Staphylococcus aureus: significance, control and rapid detection across milk chain

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Abstract: *Staphylococcus aureus* is a major pathogen of public health concern with dominant role in food poisoning outbreaks, nosocomial and community-acquired infections, and also in bovine mastitis. Milk being a rich nutritious source for growth and proliferation of pathogenic species is prone to bacterial contamination, which can lead to spoilage and food poisoning. Toxin production, heat resistance, biofilm formation, antibiotic and lysozyme resistance are among the characteristics that contribute to *S. aureus* pathogenicity. *S. aureus* and its enterotoxins in food remains a daunting challenge, despite global efforts towards its mitigation. There is a need for rapid and cost-effective on-site detection of *S. aureus* in milk to prevent its transmission. The article outlines the significance of *S. aureus* in milk chain, its ability to adapt to environmental stresses, possible mitigation and rapid detection strategies; that may help to curtail its presence in milk chain and linked food poisoning episodes; besides ensuring food quality and consumer safety.

Keywords: Dairy; Pathogen detection; Food-poisoning; Food-borne illness; Food safety; Mastitis; Milk; *Staphylococcus aureus*; Antimicrobial resistance

Abbreviations: AuNPs, gold nanoparticles; Aw, Water activity; CFU, colony forming unit; D value, decimal reduction value; dsDNA, double stranded DNA; FDA, Food and Drug Administration; LAMP, Loop Mediated Isothermal Amplification; MRSA, Methicillin Resistant *Staphylococcus aureus*; *S. aureus*, *Staphylococcus aureus*; WHO, World Health Organization

Introduction

With globalization and growing awareness among consumers for variety and quality in foods, there is surge in food trade across borders resulting in need for processed and packaged food with extended shelf life. With this expanding food supply chain, the potential for spread of food borne pathogens is high and demands attention. Unsafe food can lead to episodes of food poisoning, malnutrition, economic losses due to rejections, withdrawals etc. and consumer dis-satisfaction. Food safety remains one of the most important global health issues, and food-borne diseases caused by microbes are a widespread public health concern. *S. aureus* is amongst one of the leading cause for food poisoning and other infections and is a major focus for public health programs worldwide (Xihong et al. 2013). *S. aureus* mediated food poisoning episodes are routinely witnessed globally. Recently, Fusco et al. (2020) documented past 20 years data for the food poisoning outbreaks linked to consumption of milk and milk products contaminated with *Staphylococcus* spp. Although *S. aureus* has been associated with food poisoning in various foods, but Milk has been shown to be at risk for *S. aureus* contamination (Gill et al. 1994a; Xie et al. 2021). This is because milk acts as rich media for growth and proliferation of pathogenic species, which can lead to spoilage and food poisoning (Girma et al. 2014). Intoxication by staphylococcal enterotoxins is one of the most common causes of food poisoning outbreaks originating from consumption of raw milk or products made from it (Necidová et al. 2019). The global dairy industry is transforming and it relies heavily on the implementation of strategies to improve and strengthen milk process optimization. Different standards have been laid down by global regulatory agencies for *S. aureus* permissible limits in milk and milk products. For example, as per European Commission, the limit of *S. aureus* in raw milk for drinking and production is <500 cfu/ml and <2000 cfu/ml, respectively (<https://ec.europa.eu/food/safety/>)

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international_affairs/trade_en; Hillerton et al. 2004). The permissible limits for *S. aureus* in milk and milk products by various regulatory bodies have been shown in Table 1.

