation of nutrient transport channels [134,135]. PSMs exist both in soluble form and as amyloid fibers, which provide structural stability to the biofilm [136,137].

The structural and functional complexity of biofilms increases as cells divide and the matrix becomes denser, creating physiological heterogeneity within the biofilm. This heterogeneity is characterized by gradients of nutrients and oxygen [138]. Within a biofilm, bacterial cells can be categorized into four distinct metabolic states: (i) aerobic cells, located in the oxygen- and nutrient-rich outer layer; (ii) fermentative cells, found in the oxygen- and nutrient-poor inner layer; (iii)

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dormant cells, residing in the anoxic layer with slow growth and inactive metabolism; and (iv) dead cells [139–141]. Dormant cells exhibit decreased intracellular adenosine triphosphate (ATP) levels, rendering them less susceptible to antibiotics [142]. Additionally, gradients of viscosity and lipid composition within *S. aureus* biofilms contribute to biofilm dispersal by facilitating the detachment of loosely bound bacteria while preserving a stable core layer [143,144].

For a more comprehensive overview of biofilm formation and regulatory mechanisms in *S. aureus*, please refer to [92,93]. For *S. epidermidis* and other CoNS, please refer to [94,145].

4.2. Antibiotic Resistance

Resistance to beta-lactams in MRSA strains and many methicillin-resistant coagulase-negative staphylococci (MRCNS) strains is associated with the presence of transferable genomic islands (GI) in the bacterial genome, known as staphylococcal chromosomal cassette *mec* (SCC*mec*). The *mecA* gene, carried by the SCC*mec*, encodes penicillin-binding protein 2a (PBP2a), a transpeptidase with low affinity for beta-lactams, thereby conferring resistance to methicillin [150,153,154]. From an evolutionary perspective, the *mecA* gene found in *S. aureus* likely originated from a group of bacteria previously classified as CoNS, now reclassified under the genus *Mammaliicoccus* [150,155]. Different SCC*mec* types may harbor the *mecA* gene or others, along with resistance determinants for other antibiotic classes, such as aminoglycosides, macrolides, lincosamides, streptogramins B, and tetracyclines (MLS-B) [150,153].

The emergence of MRSA and MRCNS strains has left only a few antibiotics effective for treating infections. Even the use of glycopeptides, so-called last-resort antibiotics, such as vancomycin, is at risk of becoming ineffective [156]. Intermediate-susceptible *S. aureus* to vancomycin (VISA) and glycopeptides (GISA), as well as vancomycin-resistant *S. aureus* (VRSA; vancomycin MIC ≥ 16 mg/L), have also been reported [149]. Changes in the cell wall and metabolic pathways can lead to intermediate resistance to vancomycin [156], while the acquisition of the *vanA* resistance determinant results in high-level resistance to vancomycin [157]. Glycopeptide resistance, encoded by the *vanA* operon, is more frequently expressed in *S. aureus* strains with mutations in the modification-restriction system, the presence of the *pSK41*-like conjugative plasmid and/or the Tn1546 transposon, both of which enhance the frequency of *vanA* operon conjugation [149,158–161].

CoPS and CoNS have also developed resistance to other classes of antibiotics, including aminoglycoside, diaminopyrimidine, fusidane, lincosamide, macrolide, nucleoside, phenicol, phosphonic acid, quinolone, streptogramin, tetracycline, and trimethoprim [149,150,162,163]. Other anti-MRSA antimicrobials have been developed, including daptomycin, linezolid, telavancin, tigecycline, quinupristin/dalfopristin, cephalosporins, and ceftobiprole. However, some strains have already developed resistance mechanisms to these new drugs [149,150,164].

Table 3 provides an overview of various resistance genes associated with different antibiotic classes and their corresponding encoded proteins in *Staphylococcus* spp. As shown in Table 3, one single gene may confer resistance to multiple antibiotics. Mlynarczyk-Bonikowska *et al.* [149], Brdová *et al.* [150] and Alkuraythi *et al.* [163] published comprehensive overviews on antibiotic resistance and the molecular mechanisms of this resistance in *S. aureus* and other CoPS and CoNS.

Table 3. Resistance genes to different antibiotic classes and their encoded proteins in *Staphylococcus* spp.