***Staphylococcus* – general features**

Genera *Staphylococcus* refers to Gram positive, cocci-shaped bacteria with characteristic grape-shaped morphology. They are ubiquitous in nature and found in air, water, and soil; but humans and animals serves as primary reservoirs (Hennekinne et al. 2018). The genus is classified into around 53 recognized species and 28 subspecies. *S. aureus*, *S. epidermidis* and *S. saprophyticus* are among the members that are most frequently associated with human infections (PHE-NHS, 2020). Staphylococci are grouped into coagulase and non-coagulase producers. Coagulase negative staphylococci are largely non-pathogenic in nature (Argaw et al. 2015). *S. aureus*, one of the most popular members of *Staphylococcus* genera is a non-motile, non-spore forming, facultative anaerobic, catalase and coagulase producing potential human and animal pathogen having an inherent ability to adhere to epithelial surfaces. It is also among one of the leading causes of bovine mastitis (Sheet et al. 2016). In addition to the coagulase, *S. aureus* produce several heat stable enterotoxins (eg. enterotoxin A) responsible for food poisoning; several cell membrane targeting toxins (alpha, beta, gamma, and delta) (Kashif et al. 2019); and other extracellular proteins, such as, hemolysins, and leukocidins. Classical staphylococcal toxins (SE-A to SE-E) are responsible for more than 90% of *S. aureus* food poisoning outbreaks (Wang et al. 2018). Besides enterotoxins, other toxins like exfoliative toxin A and B, and toxic shock syndrome toxin, are also produced by *S. aureus* (Fagundes and Oliveira, 2004). Further, they possess many surface proteins, which initiate infection by sticking (adhesion phase) to the tissues; and a variety of enzymes, such as proteases, lipases, and hyaluronidases that allow the bacteria to enter (invasion phase) and destroy tissues and spread (evasion phase) to nearby tissues during the infection process (Yaniarti et al. 2017; Maikranz et al. 2020). Heat resistance, toxin production, biofilm formation, antibiotic resistances are among the many characteristics that contribute to pathogenicity of *S. aureus*. In healthy host, *S. aureus* colonizes the nasal mucosa and skin but its opportunistic entry into the bloodstream or internal tissues can cause several lethal infections including hard-to-treat hospital acquired infections (Abril et al. 2020). Presence of lactose phosphotransferase systems, as revealed by genome sequence analysis enables *S. aureus* growth in milk. Staphylococci also requires B vitamins (thiamine and nicotinic acid) and inorganic salts for efficient growth. Glutamic acid, leucine, and tyrosine are essential for enterotoxin production, however, are not required for growth (Medvedova and Valik, 2012).

Prevalence and significance of *S. aureus* in dairy sector

Milk chain right from production to processing and storage involving milking animal, animal handlers, milking environment,

utensils, industry pipe lines, packaging and storage environment can contribute to entry of *S. aureus* in raw and processed milk. Presence of *S. aureus* in milk chain has considerable impact on overall quality, consumer safety, demand and global supply chain. Animals and animal derived food products have a considerable impact on public health. Animal derived food may be infected with one or more preformed staphylococcal enterotoxins, which can cause human disease (Pal et al. 2020). *S. aureus* can make its way to milk and milk products through air, dust, waste, water, milk, human personnel or animal udders. A large number of factors such as direct usage of raw milk, improper or sub-pasteurization treatment, post-pasteurization contamination, resistance development etc. may contribute to reporting of *S. aureus* in processed milk products. Milk serves as a rich nutrient source for *S. aureus* growth and enterotoxin production. *S. aureus* including multi drug resistant variants are frequently reported in raw milk, processed milk and milk products (Fletcher et al. 2015; Yehia et al. 2019; Zeinhom and Abed, 2021; de Silva Abreu et al. 2021) indicating prevalence of *S. aureus* in milk chain. Frequent reports of *S. aureus* in milk products from across the globe indicate its capability to easily transfer from raw milk to processed milk products.

Staphylococcus enterotoxins related foodborne outbreaks revealed food handling as the most likely contamination source because the isolated *S. aureus* strains were common between food handlers, foods, and/or patient specimens (Johler et al. 2013). Biotyping in combination with phage typing has been proposed to be a useful tool for tracing origin of *S. aureus* strains from food of animal origin (Gill et al. 1994b). Cows suffering from subclinical mastitis are increasingly considered as alternative reservoirs of *S. aureus* leading to contamination of dairy production and processing chain. The *S. aureus* infections in cattle (clinical and subclinical mastitis) are responsible for the reduced milk yield, spoiled milk, poorer milk content, unstable taste, reduced milk processing, lower shelf life, and decreased yield of milk products (Pal et al. 2020). These outcomes lead to huge damage to economy and livestock owners. As recently reported by Giri et al. (2020), mastitis causes nearly 2.37 billion INR losses annually to India's dairy industry. Subclinical mastitis accounted for roughly 70% of the total loss.

S. aureus along with other staphylococci is known to produce biofilms, which contributes to its tolerance to host defense mechanisms and environmental stress. Antimicrobial treatment primarily employed to control *S. aureus* in milking animals contributes to development of antimicrobial resistance in *S. aureus* and other pathogenic strains. *S. aureus* readily acquires resistance to antimicrobials, resulting in persistent non-curable udder infections that often lead to culling of infected animals. Because of its notorious ability to develop resistance to the commonly used as well as last resort antimicrobials and development of multi drug resistant strains, antimicrobial resistance in *S. aureus* is of principal value in human and animal

Table 1 Overview of microbiological criteria/specifications for milk and milk products in context to *Staphylococcus aureus*

Products	FSSR, 2017		FDA Republic of Philippines, 2013		EC, 1992		BIS IS 1165:2002; IS 14433:2007; IS 1806: 2018; IS 1656: 2007		FSANZ, 2018	
	m	M	m	M	M	M	M	M	M	M
Skimmed milk powder	-	-	-	-	-	-	-	10 ³ /g	-	-
Pasteurized Milk/Flavoured Milk	Methylene Blue Reduction Test (MBRT) applicable at manufacturing unit shall not decolorized in 5 h	-	-	-	-	-	-	-	-	-
Pasteurized cream	-	-	-	-	-	-	-	-	-	-
Sterilized/ UHT/ Flavoured milk/ Evaporated milk	Absent/1g	-	-	-	-	-	-	-	-	-
Sterilized/ UHT cream	-	-	-	-	-	-	-	-	-	-
Sweetened condensed milk	<10/g	-	-	-	-	-	-	-	-	-
Butter (Unpasteurized milk) and/or Unpasteurized milk products	Absent/1g	-	-	-	-	-	-	-	10/g	10 ² /g
Pasteurized Butter	10/g	50/g	10 ² cfu/ml	-	-	-	-	-	-	-
Milk powder; SMP, Dairy Whitener; Cream powder; Ice Cream Mix powder; Lactose; Whey based powder; Butter Milk powder; Casein powder	10/g	10 ² /g	-	-	10cfu/g	10cfu/g	Absent/0.1g *	-	-	-
Infant Milk Food, Infant Formulae, Infant Milk Substitute	Absent/0.1g	-	-	-	-	-	Absent/0.1g	-	-	-
Ice Cream, Frozen Dessert, Milk Lolly, Ice Candy	10/g	10 ² /g	10cfu/g	10 ³ cfu/g	10cfu/g	10cfu/g	-	-	-	-
Processed Cheese/ Cheese Spread	10/g	-	10cfu/g	10 ³ cfu/g	-	-	-	-	-	-
Fresh Cheeses/ Cheddar/ Cottage /Soft, Semi Soft cheese from heat treated milk	10/g	10 ² /g	10 ² cfu/ml	10 ³ cfu/g	10 ² cfu/g	10 ³ cfu/g	-	-	10 ² /ml	10 ³ /ml
Fermented milk products: Yoghurt, Dahi, Chakka, Shrikhand	10/g	10 ² /g	10cfu/ml	10 ² cfu/ml	-	-	-	-	-	-
Paneer/ Chhanna/ Chhanna based sweets	10/g	10 ² /g	-	-	-	-	-	-	-	-
Khoa/ Khoa based sweets	10 ² /g	10 ² /g	-	-	-	-	-	-	-	-
Malted milk food	-	-	-	-	-	-	Absent/0.1g (with cocoa powder / without cocoa powder)	-	-	-
Milk cereal based complementary foods/ follow-up formula – complementary food	Absent/0.1g	-	-	-	-	-	Absent/0.1 g	-	-	-