Antibiotic class	Resistance gene	Encoded protein
Aminoglycoside	*aacA-aphD*	6'-aminoglycoside N-acetyltransferase/2''-aminoglycoside phosphotransferase
	aadA2	Spectinomycin 9-adenylyltransferase
	aadA5	Aminoglycoside-3'-adenylyltransferase
	ant(4′)-Ia	Aminoglycoside adenyltransferase
	aph(2′′)-Ih	Aminoglycoside 2′′-phosphotransferase
	aph(3′)-IIIa	Aminoglycoside 3′-phosphotransferase

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Beta-lactam	*mecA* *mecA1* *blaZ* *blaTEM*	Penicillin-binding protein 2a (PBP2a) Beta-lactamase
Diaminopyrimidine	*dfrG*	Dihydrofolate reductase
Diaminopyrimidine	*dfrG*	Dihydrofolate reductase
Fusidane	*fusB* *fusC*	2-domain zinc-binding protein
Glycopeptide	*bleO* *vanA*	Bleomycin resistant proteins Vancomycin/teicoplanin A-type resistance protein
Lincosamide	*lnuA*	Lincosamide nucleotidyltransferase
Lincosamide/ macrolide/ streptogramin	*ermC*	rRNA adenine N-6-methyltransferase
Lincosamide/ pleuromutilin/ streptogramin/	*salA* *vgaA-lc*	Iron-sulfur cluster carrier protein ABC transporter
Macrolide	*mphC*	Macrolide 2′- phosphotransferase
Macrolide/streptogramin	*msrA*	Peptide methionine sulfoxide reductase
Nucleoside	*sat-4*	Streptothricin N-acetyltransferase and streptothricin
Phenicol	*fexA* *cmlA1*	Chloramphenicol/florfenicol exporter Bcr/CflA family efflux transporter
Phosphonic acid	*fosB-saur*	Metallothiol transferase
Quinolone	*gyrA*	DNA gyrase subunit A
Tetracycline	*tetK* *tetL* *tet38*	Tetracycline resistance protein Tetracycline efflux MFS transporter
Trimethoprim	*dfrA12* *dfr17*	Dihydrofolate reductase

Adapted from Alkuraythi et al. [163].

Thus, *S. aureus*, throughout its evolution, has acquired resistance to nearly all antibiotics developed so far. The presence of populations exhibiting multiple antibiotic resistances, which are highly prevalent in the environment, is a serious concern as it compromises the effectiveness of treatments for staphylococcal infections [83]. Furthermore, their antimicrobial resistance determinants may also be transferable to other commensal or potentially pathogenic bacteria in foodstuff [52,148,165]. Similarly, CoNS have also acquired resistance to various antibiotics throughout their evolution and may be present in cheeses, contributing to the transfer of resistance genes [63,86,166]. It is noteworthy that Fontes *et al.* [86] found high counts of CoNS in Brazilian soft cheeses, ranging from 10^6 to 10^7 CFU/g.

Gajewska *et al.* [167] conducted a study in Poland in which they tested 180 *S. aureus* isolates collected from various stages of artisanal cheese production using unpasteurized milk. The study revealed notable levels of antimicrobial resistance among the isolates: penicillin (58.1%), tobramycin (34.4%), azithromycin (18.3%), clarithromycin (16.1%), erythromycin (22.6%), cefoxitin (12.9%), and oxacillin (9.7%). The *blaZ* gene, which encodes penicillin resistance, was the most common antibiotic resistance gene among the tested isolates. All isolates showing phenotypic resistance to cefoxitin carried the *mecA* gene. Allaion *et al.* [168] evaluated *S. aureus* isolates from Minas artisanal cheeses, and at least one antibiotic resistance gene was detected in 83.0% of the isolates. Nearly half (47.1%) carried more than one resistance gene. The most frequently detected resistance genes were *tetK* (54.4%) and *mecA* (52.2%), followed by *aacA-aphD*, which was found in 30.0% of the isolates. Aguiar

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et al. [152] characterized 57 *S. aureus* isolates from artisanal colonial cheese, with penicillin resistance being the most prevalent (33%), followed by resistance to clindamycin (28%), erythromycin (26%), and tetracycline (23%). The evaluated strains also exhibited inducible resistance to clindamycin, with nine isolates classified as MDR.

Pineda *et al.* [88] characterized the genomes of several *S. aureus* strains isolated from raw milk artisanal cheese in Brazil, identifying antimicrobial resistance genes with phenotypic confirmation of methicillin and tetracycline resistance. The authors also discovered a rich virulome encoding iron uptake systems, immune evasion mechanisms, and an extensive arsenal of toxins, along with the capacity to form biofilm. These findings suggest that multiple strains circulating in the cheese-producing region pose a potential health risk.

Fontes *et al.* [86] isolated 227 CoNS from soft cheese in Brazil, and high percentages of antimicrobial resistance were observed for penicillin (78.5%), oxacillin (76.2%), erythromycin (67.8%), gentamicin (47.2%), clindamycin (35.7%), rifampicin (26.8%), azithromycin (14.7%), tetracycline (14.7%), levofloxacin (14.2%), and sulfamethoxazole-trimethoprim (11.9%). All isolated CoNS were susceptible to vancomycin and linezolid. A multiple antibiotic resistance (MAR) index of >0.2 was observed in 80.6% of the isolates. In addition, 81.5% of the isolates carried the *mecA* gene, and 76.2% of these were phenotypically resistant to oxacillin. Nunes *et al.* [63] isolated CoNS from Minas Frescal cheese in southeastern Brazil, and the strains showed multiresistance to antimicrobial agents such as beta-lactams, vancomycin, and linezolid. Klempt *et al.* [166] evaluated 53 CoNS isolates from different cheeses, some of which exhibited resistance to cefoxitin, penicillin, and tetracycline. In addition, several carried genes encoding antibiotic resistance, such as *mecA, mecB, mecD, blaTEM, tetK,* and *tetL*.