m = Represents an acceptable level and values above it are marginally acceptable in terms of the sampling plan. M = A microbiological criterion which indicates unsatisfactory or potentially hazardous quality. Values above M are unacceptable in terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection. * = Spray dried milk powder from standardized milk.

FSSR, Food Safety and Standards Regulations; FDA, Food and Drug Administration; EC, European Commission; BIS, Bureau of Indian Standards; FSANZ, Food Standards Australia New Zealand

medicine (Abdi et al. 2018). Methicillin resistant *Staphylococcus aureus* (MRSA) causes mild to severe infections in humans and animals worldwide (Boswihi and Udo, 2017). Reports of presence of MRSA in foods of animal origin raise concerns about its transmission to humans (Basanisi et al. 2017). Dairy cows having subclinical mastitis can transmit MRSA to milk even without changing the milk organoleptic properties and thus can be linked to its spread to people associated with cattle and milk processing (Basanisi et al. 2017) and also consumers.

Resistance to processing conditions and microenvironment

S. aureus has been shown to survive across wide range of environmental stresses (cold, low water activity (Aw) etc.) and its ability to rapidly adapt to environmental fluctuations determines its pathogenicity (Alreshidi et al. 2015). Each environmental stress is known to influence the expression of several cellular processes, virulence factors and antimicrobial resistant determinants that endows the organism with an ability to survive under stress conditions (Anderson et al. 2006). Thermal tolerance among *S. aureus* is strain specific and varies with many factors. The 'D' value (decimal reduction value) for *S. aureus* has been shown to vary with the media/food matrix in which it is present. Likewise, *S. aureus* was reported to display thermal resistance in order: skim milk > Cheddar cheese whey > phosphate buffer > whole milk (Walker and Harmon, 1966). Recently, Yehia et al. (2019) reported heat resistant *S. aureus* strains from pasteurized camel milk sold in Riyadh City, Saudi Arabia. The enterotoxin producing strain survived heat treatment at 90°C for 2 minutes. Earlier, Montanari et al. (2015) too reported *Staphylococcus sp.* survival at 80°C for 20 minutes. Another study documented that the pasteurization parameters failed to eliminate *S. aureus* from different dairy products. Optimally, 80°C/20 minutes is required to kill *S. aureus* in dairy products (Yaniarti et al. 2017).

S. aureus is resistant to freezing and survives well in food stored below -20°C. Prolonged cold stress induced different metabolomic and proteomic profile at the mid-exponential growth phase of *S. aureus*, compared to those incubated at 37°C. Several (nine) cytoplasmic ribosomal proteins and citric acid were up-regulated in cells adapted to cold-stress. Changes in metabolic homeostasis and protein profile are critical for survival under cold stress (Alreshidi et al. 2015). Pathogens including *S. aureus* possess several additional structural and biochemical features that allow them to resist the host gastrointestinal defense stress (Bera et al. 2005; Panwar et al. 2020). According to recent reports, oral cavity has a high prevalence of *S. aureus* and MRSA (Donkor et al. 2020). Earlier, Vanzato et al. (2010) reported *S. aureus* and MRSA from the oral cavity of healthcare workers. Biofilm forming potential of *S. aureus* may be one of the factors contributing towards prevalence of *S. aureus* in oral cavity.