4.3. Expression of Enterotoxins Genes

Staphylococcal food poisoning (SFP) is caused by one or more enterotoxins produced by some species and strains of *Staphylococcus*. Although enterotoxin production is associated with CoPS and thermonuclease-positive *S. aureus* (TPS), some CoNS and species that are thermonuclease-negative also produce enterotoxins [46,169]. Within *S. aureus*, the regulation of virulence factors is subject to a complex network that integrates host and environmentally-derived signals into a coordinated response [170].

The genome of *S. aureus* harbors numerous toxin-encoding genes, which are primarily located on mobile genetic elements [148,171]. This arrangement results in significant variability in toxin production among different *S. aureus* strains [148,172]. Among the various known or strongly suspected toxins and virulence factors that cause specific diseases or symptoms, staphylococcal superantigens (SAgs), comprising SEs (*Staphylococcus* enterotoxins), SEls (*Staphylococcus* enterotoxin like), and TSST-1, are the most prominent [148,171,172].

SAgs are a group of potent immunostimulatory toxins produced by *S. aureus.* SAgs are characterized as pyrogenic toxin superantigens, with the ability to induce SFP and an infection known as Toxic Shock Syndrome (TSS). SAgs share many structural and functional similarities but have distinct characteristics. They are relatively resistant to heat and to proteolytic gastric enzymes such as pepsin and trypsin, allowing them to pass through the digestive tract and head to the site of action. In SFP, SAgs stimulate the vagus nerve endings in the stomach lining that control the emetic response, causing nausea, cramping, vomiting, and diarrhea, appearing abruptly 2–8 h after ingesting food containing these toxins. TSS is a potentially fatal disease characterized by fever, erythematous rash, hypotension, shock, multiple organ failure, and skin desquamation. This toxin-mediated systemic disease was first observed in non-systemic infections by SE producing *S. aureus.* Subsequently, another *S. aureus* toxin, designated as TSST-1 (formerly SEF), was shown to be associated with TSS in menstruating women and in non-menstrual cases [171].

Superantigens (SAgs) are single-chain proteins that interact with variable regions of T-cell receptors (TCR Vα or TCR Vβ), activating a large number of T-cells. This activation triggers massive proliferation and release of pro-inflammatory cytokines, such as IL-1, IL-2, IL-6, γ-interferon, and TNF, potentially leading to lethal TSS. SAgs can also interact with epithelial cells, promoting

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transepithelial transport and an inflammatory state. Due to their effects on the immune system and ability to induce SFP and TSS, SAgs are classified as pyrogenic toxin superantigens. Similar to TSST-1, they are super antigenic toxins, that activate T-cells in a predominantly nonspecific manner, resulting in an excessive immune response that includes polyclonal T-cell activation and massive cytokine release [3,171,173].

SEs belong to a large family of staphylococcal and streptococcal pyrogenic exotoxins, sharing common phylogenetic relationships, structure, function, and sequence homology. SEs are potent gastrointestinal toxins that cause emesis in a not completely understood manner that involves the induction of histamine release from intestinal mast cells [3,173].

SEs are heat-stable, low molecular weight (19,000–29,000 Da), single-chain proteins primarily produced by *S. aureus*, though not exclusively. These toxins belong to a major family of serological types, including SEA to SEE and SEIG to SEIJ. The classical enterotoxins, SEA, SEB, SEC1-3, SED, SEE, and SEH, are the main agents responsible for SFP [9,169,173,174]. These toxins function as SAgs, originally identified due to their emetic activity in SFP. This group includes SEs A, B, C, D, E, G, H, I, R, and T, as well as SElJ to SElX, which do not cause emesis or have not been tested in non-human primates. TSST-1, a pyrogenic exotoxin previously known as SEF, is also part of this SAgs group [23,175]. Although the role of certain SEs, SEls, and TSST-1 in foodborne diseases remains unclear and therefore cannot be ruled out, they share structural and functional similarities and have been associated not only with SFP- and TSS-like syndromes, but also with allergic and autoimmune disorders [9,169,173,174].

The expression of genes encoding SEB occurs primarily at the end of the stationary phase, while the production of SEA, SED, and SEE takes place throughout the logarithmic growth phase (Figure 1; [176]). The production of SEA and SEE is not regulated by the accessory gene regulator (*agr*) system [169,173]. In contrast, SEB, SEC, and SED require a functional *agr* system for maximal expression [173]. The *agr* system facilitates cell-to-cell communication through a quorum sensing mechanism, using autoinducing peptides (AIPs) as signaling molecules. Activation of the *agr* system leads to the expression of exo-toxins and exo-enzymes and is essential for virulence in animal models of skin infection, pneumonia, and endocarditis [170].

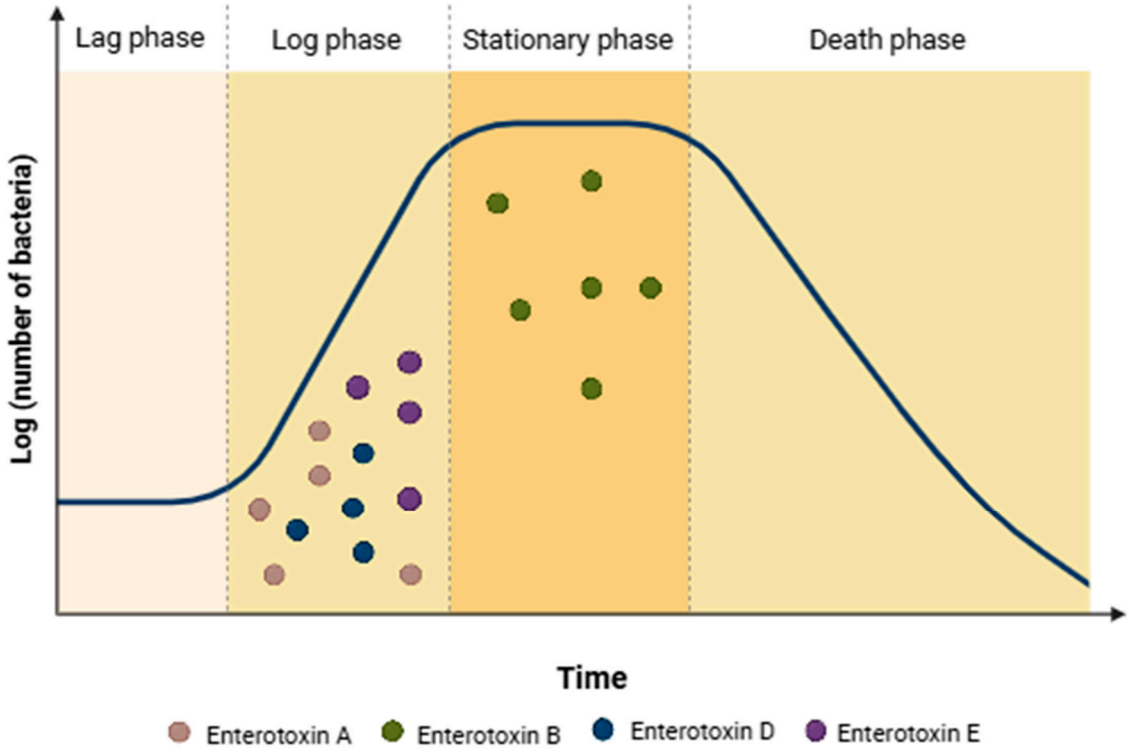

Figure 1. Regulation of enterotoxin production during bacterial growth phases. Based on Derzelle et al. [176].

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The synthesis of SEs depends on temperature, pH, water activity, and the presence/activity of other microorganisms with beneficial or antagonistic interactions. Generally, the production and accumulation of enterotoxins in food occurs when enterotoxigenic staphylococci are capable of proliferating, normally when populations are above 10^5 CFU/g [169,174,177].

The successful growth of *S aureus* in diverse environmental conditions is partly due to its ability to express different genes in response to changing conditions. Additionally, *S. aureus* has extraordinary adaptive power to ensure its success as a pathogen [171,178]. This microorganism is capable to detect different environmental signals and adjust the production of virulence factors critical for survival in the host, such as cell surface adhesins, extracellular enzymes, and toxins [24,170]. A virulence gene is often susceptible to transcirculatory control by more than one regulatory system and, there is cooperation or even competition between these systems to modulate the expression of a given virulence gene [179]. The accessory gene regulator (*agr)* quorum sensing mechanism is an important regulatory system of *S. aureus* and contributes to its pathogenicity, playing a key role in the expression of enterotoxins genes [170,179].

SaeRS, a two-component system in *S. aureus* responsible for the production of toxins, immunomodulators, and enzymes, was proven to be essential for virulence in animal models of skin infections and pneumonia [170,180]. The staphylococcal respiratory response regulator (SrrAB) is an oxygen-responsive two-component system that induces the expression of *plc* and *ica*, while repressing *agr*, TSST-1, and *spa*. It is essential for defense against neutrophils [170,181]. SrrAB activates *ica* operon transcription and promotes the expression of polysaccharide intercellular adhesin, helping *S. aureus* evade neutrophil-mediated killing during anaerobic growth conditions [181]. ArlRS, another two-component system that regulates autolysis and cell surface properties, promotes MgrA expression while repressing *agr* and autolysis. It is crucial for virulence in animal models of skin infection and endocarditis [182].