Staphylococcus sp. has above average pH tolerance and grows over pH range of 4.0-10.0, with an optimum of 6-7. Genes for urease enzyme such as *ure* have been found to be essential for survival under acid stress, which might act by regulating pH homeostasis and urea utilization (Zhou et al. 2019). During acid stress, genes for the arginine deiminase pathway such as *arcA/B/C* becomes activated and lead to concomitant production of ammonia which maintains pH homeostasis and ATP which drives intracellular proton transport (Grosser et al. 2018). Similar function is performed by F_1F_0 -ATPase which actively exports protons and regulates pH balance (Pi et al. 2009). Staphylococci also show bile resistance but the mechanisms are not well explored. Recently, *S. aureus* has been shown to have *mnhF* gene, which has a role in providing bile resistance. This operon is known for exchange of protons with Na^+/Li^+ or K^+ and thus *mnhF* gene is supposed to confer bile resistance by active transport of the bile salts (cholate) from *S. aureus* (Vaish et al. 2018).

Staphylococcus species displays complete lysozyme resistance, which helps them persist and successfully colonize the skin and mucosal areas of humans and animals. In *S. aureus*, an integral membrane protein *OatA* codes for O-acetylation at C6-OH of peptidoglycan muramic acid. *OatA* was identified as the molecular basis for high lysozyme resistance in staphylococci (Bera et al. 2005). *S. aureus* is able to survive in potentially dry (dessication resistance) and stressful environments, such as skin, nose, and inanimate surfaces. Chaibenjawong and Foster (2011) identified several genetic determinants (*clpX*, *sigB* and *yjbH*), playing role in dessication tolerance. *S. aureus* being highly osmo-tolerant can thrive well in foods with reduced Aw (Shebuski et al. 2000).

S. aureus is halotolerant and can grow well in the presence of high salt concentrations, such as on skin surfaces which often have high NaCl concentration (10%). Under low salinity, immediate influx of small solutes relieve physical stress; whereas under high salinity, water efflux is counterbalanced by an increase of compatible solutes such as proline, glutamate, glycine betaine, ectoine and trehalose (Omotoyinbo et al. 2017). *S. aureus* can cope with osmotic environments by accumulating osmoprotectants such as, proline and glycine betaine (Hajmeer et al. 2006).

S. aureus control in the milk chain

As discussed and established by scientific data, *S. aureus* is prevalent in dairy environment, animals, animal handlers, raw milk and even makes its way to processed milk and milk products due to its adaptive nature. In order to check *S. aureus* and its enterotoxins in dairy chain, antibiotics are employed which further adds to developing antibiotic resistance. Moreover, available and popular mitigation strategies cannot be applied to milk and milk products without compromising consumer preferences and safety guidelines. Hence, there is a need for natural, safe and cost-effective strategies that can help to minimize *S. aureus* in

milk chain without selecting resistant strains, without targeting commensal flora and without compromising general food sensory characteristics. The section discusses few such emerging promising strategies in brief.

Several probiotic strains have been shown to be effective against mastitis causing pathogens (Assis et al. 2015). Probiotics can also stimulate the immune response in cattle and can modulate internalization of *S. aureus* within the host cells (Zatout et al. 2019). Some *Lactobacillus* spp. strains produce metabolites that prevent *S. aureus* adhesion to udder tissues; resist pathogenicity by producing hydrogen peroxide, altering the host immunity, and competition for nutrients. Sharma et al. (2017) documented the antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against several food-borne and human pathogens. Probiotics and their metabolites can also prevent biofilm formation and dissociate early stage and mature pathogen biofilms. In one such study, *Lactobacillus* spp. strains from goat milk origin showed antagonistic activity against growth and biofilm formation by *Pseudomonas aeruginosa* and *S. aureus* (Singh et al. 2018). Few probiotic based formulations have been recently introduced for management of mastitis in dairy animals. Provilan ANNAA+ Optimum Care spray™, a plant origin probiotic strain formulation have been introduced for preventing mastitis in dairy cows (<https://ingenious-probiotics.com>). Another probiotic formulation, Aptamama (*L. salivarius* PS20, recently launched by Danone promises to reduce mastitis incidence amongst healthy lactating mothers by 59% (<https://www.nutraingredients-asia.com>).