SarA is a cytoplasmic regulator that promotes the expression of extracellular proteins and represses *spa*, which encodes staphylococcal protein A. SarA is required for virulence in biofilm infection models [183]. Rot is a cytoplasmic regulator that controls the production of toxins and extracellular proteases in *S. aureus*. Its expression is regulated by the *agr* system, which, when active, prevents Rot from being translated. Interestingly, in certain conditions where *agr* is inactive (e.g., *agr*-null mutants), mutations in *rot* can restore the virulence of the bacteria. This was demonstrated in a rabbit model of endocarditis [184].

The transition of *S. aureus* from a commensal organism to a pathogen is strongly influenced by host-derived environmental signals, as described by Choueiry *et al.* [185]. In this study, significantly lower growth of the MRSA strain was observed under aerobic conditions, suggesting that these bacteria were subjected to oxidative stress, which impaired growth. Furthermore, supplementation of culture media with energy substrates and addition of carbon sources facilitated the ability of *S. aureus* to overcome environmental stress and grow, demonstrating a more robustly adaptive metabolism. These authors also noted that changes in growth environments may drive the regulation of virulence in *S. aureus* with the associations of changes in their metabolism with its virulence. Increased expression of the virulence factors *agr-I*, *sea*, *seb*, and *eta* was apparent in the supplemented *S. aureus* cultures [185].

Signal transduction systems that sense cell density, energy levels, and external stimuli facilitate *S. aureus*'s remarkable adaptability to diverse environmental conditions [178,183]. These host environmental signals are crucial in promoting *S. aureus* colonization, allowing bacteria to adapt to different conditions and potentially switch to a pathogenic state when conditions are favorable. Understanding these host-pathogen interactions is critical for managing *S. aureus* infections in clinical settings and understanding enterotoxin expression in food [186].

5. Staphylococcal Food Poisoning from Cheese Consumption

SFP occurs due to the consumption of food containing preformed SEs, typically produced when *S. aureus* reaches concentrations of 10^6 CFU/g or mL in food matrix [187]. Although Bastos *et al.* [187]

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cite 100 ng as a general threshold dose to cause illness, other studies suggest that, for example, even 20–100 ng of SEA may be sufficient [188]. However, the dose response depends on the individual's sensitivity, body weight, and the specific SE involved [188–191].

Documented SFP outbreaks associated with cheese consumption, categorized by country and year of occurrence, are summarized in Table 4. No further outbreaks related to cheese consumption were identified in the literature.

Table 4. Occurrence of staphylococcal food poisoning (SFP) associated with cheese consumption in different locations.

Year	Location	Product	Enterotoxin type	Symptoms	Number of patients (deaths)	Reference
1980	Canada	Curd Cheese	SEA, SEC	Nausea, vomiting, abdominal cramps and diarrhea	62 (0)	[196]
1981	United Kingdom	Halloumi cheese	SEA	Nausea, vomiting, abdominal cramps and diarrhea	4 (0)	[198]
1981	France	Raw milk semi-hard cheese	SEA	Unknown	4 (0)	[199]
1983	France	Raw milk semi-hard cheese	SEA, SED	Vomiting and abdominal cramps	20 (0)	[199]
1983	France	Raw milk soft cheese	Absent	Vomiting and diarrhea	4 (0)	[199]
1985	France	Soft cheese	SEB	Vomiting and diarrhea	2 (0)	[199]
1985	France	Soft cheese	SEB	Vomiting and diarrhea	3 (0)	[199]
1985	United Kingdom	Raw ewe's milk cheese	SEA	Nausea, vomiting, abdominal cramps and diarrhea	27 (0)	[197]
1986	France	Sheep's milk cheese	SEB	Unknown	Unknown	[199]
1988	Brazil	Fresh Minas cheese	SEA, SEB, SED, SEE	Nausea, vomiting, abdominal cramps and diarrhea	4 (0)	[192]
1995	Brazil	Minas cheese	SEH	Vomiting and diarrhea	7 (0)	[193]
1997	France	Raw milk cheese	Present but not specified	Unknown	43 (0)	[199]
1998	France	Raw milk cheese	Present but not specified	Vomiting, abdominal cramps and diarrhea	47 (0)	[199]
1998	France	Raw milk semi-hard cheese	Absent	Vomiting and abdominal cramps	10 (0)	[199]
1999	Brazil	Minas cheese	SEA, SEB, SEC	Vomiting, dizziness, chills, headaches and Diarrhea,	378 (0)	[194]
2000	France	Raw sheep's milk cheese	SEA	Unknown	Unknown	[199]
2001	France	Sliced soft cheese	SEA	Nausea, vomiting, abdominal cramps and diarrhea	2 (0)	[199]

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2001	France	Raw milk semi-hard cheese	SED	Vomiting	17 (0)	[199]
2002	France	Raw sheep's milk cheese	SEA	Nausea, vomiting, abdominal cramps and diarrhea	43 (0)	[199]
2007	Switzerland	Robiola cheese	SEG, SEI, SEM, SEN, SEO	Nausea, vomiting, abdominal cramps and diarrhea (in some cases)	5 (0)	[188]
2009	France	Soft cheese	SEE	Nausea, vomiting, abdominal cramps and diarrhoea and fever (in some cases)	23 (0)	[200]
2014	Switzerland	Tomme cheese	SEA, SED	Vomiting, Abdominal cramps, severe diarrhea and fever	14 (0)	[188]
2018	Italy	Alm cheese	SED	Vomiting, abdominal cramps and diarrhea	3 (0)	[201]
2022	Italy	Raw milk cheese	SEA, SEB, SEC, SED	Vomiting and diarrhea, headaches	8 (0)	[202]

Regarding SFP outbreaks linked to cheeses, [192] reported that four individuals from the same family became ill after consuming fresh Minas cheese, in Brazil. The cheese contained high counts of *S. aureus* ($9{,}3\times10^{7}$ CFU/g), and the strains were capable of producing SEA, SEB, SED and SEE. which were likely responsible for the outbreak, the main symptoms were nausea, vomiting, diarrhea and abdominal pain, with no hospitalizations. The average incubation period was approximately one hour.

Pereira *et al.* [193] reported an outbreak that occurred in 1995 due to consumption of cheese produced in the Minas Gerais state, Brazil. A family of seven individuals consumed the cheese and began to present symptoms of vomiting and diarrhea approximately 4 hours later. Analysis of the cheese revealed a high population of *S. aureus* (2.9×10^{8} CFU/g) and the presence of SEH. There were no hospitalizations or deaths. In 1999, two additional outbreaks involving *Staphylococcus* and cheeses occurred in Minas Gerais state, Brazil, affecting around 700 people. One outbreak was linked to the consumption of Minas cheese and the other to raw milk. In the first outbreak, analysis of the cheese revealed *S. aureus* levels ranging from 2.4×10^{3} to 2.0×10^{8} CFU/g, with the production of SEA, SEB, and SEC. In the second outbreak, raw milk samples contained CoNS at counts exceeding 2.0×10^{8} CFU/g, along with the production of SEC and SED [194].

From 2014 to 2023, 6,874 foodborne disease outbreaks were reported in Brazil, leading to 110,614 illnesses and 12,346 hospitalizations and *S. aureus* was the second leading etiological agent, responsible for 9.7% of cases [195]. In these outbreaks dairy products were responsible for 6.7% of the total number of outbreaks. Unfortunately, no data is available on enterotoxins in these samples.

In Canada, in 1980, 62 individuals presented symptoms of nausea, vomiting, abdominal cramps, and diarrhea after consuming curd cheese, which was present in both boxed lunches and cheeses purchased at retail stores in cities near Montreal. The curd cheese was mainly produced in a cheese factory and distributed to retail stores for preparation of boxed lunches. When analyzed, the curd cheeses contained between 2.0 and 8.0×10^{7} *S. aureus*/g, in addition to SEA and SEC. No deaths were reported [196]. In United Kingdom, in 1981, a family of four consumed Halloumi cheese in brine imported from Cyprus. This cheese, traditionally made with goat's and sheep's milk, may also include cow's milk in some cases. After consumption, all family members developed symptoms typical of SFP. Although *S. aureus* was not isolated, SEA was detected in both the cheese and the brine [197]. Between December 1984 and January 1985, cheese made from raw ewe's milk at a dairy farm

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was linked to three outbreaks involving 27 people in the United Kingdom. The people who got ill had severe symptoms, such as violent vomiting and severe diarrhoea. SEA was detected in the cheese, although *S. aureus* was not identified. Subsequent testing of milk samples from the dairy revealed the presence of a SEA-producing strain [198].

Kérouanton *et al.* [199] investigated outbreaks associated with *S. aureus* in France, reporting that between 1981 and 2002, there were 13 incidents involving cheese. The analyzed matrices included raw milk semi-hard cheeses, raw milk soft cheeses, soft cheeses, raw milk cheeses, and sheep's milk cheeses (raw and pasteurized). The enterotoxins detected in the cheeses were SEA, SEB, and SED. *S. aureus* populations ranged from 1.0×10^4 to 3×10^8 cfu/g, depending on the outbreak and cheese type. Reported symptoms among patients included nausea, vomiting, abdominal cramps and diarrhea, with no deaths. Ostyn *et al.* [200] reported six outbreaks occurred in France between October and November 2009 due to SEE in soft cheeses, resulting in 23 cases. The people got ill after consuming soft cheese from one producer with 1.5×10^5 CFU/g and the only type of SE detected in all food samples was SEE (between 0.36 to more than 1.14 ng/g of cheese).

Filipello *et al.* [201] reported an outbreak in Lombardy, Italy, in 2018, caused by the consumption of artisanal Alm cheese containing SED. This outbreak involved three patients, and all individuals presented abdominal cramps, vomiting, and diarrhea.