Phytocompounds have also found to be effective in controlling bovine mastitis causing *S. aureus* isolates (Mordmuang et al. 2019). Due to the presence of phenolic and ethanolic compounds, plant extracts displays effective antioxidant and antimicrobial effects. The combination of these phenolic plant extracts displayed potent bactericidal effect against *S. aureus* biofilms (Gomes et al. 2019). Also, plant ethanolic extracts reduced *S. aureus* internalization into bovine mammary cells (Mordmuang et al. 2019). Active component of *Eucalyptus globulus* has been shown to display potent anti-biofilm and anti-quorum sensing activities against MRSA (Merghni et al. 2018). Co-application of phytocompounds and antibiotics can also be an effective strategy against *S. aureus* due to synergistic action of the two. Bacteriophage presents a promising approach for controlling *S. aureus* infection in dairy (Iwano et al. 2018). Phage K can efficiently lyse staphylococci and can be used prophylactically against *S. aureus* infections. Another phage MSA6 is a potential universal agent against *Staphylococcus* spp. (Kwiatkiewicz et al. 2012). Nanoparticles have gained attention as effective antimicrobial against *S. aureus* and mastitis in dairy animals. Encapsulation of antibiotics with nanoparticles enhances their activity and targeted site delivery. Lysostaphin, a potential *Staphylococcus* inhibiting enzyme cleaves penta-glycine bridge of *S. aureus* cell wall.

Recently, recombinant lysostaphin has been found to cure 95% *S. aureus* udder infection (Aqib et al. 2021).

Vaccination is used as a powerful strategy for prevention and even eradication of infectious diseases; besides restraining MDR bacteria including *S. aureus*. The strategy is promising as a valid alternative therapeutic to antibiotics in animals and humans; besides possessing an added advantage of being free from resistance development (Sharma et al. 2018). Vaccines turn out to be the most effective measure to prevent bovine mastitis. Commercially available vaccines against *S. aureus* include Lysigin® and Starvac®. Besides the above mentioned strategies, proper precautions like maintenance of hygiene during milking and other processing steps can considerably reduce *S. aureus* entry in milk and milk products.

***S. aureus* – traditional and rapid identification approaches**

Identification and characterization of *S. aureus* is primarily carried out on basis of characteristic morphological and biochemical properties during its culture. Isolation of staphylococci from milk involves initial enrichment in common enrichment media (eg. Giolotti-Cantoni Broth, Tryptic Soy Broth) followed by plating over selective and differential media (eg. Baird Parker). Candidate staphylococci displaying typical colony characteristics over differential media are subjected to staining for morphological identification as non-motile, Gram positive cocci with grape like cluster arrangement. Biochemical identification is primarily based on catalase, and coagulase production, agglutination tests (clumping factor, protein A), and sugar fermentation tests that besides identifying *S. aureus*, also differentiates it from other closely related staphylococcal species. *S. aureus* gives positive reaction for catalase, citrate, urease, coagulase production, lipid hydrolysis, and mannitol fermentation; and have thermostable deoxyribonuclease (Tang and Stratton 2010).

Rapid detection of any potential food pathogen with high sensitivity and reproducibility is of significance for ensuring food quality and safety. Polymerase Chain Reaction (PCR) methods (PCR, Real Time Quantitative PCR) offers ability to rapidly identify *S. aureus* on basis of unique DNA sequences targeted *via* single-plex and multi-plex PCR reactions. In recent years, Loop mediated isothermal amplification (LAMP) assay has emerged as a simple, rapid and cost-effective DNA based tool for detection of pathogens. The results of LAMP assay can be visualized by naked eyes (Tian et al. 2018). Although the DNA based identification is quick, sensitive and reproducible; it requires skills, high-end infrastructure; besides need for enrichment, growth in liquid or solid media, and selection of pure colonies. This is followed by DNA isolation, PCR amplification and electrophoresis (not required for qPCR) steps, which slows the rapid identification, especially when it comes to a perishable commodity, such as milk. Some other rapid identification strategies have been

proposed and are likely to have application in early detection of *S. aureus* in milk.