In Northern Italy, in 2022, a family of eight reported gastrointestinal symptoms such as vomiting and diarrhea, as well as headaches, after eating sandwiches at a small local restaurant in the Alps region of Piedmont. Food safety agency inspectors collected samples of ham and cheese made with raw milk that the family members had consumed and found CoPS varying between 1.3×10^3 and 8.1×10^3 cfu/g in the cheeses, in addition to enterotoxins A to E, with SED estimated at 0.649 ng/g. There were no deaths [202].

In Switzerland, in 2007, five individuals presented nausea, abdominal cramps, vomiting and diarrhea after ingestion of a fresh goat milk cheese (Rabiola). The samples presented counts of CoPS between 6.7×10^6 and 2.6×10^7 CFU/g. The strains were positive for genes (*seg*, *sei*, *sem*, *sen*, and *seo*; [188]). Also in Switzerland, another outbreak affected 14 people, including children and adults, after consuming soft cheese produced from raw cow milk (Tomme cheese). The soft cheese contained SEA (>6 ng/g) and SED (>200 ng/g). Counts of 10^7 CFU/g of CoPS were detected. No deaths were reported [188].

According to the European Food Safety Authority and European Centre for Disease Prevention and Control, in 2022, *S. aureus* toxins were responsible for 137 outbreaks (0.02% of the total of 5763 reported cases), resulting in 148 hospitalizations and 4 deaths [203]. Between 2007 and 2018, 8,730 foodborne disease outbreaks caused by seven pathogens were reported in Japan. Among these, *S. aureus* was responsible for 448 outbreaks, none of which resulted in fatalities. Additionally, 2.6% of outbreaks linked to dairy product consumption were attributed to *S. aureus* [204].

Abiotic factors such as temperature, pH, water activity, redox potential, NaCl concentration and oxygen availability, in addition to bacterial antagonism, influence the growth and enterotoxin production by *S. aureus* in food [205,206]. These factors may help explain the relatively low number of *S. aureus* outbreaks associated with cheese. The optimal temperature range for both growth and enterotoxin production by *S. aureus* is 34–40 °C. The optimal pH for growth is 6 to 7, while for enterotoxin production it is 7 to 8. The ideal water activity for both growth and enterotoxin production is 0.99, although reports indicate enterotoxin production can occur between 0.86 and 0.99 [205]. Additionally, Schelin *et al.* [205] reported that the presence of lactic acid bacteria in cheese, such as *Lactococcus lactis*, can inhibit the transcription of genes responsible for enterotoxin production, such as *sec, selk, seg,* and *seh*. These characteristics suggest that although cheeses may provide conditions that support growth and toxin production, it does not offer the ideal environment. This likely contributes to the infrequent association of cheese with *S. aureus*-related foodborne outbreaks. While the characteristics of the cheese matrix may not be favourable for enterotoxin production the number of reported cases of staphylococcal foodborne diseases could also be attributed to underreporting.

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Given that the symptoms are often mild and the illness is self-limiting, affected individuals may not seek medical attention, making the true number of cases difficult to determine.

6. Control of Staphylococci in Cheeses

Controlling pathogens like enterotoxin-producing *S. aureus* is essential to ensure the safety of cheeses. At the farm level, maintaining animal health and adopting hygienic milking practices are critical to minimizing microbiological contamination of raw milk [207]. Preventing mastitis and implementing good agricultural practices significantly reduces the risk of contamination with spoilage and pathogenic bacteria, including those from the *Staphylococcus* group [88]. In cheese production facilities, strict hygiene protocols, good manufacturing practices (GMP), proper equipment design and maintenance, adequate production flow, as well as monitoring of production surfaces, raw materials and final products are important barriers to reducing cross-contamination with harmful bacteria. Additionally, the adoption of a hazard analysis and critical control points (HACCP) plan and a proactive food safety culture can minimize contamination, ensure product safety and promote a better working environment [208]. Controlled ripening conditions, such as proper temperature and pH, also help prevent *S. aureus* proliferation, while regular health checks for food handlers mitigate risks of contamination during handling.

Further down the supply chain, appropriate storage conditions at retail are important to hinder bacterial growth. Preventing cross-contamination during slicing and repackaging ensures that cheese remains safe for consumers. At home, proper handling, hygiene, and storage practices are key to reducing contamination and microbial growth, spoilage and to keep the product safe [209]. A comprehensive approach, from the farm to table, is necessary to effectively control *S. aureus* and other foodborne pathogens and safeguard public health.