Recently, a one-step enzyme free, label free, fluorometric strategy (Target Inhibited Fluorescence Signal Recovery) based on nanometal surface energy transfer between carbon dots and gold nanoparticles (AuNPs) has been developed for facile detection of *S. aureus*. The proposed strategy has enhanced detection limit (10 cfu/mL) for *S. aureus* (Yao et al. 2021). Hu et al. (2021) developed a Nanobodies (Nbs) sandwich ELISA based immunoassay for screening of *S. aureus*. The detection ability was verified by detection of 10 cells per ml of *S. aureus* in 8hr enriched milk samples.

Phage endolysins contains N-terminal catalytic domain and C-terminal cell wall binding domains. Lysin cell wall binding domains (CBD) are substrate specific and bind bacterial cell wall receptors by non-covalent bonds. In a recent study, phage lysin cell wall binding domains along with immunomagnetic particles were used for *S. aureus* detection in milk (Yu et al. 2016). M13 phage has also been explored for early pathogen detection. In one such study, surface enhanced Raman scattering gold nanoprobe based on pIII protein of M13 phage was used for early detection and de-activation of *S. aureus*. The probe could quantify *S. aureus* loads within range of 10^1 - 10^6 cells/mL. Furthermore, this probe has antibacterial potential towards *S. aureus* (Wang et al. 2021).

Recently, a new strategy using modified propidium monoazide (PMA) dye combined with recombinase aided amplification (RAA) was proposed for the rapid and real-time detection of viable *S. aureus* in milk. Xie and co-workers (2021) developed a PMAxx-RAA combination in a microplate assay for *S. aureus* detection in milk. Following enrichment, limit of detection for viable *S. aureus* ranged from 10^2 cfu/mL (3 h enrichment) to 10^1 cfu/mL (6 h enrichment) in spiked milk samples (Xie et al. 2021). Liu et al. (2021) standardized a flow cytometry based method for *S. aureus* detection in milk and milk powder. In this methodology, fluorescently labeled antibodies and propidium iodide were used for selective detection of viable *S. aureus*. Following a 5 hour enrichment period, the method could detect around 7 *S. aureus* cells/mL in milk. In another approach, Yang et al. (2021) developed magnetic aptamer biosensor based on personal glucose meter for food pathogen detection. The biosensor could detect *S. aureus* with least detection count of 2 cells/mL. Another biosensor based approach involved design of an electrochemical biosensor based on triple helix molecular switch for detection of various pathogens in food and environment. This electrochemical biosensor can broadly detect *S. aureus* within dynamic range from 30 to 3×10^8 CFU/mL and having least detection count of 8 cell per unit sample (Cai et al. 2021).

Conclusions

Staphylococcus aureus has diverse metabolic potential and it often causes diseases in both animal and humans. *S. aureus* resides in different host body parts and food types, but one of its important sources is milk, which is widely consumed, and thus *S. aureus* becomes an important food pathogen. It manifests disease through its toxins which vary in their nature and action depending and they frequently have mechanisms to either evade or overwhelm the host immune system. Additionally the pathogen is resistant to different types of external stress conditions and thus spoils a range of food items including the processed ones. Due to its genomic plasticity and multiple drug resistance, it features in WHO's top list of microbes for which antibiotics are urgently needed. Recently, non-antibiotic interventions are being explored as alternative strategies against it, which are yet to become pharmacological practice. High throughput, on-site rapid detection of *S. aureus* present in low cell counts in milk is also very important to prevent food poisoning outbreaks and other clinical conditions. Multi-dimensional understanding of *S. aureus* pathology, reservoir-transmission dynamics and rapid detection would help in careful mitigation of the issue of *S. aureus* inside hosts and in overall dairy sector.

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