In recent years, a growing number of studies have investigated both conventional and innovative strategies to reinforce these control measures, especially in response to the outbreaks involving SEs in cheeses. In addition to established interventions, such as cleaning-in-place (CIP) systems combined with peracetic acid or sodium hypochlorite sanitizers, novel approaches have emerged. These include the application of lytic bacteriophages targeting *S. aureus* [210], the use of competitive probiotic strains to reduce pathogen colonization [211], and nanotechnological solutions, such as functionalized magnetic microrobots capable of selectively removing *S. aureus* from milk without disrupting beneficial microbiota [212]. Furthermore, recent advances in rapid detection techniques, including real-time PCR, chromogenic media, and biosensors, have enhanced the early identification of enterotoxigenic strains, improving traceability and enabling more timely interventions [213]. While several of these innovations are still undergoing validation, their integration into the existing framework of GMP presents a promising avenue for strengthening microbial safety in both artisanal and industrial cheese production.

7. Microbiological Criteria for Staphylococcus and Enterotoxins in Cheeses

Microbiological criteria for CoPS, including *S. aureus* and their SEs in cheeses, are determined by regulatory bodies and differ among countries. In Brazil, the National Agency for Sanitary Vigilance (ANVISA) aligns its regulations with international standards, particularly those from Codex Alimentarius, focusing on controlling both CoPS and SEs in dairy products (Table 5). Detection of SEs in cheeses triggers corrective actions, including product destruction and potential product recalls.

Table 5. Microbiological criteria for coagulase positive staphylococci (CoPS) and *Staphylococcus* enterotoxins (SEs) in different countries or regions of the world.

Country or region	Microbiological criteria						
	CoPS or enterotoxins	n	c	m	M	Notes	Reference

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Australia	CoPS	5	2	100	1000	All types of cheese	[214]
Brazil	CoPS	5	2	100	1000	All types of cheese	[215]
	Staphylococcus Enterotoxin (SE)	5	0	absence	-	All types of cheese	
Canada	*S. aureus*	5	2	1000	10,000	Cheese made from an unpasteurized source	[216]
China	*S. aureus*	5	2	100	1000	All types of cheese	[217]
European Union	CoPS	5	2	10,000	100,000	Cheese made from raw milk	[218]
European Union	CoPS	5	2	100	1000	Cheese made from mild heat treated milk	[218]
European Union	CoPS	5	2	10	100	Unriped soft cheese made with pasteurized milk	[218]
United States	*S. aureus*	-	-	-	10,000	All dairy products	[219]
	SE	-	-	not detected	-	All dairy products	

In the European Union, Commission Regulation (EC) No 2073/2005, amended by Regulation (EC) No 1441/2007, specifies that the presence enterotoxins in cheese used as raw material should be monitored, especially when levels of CoPS exceed 10^5 CFU/g of product [218]. This threshold is considered critical as it marks the potential for enterotoxin production, which can lead to foodborne illness outbreaks. The EFSA's guidelines for the detection of SEs focus on preventing contamination, particularly in raw milk cheeses, which are more susceptible to staphylococcal contamination during the production and ripening stages.

The FDA, through its Bacteriological Analytical Manual (BAM), provides specific methods for detecting SEs in foods, including cheese. While it does not set explicit limits for CoPS in cheeses, it emphasizes the importance of testing for enterotoxins, given their heat stability, which allows them to withstand pasteurization processes that eliminate the bacteria themselves [219]. The USDA recommendation is similar to the FDA.

These regulations aim to reduce the risk of SFP through a combination of good hygiene practices, appropriate processing conditions, and regular testing at various stages of cheese production, storage, and distribution.

8. Conclusions

As discussed in this review, the genera *Staphylococcus* spp. exhibit a dual role in cheese production, where certain CoNS can be seen as beneficial microbes contributing to ripening by enhancing flavor and texture, while pathogenic strains, especially *S. aureus* and other CoPS, pose food safety risks due to enterotoxin production. The persistence of antibiotic-resistant strains, including MRSA, are additional issues within the cheese production chain. At the farm level, pathogenic staphylococci, including CoPS and CoNS, are also of concern due to the potential to cause several diseases, including mastitis in the herd. Furthermore, given that low concentrations of SE can lead to food poisoning, strict control measures need to be put in place. This includes advancing research on the regulatory mechanisms of enterotoxin expression under various production conditions, so that better control measures are created, implementing stringent hygiene and GMP protocols, and maintaining rigorous temperature control throughout the production chain. Additionally, monitoring raw materials and livestock health is critical to prevent contamination, particularly reducing the risk of mastitis. Finally, balancing the applications of beneficial CoNS with the risks posed by pathogenic staphylococci requires ongoing research and improved control measures to ensure both product quality and food safety.

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Abbreviations

The following abbreviations are used in this manuscript:

CoNS	coagulase-negative *Staphylococcus* species
CoPS	Coagulase-positive *staphylococci*
CoPS	coagulase-positive
CoVS	coagulase-variable staphylococci
MRSA	methicillin-resistant *S. aureus*
MSSA	methicillin-susceptible *S. aureus*
SEs	staphylococcal enterotoxins
SFP	staphylococcal food poisoning
TSS-like	toxic shock syndrome
TSST-1	like toxic shock syndrome toxin-1

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